



UNIVERSITY OF NAIROBI

FACULTY OF SCIENCE AND TECHNOLOGY

**SPECTROSCOPIC AND VOLTAMMETRIC DETERMINATION OF BISPHENOL A
EXPOSURE TO POLYCARBONATE PLASTIC USERS**

BY

SERAH WANJIKU NJOROGE

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Degree of Masters of Science in Analytical Chemistry of the University of Nairobi**

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DECLARATION

I declare that this thesis is solely my original work. It has not been submitted to any other university for the purposes of research. In instances where reference to other scholars' work has been used, clear acknowledgment, as well as referencing, has been done following the guidelines set by the University of Nairobi.

Signature..... 

Date...12/08/2023.....

Serah Wanjiku Njoroge

I56/35917/2019

The thesis has been submitted with our approval as university supervisors:

	Signature	Date
Dr. Immaculate N. Michira Department of Chemistry University of Nairobi P.O Box 30197-00100 Nairobi, Kenya		13/08/2023
Dr. Deborah A. Abong'o Department of Chemistry University of Nairobi P.O Box 30197-00100 Nairobi, Kenya 	14/08/2023
Dr. Peterson Guto Department of Chemistry University of Nairobi P.O Box 30197-00100 Nairobi, Kenya	... 13/08/2023.....

DEDICATION

This thesis is a special dedication to my dear sister Beth Wambui Njoroge, my friend Patrick Mwangi, my other siblings Anthony, Judy, Mary and Jane for their moral and financial support, throughout my studies.

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Above all, I specially thank Almighty God for giving me the gift of life, wisdom, knowledge and grace to pursue and conduct my research project

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ABSTRACT

Recently endocrine disorders have become a big concern. Endocrine-disrupting chemicals are contaminants interfering with hormone biosynthesis, metabolism, or change how the endocrine system works. Bisphenol A is an endocrine disruptor having weak estrogenic activity and the body easily mistakes it for the native estrogen. Continued use of products made using bisphenol A exposes user to risk of exposure above threshold levels leading to endocrine disruption. Polycarbonate containers are known to be major sources of bisphenol A that leaches to the container's content through hydrolysis. This study aimed at monitoring levels of bisphenol A exposure to polycarbonate users through two novel techniques namely Spectrophotometric and Voltammetric techniques. The polycarbonate samples used were baby feeding bottles and portable water bottles. The Spectroscopic method employed a diazotization reaction procedure while the voltammetric one involved monitoring electrochemical response to varying bisphenol A concentration on a multiwalled carbon nanotube modified glassy carbon electrode. Polymer classification was done using Fourier transform infrared spectroscopy. The Fourier transform infrared spectra of thin plastic solids were identified by scanning between, 500-4000 cm^{-1} for characterization. The resulting spectra were compared with those of standard polycarbonate plastics reported in the literature. Characteristic polycarbonate peaks at 2970, 1970, 1504, 1187, 1013, 1079 cm^{-1} associated with the $-\text{C}-\text{H}$, $\text{C}=\text{O}$, $\text{C}=\text{O}$, $\text{O}-\text{C}-\text{O}$, $-\text{CH}_3$ stretching vibrations respectively confirmed sampled bottles were polycarbonates. Temperature effect, contact time and food type on the extent of Bisphenol A leaching was determined. Microsoft Excel and Origin software 2019b were used to analyze the results. Using the diazotization technique, the bisphenol A levels for all the sampled polycarbonate bottles in the various environments ranged between 0.14 – 3.23 $\mu\text{g}/\text{mL}$. Under the voltammetric techniques, electrode modification with the multiwalled carbon nanotube enhanced the bisphenol A oxidation current signals in phosphate buffer systems by more than 50% as compared to 5.8% in the ferricyanide systems. The bisphenol A levels obtained via the voltammetric methods for all the sampled bottles subjected to different environments were in the range 0.1 – 2.3 μM . All the samples surpassed the tolerable daily intake for BPA of 4 $\mu\text{g}/\text{kgbw}/\text{day}$ recommended by the European Food Safety Authority and this calls for serious consideration.

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UNITS OF MEASUREMENT

Measurement	Unit	Symbol
Amount of substance	Mole	Mol
Temperature	Kelvin	K
Current	Ampere	A
Time	Seconds	S
Mass	Kilograms	Kg
Mass	Grams	G
Mass	Micrograms	Mg
Potential	Volts	V
Temperature	Celsius	°C

LIST OF ABBREVIATIONS

BFBs	Baby feeding bottles
BPA	Bisphenol A
BPF	Bisphenol F
BPS	Bisphenol S
CV	Cyclic voltammetry
DMF	Dimethylformamide
DPV	Differential pulse voltammetry
EDCs	Endocrine disrupting chemicals
EFSA	European Food Safety Authority
FTIR	Fourier-transform infrared spectrometry
GCE	Glassy -carbon electrode
HPLC	High performance liquid chromatography
KNBS	Kenya National Bureau of Statistics
LOD	Limit of detection
LOQ	Limit of quantification
MWCNTs	Multi walled carbon nanotubes
PBS	Phosphate buffer
PC	Polycarbonate

PE	Platinum electrode
PVC	Polyvinyl chloride
SEM	Scanning Electron Microscope
USA	United States of America
UV-Vis	Ultra violet-visible
TDI	Tolerable Daily Intake
t-TDI	temporary Tolerable Daily Intake
WBs	Water bottles

CHAPTER 1: INTRODUCTION

1.1: Background information

Many substances ensuing from anthropogenic activities interferes with physiological roles of living organisms, and later on adversely affect the status of their health. Bisphenols, which are predominantly manufactured worldwide, are the primary substances within this category of compounds. The main type of Bisphenol is known as Bisphenol A (BPA), which is a chemical found in high quantities globally and has an impact on the environment. Bisphenol A, first manufactured in 1891 by a Russian chemist Alexander P. Dianin through chemical condensation reaction, was not utilized until its uses in the plastics business were distinguished during the 1950s (Goodman & Peterson, 2014). It is widely used in plastic containers production that are used for packaging of beverages and food, and this makes differently aged populations to be exposed to BPA in their usual activities of the day. Bisphenol A disrupts the endocrine system mimicking estrogen and thyroid hormone, acting as metabolic and immune disruptor. This implies that the health effects of BPA are adverse and this can be attributed to its potential to cause hormonal imbalance in natural systems (Chapalamadugu *et al.*, 2014). Because humans are continually exposed to BPA through various routes such as consumption, inhalation, and contact with the skin, it leads to negative health effects. Consequently, it is essential to conduct more extensive studies and make advancements in order to protect human health and ensure the environment is not compromised.

Many techniques have been employed for determining the levels of BPA in the environment, its fate and BPA's behavior, among the techniques include, capillary electrophoresis, gas chromatography coupled with mass spectrometry (GC-MS) (Tan & Mustafa, 2003), high performance liquid chromatography mass spectrometry (HPLC-UV-Vis) (Rodriguez *et al.*, 2019), enzyme linked immune sorbent assay (ELISA) and electrochemical methods. Pretreatment procedures have also been done for extraction in solid-phase, liquid-liquid extraction and immunoaffinity chromatography, HPLC-MS and ELISA for precisely determining BPA levels in complex matrices. These techniques are effective as they are rapid but are hindered by few limitations such as; they involve complex pretreatment process, they are time consuming, the instruments are not portable so not easy to carry out *in situ* analysis, expensive equipment's and

also they require trained personnel to operate the instruments (Vilarinho *et al.*, 2019). For these reasons electrochemical techniques are used as alternatives as they are less expensive, and portable.

1.2: Source and uses of Bisphenol A

When BPA was first synthesized in 1895, it was found to be a synthetic oestrogen, but currently the compound is utilized in hard polycarbonate plastics and epoxy resins (Prioleau & Gray, 2010). During the production process of polycarbonates, a monomer of BPA is mainly used (Bashir & Audu, 2020), with plastic additives. Approximately 95% of BPA manufactured is utilized in making polycarbonates and epoxy resins (Flint *et al.*, 2012). The remaining 5% is utilized in the ramification of merchandise, that consist of phenoplast resins, phenolic resins, unsaturated polyester resins, antioxidants and inhibitors for polyvinyl chloride manufacturing, ethoxylated BPA, components used in thermal paper manufacturing, changed polyamide, compounding factor used in manufacturing of automobile tires, flame retardants, automobile system, optical media consisting of digital video disks, electric gadget, production, and so forth (Huang *et al.*, 2012). Polycarbonates mainly find use as food contact products i.e., recyclable beverage bottles, feeding bottles for children and storage containers. Internal coatings for various food and containers for beverages and electronic laminates, use epoxy resins, as they have a high ability to resist heat and chemicals (Abraham & Chakraborty, 2020). The chemical formula for Bisphenol A is $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OH})_2$ shown in (Figure 1.1).

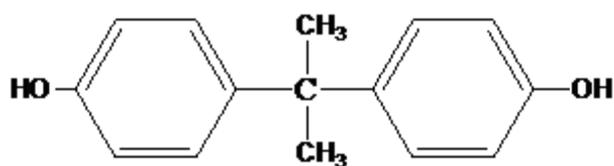


Figure 1:1 Chemical formula for Bisphenol A

Source (Krishnan *et al.*, 1993)

1.3: Production of Bisphenol A and physical properties

Bisphenol A (Figure 1.1), IUPAC name: 4,4'-Dihydroxy-2,2-diphenylpropane 2,2-bis(4-Hydroxyphenyl)propane 4-[2-(4-Hydroxyphenyl)propan-2-yl]phenol, EINECS name: 4,4'-Isopropylidenediphenol, CAS name: Phenol, 4,4'-(1-methylethylidene) bis- (EFSA, 2015) Is a

chemical compound that is not produced naturally and its presence in the environment is in consequence of anthropogenic activities. Therefore, BPA is synthesized through condensation of acetone (hence the suffix A in the name) and two phenol equivalents catalyzed by a strong acid (Figure 1.2), for example, Sulfonated polystyrene resin or Hydrochloric acid (Geens *et al.*, 2011).

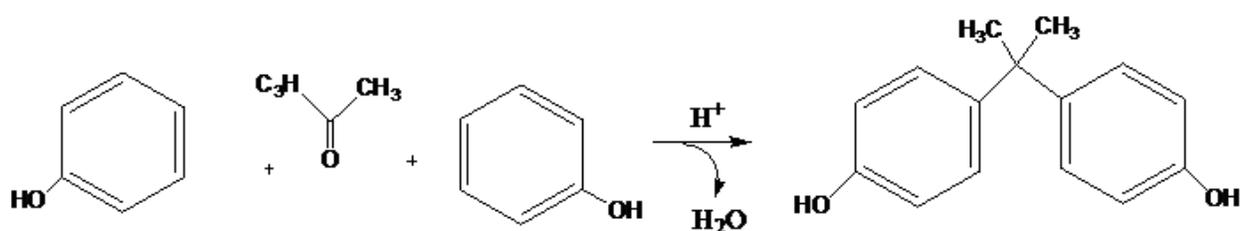


Figure 1:2 Synthesis of Bisphenol A

The National Institutes of Health Hazardous Substances Data Bank in the United States of America identified a chemical with a log Kow of 3.64 ± 0.32 that quickly degrades in air owing to photo-oxidation, resulting in a short half-life (Lu *et al.*, 2013). Reports indicate BPA detection in water, air, soil, wildlife and humans in spite of having a short half-life and its slight bioaccumulation ability (Corrales *et al.*, 2015). Literature indicates a low vapor pressure, high melting point and BPA has a slight dissolution in water, resulting in its low volatility (Odera, 2019). With a pKa value of between 9.59 and 11.30, BPA majority exists in a non-ionized state in a liquid media having a pH less than 7. With a fairly strong fluorophore, BPA molecule can be detected using its fluorescence. It is a relatively weak chromophore, and being ultraviolet (UV) sensitive, detection is lower than that of fluorescence detection (EFSA, 2021). The BPA physical properties are shown in table 1.1 below.

Table 1:1 Bisphenol A physical properties

Physical properties	Values
Melting point	150 -157°C
Boiling point	398°C at 760mm Hg
Liquid molar volume (cm ³ /mol)	398°C at 760mm Hg
Water solubility at 20-25°C	120 -300 (mg/L)
Henry constant	8.7 x 10 ⁻¹⁰ to 3.96 x 10 ⁻⁷ mm Hg at (20-25) °C
Henry constant	1.0 x 10 ⁻¹⁰ Atmosphere cubic meter /mol (Atm.m ³ /mol)
Diffusion coefficient (in water)	6.34 × 10 ⁻⁶ cm ² /s
Specific gravity	1.060 -1.195 mg/L at (20-25)°C
Logarithm octanol-water partition coefficient (Log K _{ow})	2.20 -3.82

Source: Staples *et al.*, 1998

1.4: Statement of the problem

Bisphenol A, is a major ingredient in the plastic making industry and it's utilization has risen steadily throughout the world, hitting to more than 10 million tonnes annually (Fonseca *et al.*, 2022). In particular, it is used in the manufacture of the class of plastics called polycarbonates (Vandenberg *et al.*, 2007). These plastics are light in terms of weight, resistant to shatter and electricity, highly durable, have good optical clarity and have high tolerance to heat (Shrinithiviahshini *et al.*, 2014). Polycarbonate plastics are commonly used to make food containers, water and juice jags among others (EFSA, 2015). Research has shown that BPA is an endocrine disruptor (Krishnan *et al.*, 1993) and is able to mimic body estrogen leading to adverse effects. Obesity, early girl maturity, cancer is among some of the health effects of this compound (Lang *et al.*, 2008). Studies have shown BPA can leach from PC materials through hydrolyzation processes. Bisphenol A has caused most concern because it exhibits oestrogenic activity, specifically at tender stages of life. It interferes with foetal development leading to low birthweight and inhibited maturation in body organ systems as a result of interference with organogenesis and histogenesis processes (Quirós *et al.*, 2019). In fact, more than 40 countries are on the verge or have phased out the use of plastics made using BPA to feed young children in their countries (Mahamuni &Shrinithiviahshini, 2017).

Unfortunately, in developing countries such as Kenya, polycarbonate plastics are still being used to manufacture polycarbonate objects. A popular use of polycarbonate materials is in the manufacture of baby feeding bottles, portable water-carrying bottles of different sizes and shades. (Rotimi *et al.*, 2020). They are also used for pure water used in dispensers. Such usage and the fact that there are no regulatory measures in place exposes the innocent PC users to the dangerous xenoestrogenic compound due to possible leaching into the bottle contents.

Further, the extent of BPA leachability depends on the pH, temperature, contact time and is likely to aggravate the extent of exposure (Johnson *et al.*, 2015). It is a common habit to subject baby feeding bottles to high temperatures for sanitization. It is also common practice to use PC portable water carrying bottles for juices. These practices can easily enhance BPA leachability. Consequently, it is imperative to monitor levels of exposure of BPA to polycarbonate plastic users depending on the usage of the said bottles and to advise the users accordingly.

1.5: Overall objective

The main aim of this study was to monitor the extent of bisphenol A leachability from polycarbonate plastics into water, beverages and foods using spectroscopic and voltammetric techniques.

1.5.1: Specific objectives

Were to:

1. Characterize sampled bottles as polycarbonate or not.
2. Determine the levels of BPA leached into different matrices at different temperatures, pH levels and contact times using UV-Vis spectrometry.
3. Modify glassy carbon electrode using multiwalled carbon nanotubes
4. Apply the modified glassy carbon electrode to determine the levels of BPA leached into different matrices at different temperatures, pH levels and contact times using voltammetry.

1.6: Justification of the study

Good health is invaluable for all human beings and has direct impact on any nation's social-economic and political growth and for this reason it is a major part of the sustainable development Agenda. Previous studies indicate an increased BPA exposure risk to humans (Vandenberg *et al.*

2013). The public interacts with polycarbonate plastic goods containing BPA and its polymeric forms in any everyday operation such as in compact disks (CDs), digital video disks (DVDs) and polythene bags, (Johnson *et al.*, 2015) which the average individual handles on a regular basis. Due to advanced Bisphenol A effects, it is important to assess its existence in baby feeding bottles and water bottles and keep a track to decrease its exposure.

Several health effects have been attributed to BPA exposure, as an example it was established that there is a substantial rise in cases of breast and prostate cancer and sexual dysfunction cases (Yin *et al.*, 2011, Castillo, 1997). Cases of thyroid gland dysfunction are also rapidly increasing, recurrent miscarriages, oxidative stress and inflammation in postmenopausal women are other conditions that affect several individuals (Rochester, 2013). There was also a situation in which baby girls developed mammary glands early, genitals, and early menstruation, this is of great concern since it is an inconsistency and these young ladies properly grow due to the exposed difficulties past their psychological capacities and can likewise be seen by their companions as freaks and that can psychologically influence them (Braun *et al.*, 2009).

Due to these already known health effects associated to BPA there is need to conduct more studies to monitor BPA leaching into different matrices and document the results in order to educate the public on dangers related to BPA.

1.7: Significance of the study

The quest to reap the maximum benefit of plastic polymers without compromising the health of humans or environment is of necessity (Siddique *et al.*, 2008). Major toxic pollutants are contained within plastic materials thus giving it the potential ability to pollute air, water, and land and human health (Teuten *et al.*, 2009). Toxic chemicals such as BPA that leach out of plastics during their usage as packaging or during drink storage are very harmful to both human health and wildlife (Meeker *et al.*, 2009). The study entails monitoring of BPA levels in polycarbonate (PC) plastics used as either baby feeding or water carrying bottles sampled from different supermarkets in Thika Town as a way of determining possible adverse exposure to the PC plastic end users.

1.8: Research hypothesis

To test whether or not bisphenol A can leach out from polycarbonate plastics under different environmental conditions such as temperature, pH and contact time.

CHAPTER 2: LITERATURE REVIEW

2.1: Bisphenol A

Bisphenol A (BPA) is an emerging environmental pollutant that appears as white crystals or pills or flakes (Abraham & Chakraborty, 2020). It is soluble in organic solvents whereas not very soluble in water (Yousif Hammad, 2015). The BPA's production and consumption worldwide is huge, with China producing the most each year at 3.8 million tons and having maintained this level since 2018. Globally BPA market is anticipated to hit 7.35 million tons by late 2023, with a growth rate of about 3% annually from 2017 to 2023 (Dong *et al.*, 2022). Bisphenol A is among the highly manufactured compounds globally (Alonso-Magdalena *et al.*, 2006). Additionally, BPA is used as a raw material in the manufacturing of polycarbonate plastics and epoxy resins (Hoekstra *et al.*, 2013). Studies done by Dodds and his colleagues in 1938, indicated the different bisphenols biological properties. In that study they studied estrogenic properties of bisphenol A, it's from that study that a conclusion was made on the presence of OH groups influencing the estrogenic capacity of bisphenols (Vilarinho *et al.*, 2019).

Thermo resistance property of polycarbonates (up to 145°C), their strength and hardness makes them to be used in the manufacture of water bottles, infant feeding bottles and household utensils (Abraham & Chakraborty, 2020). In the process of manufacturing polyvinyl chloride (PVC), BPA is used to remove extra hydrochloric acid thus performing a stabilization role. It has found extensive application in manufacturing of contact lenses (Vandentorren *et al.*, 2011). The BPA is additionally utilized in the production of food cans for packaging (Cwiek-Ludwicka and Ludwicki, 2014).

2.2: Bisphenol A leaching in baby feeding and water bottles

Bisphenol A is literally present almost everywhere thus it is used in many of our daily products lives. Because of two distinct processes, BPA can leach from polycarbonate plastics into aqueous foodstuff through diffusion. The BPA can also leach out of polycarbonates and move to the contents in the bottles when there is residual BPA, when the hydrolyzed polymer that is catalyzed by hydroxide is in contact with liquid foods and stimulants (Hoekstra & Simoneau, 2013).

Bisphenol A can also be released by aminolysis of polycarbonates by biogenic amines in the milk (Hoekstra & Simoneau, 2013).

Bisphenol A was detected to leach from the infant feeding bottles when filled with boiling water at a temperature of 95°C then at room temperature for 30 minutes. In this case, BPA was detected in the 0- 5.90 ppb range with 3.16, 5.20 and 5.90 ppb as mean amounts. Filling the infant feeding bottles with boiling water at 95°C accompanied by milk powder and held for 30 minutes, BPA leaching was greater, and it was found between the ranges of 0 - 7.06 ppb. 7.06, 3.6 and 3.2 ppb were the mean amounts detected (Johnson *et al.*, 2015). In an additional study carried out in 2019 using a biosensor using two different types of infant feeding bottles indicated that BPA leached more from plastic bottles into the water when they were subjected to higher temperatures (Lucia, 2019).

In a study conducted in 2010, It was found out that the rate of migration of BPA from new and frequently used bottles for infants made of polycarbonate indicated that temperature increase and prolonged use of the bottle improved polymer hydrolysis, leading to more extreme BPA migration into water (Nam *et al.*, 2010). Release of BPA from new infant feeding bottles at an average concentration of 0.03 and 0.13 g/dm³ at temperatures of 40 and 95 °C, respectively. There was an increase in BPA concentrations in water to 0.18 g/dm³ and as high as 18.47 g/dm³ at 40 and 95 °C, respectively, after 6 months of bottle use (Nam *et al.*, 2010). In another study the authors discovered that detergents used for washing dishes may have increased BPA release when in contact with the Polycarbonate. They also noted that PA concentrations lowered near to control levels immediately after rinsing the polycarbonate samples (Maia *et al.*, 2009).

2.3: BPA acting as an endocrine disruptor

Natural or synthetic endocrine-disrupting chemicals (EDCs) or xenoestrogens are substances which can disrupt endocrine functions by imitating or blocking endogenous hormones (Michałowicz, 2014). In doing so, the genes expressed in the body change, triggering hormonal concentration change, enzyme activity and synthesizing of proteins. A prevalent endocrine disrupting chemical (EDC) which for years has been in use for epoxy resins production and polycarbonate polymers is bisphenol A (Kawa *et al.*, 2021). Bisphenol A was reported to have the ability to bind to many types of receptors, which included estrogens receptors, androgen receptors, receptors activated by aryl hydrocarbon receptors and peroxisome proliferators, which are related

to endocrine system hormones and other body systems (Michałowicz, 2014). Endocrine disrupting chemicals have negative impacts on the health of human, among them is reproductive health, posing a worry for the entire world. Exposure at early life to EDCs can lead to adult illness and possibly even pass from one generation to the next (Kawa *et al.*, 2021). Figure 2.1 below shows a schematic representation of mechanism of endocrine disrupting chemicals.

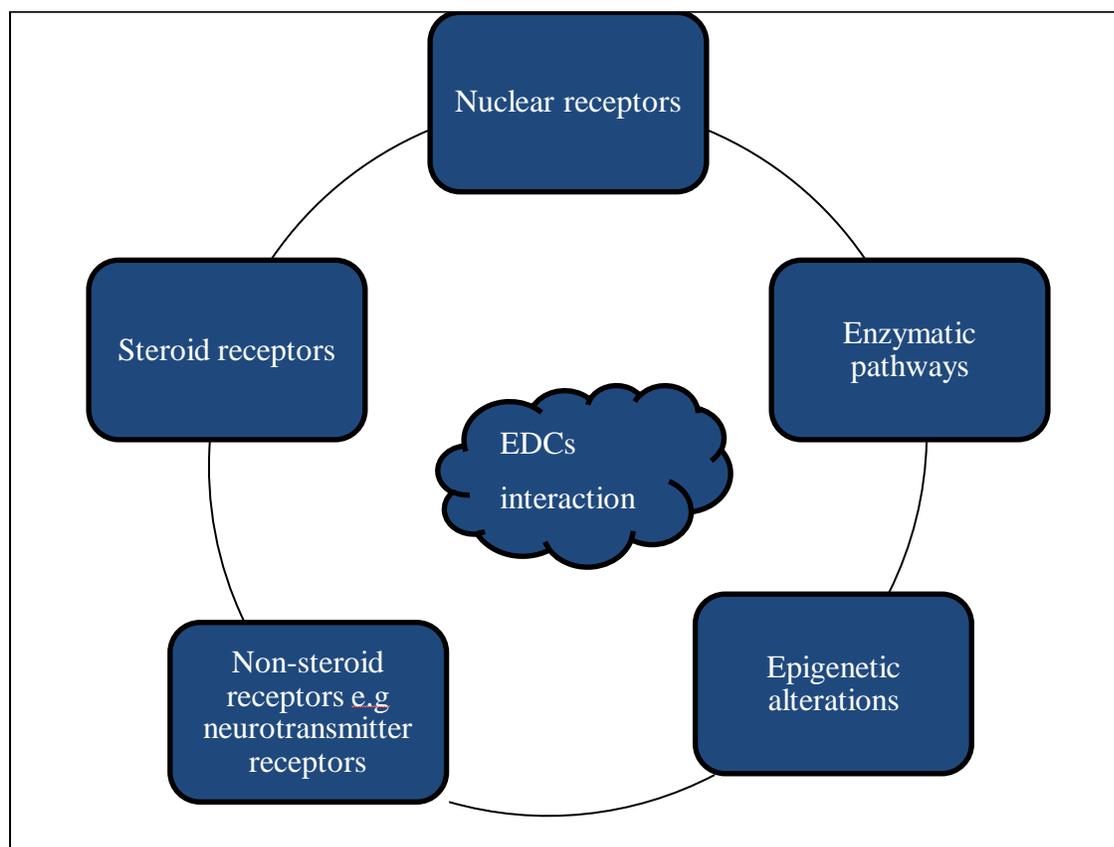


Figure 2:1 Representation of mechanism of Endocrine disrupting chemicals

Endocrine disrupting chemicals cause agonistic and antagonism effects by preventing hormones from attaching to their receptors, such as ERs and androgen receptors. EDCs also affect steroidogenesis and metabolic processes. Endocrine disrupting chemicals also use an epigenetic process to influence gene expression directly.

2.4: Exposure of BPA to humans

There are various routes of exposure which include, skin absorption, inhalation through air and water, huge populations come into contact with BPA from various industrial wastes. Commonly

BPA exposure is via the ingestion of food and water contained in containers containing BPA (Mirmira & Evans-Molina, 2014). The measure of BPA that leaches to the food substances depends on the measure of BPA that is used to produce the commodity, also the heating times and temperatures used to make the product (Mirmira & Evans-Molina, 2014). Because of the physicochemical properties of BPA, effective sources of oral intake include canned foodstuffs, polycarbonate bottles used to store water and saliva extracted from dental sealants. In cosmetics and meats, BPA is also used as an antioxidant. Therefore, it is expected that additional human exposure mechanisms include the absorption and passage of BPA by inhalation and BPA coming into contact with the skin (Vom Saal and Hughes *et al.*, 2005).

Table 2:1 Different sources that contribute to BPA intake in $\mu\text{g}/\text{kg}$ body wt/day in a population

Population	Exposure source	Mean BPA intake ($\mu\text{g}/\text{kg}$ body wt/day).
Infants 0-6 months	Mainly through Breastfed Bottles made from polycarbonates and formula powder–liquid) Formula, Bottles without polycarbonate (powder–liquid)	0.3 $\mu\text{g}/\text{kg}$ 2.0 -2.4 $\mu\text{g}/\text{kg}$ 0.01- 0.5 $\mu\text{g}/\text{kg}$
Infants 6-36months	Breastfed + solid food PC bottles and formula + solid food Formula only, without polycarbonate bottles + whole nutrients	0.1 $\mu\text{g}/\text{kg}$ 0.5 - 0.6 $\mu\text{g}/\text{kg}$ 0.01 - 0.1 $\mu\text{g}/\text{kg}$
Both children and adults	Dust (inhalation and ingestion)	1460 $\mu\text{g}/\text{kg}$ Dust
Adults	Thermal paper (through touching with hands)	1.4 $\mu\text{g}/\text{finger}$
Adults	Cosmetics	31 $\mu\text{g}/\text{kg}$

Source: World Health Organization. (2011). Joint FAO/WHO expert meeting to review toxicological and health aspects of Bisphenol A: final report, including report of stakeholder meeting on Bisphenol A, 1st-5th November 2010, Ottawa, Canada.

Foodstuffs are the most significant BPA exposure source to the population. This is attributed to the fact that both raw plant material and animals are exposed to BPA, BPA accretion in the

atmosphere and foodstuffs interaction with BPA containing polymers. Bisphenol A is known to be eaten with food every day, and the sensitivity of the human body to BPA by the food channel was estimated at 0.48 to 1.6 g/kg/body weight/day (Michałowicz, 2014)). In 2015, EFSA reevaluated the daily tolerable BPA intake from 50 µg/kg body weight/day to 4 µg/kg bodyweight/day (EFSA, 2015).

2.5: Health risks associated to BPA exposure

Bisphenol A influences the development of humans especially in the period of fetal development (Rubin & Soto, 2009). The BPA could be carcinogenic, possibly contributing to being a precursor in breast cancer (EFSA, 2006). It has also been proved that BPA leads to decrease in the number of sperms and sperm activity due to its estrogenic activity, BPA is also harmful to the liver, and may even be associated with obesity by influencing fat cell activity (EFSA, 2006). Furthermore, BPA exposure has been related to human chronic disease conditions such as cardiovascular diseases (Lang *et al.*, 2008).

In various potentially important brain regions, BPA has been found to induce biochemical changes. Several recent studies have found similar changes that demonstrate the brain growth impact of BPA. It remains to be clarified whether these changes are mechanistically linked to the observed neuro-behavior after BPA exposure (EFSA, 2014).

Effects from BPA on reproductive indices through experimental studies show that the ovary, uterus, and vaginal weights, shape of the egg; fertility rate; number of live-born neonates per litter; newborns' anal and genital pore distance, vaginal opening time which shows onset of female puberty); estrous onset (showing maturing sexually) (Catenza *et al.*, 2021).

In another research, BPA levels in urine, its effects on sperm quality, damage to DNA and aneuploidy was conducted (Catenza *et al.*, 2021) . An abnormally high chromosomes numbers in a haploid set was examined. A total of three hundred and fifteen males under the age of 45 were enlisted for this research from clinics dealing with infertility cases. In urine, the high BPA levels were associated to a rise in the proportion of immature sperm and sperm chromosomal disomy and a decline in sperm motility. Such studies indicated exposure to BPA being a crucial cause of the

deteriorating quality of male sperm (Catenza *et al.*, 2021). These studies showed that BPA exposure is a crucial cause of decreased male sperm quality.

Workers who work with plastic-based items such as polyvinyl chloride (PVC) gloves and sticky tapes have reported rare incidences of skin allergies. Rashes on the hands, legs, or face are common skin signs. Exposure to BPA during pregnancy can also cause anxiety, sadness, disruptive behavior and hyperactivity in children (Abraham & Chakraborty, 2020)

2.6: Alternatives for BPA

Considering BPA's adverse effects on human life, regulating agencies, among them the European Commission, the US Food and Drug Administration, and Health Canada, raised a ban on BPA use in baby feeding bottles production (Huang *et al.*, 2012; von wt. Reppert-Bismarck, 2010). With such confinements and insistence from the society, "BPA free" products have been produced from manufacturers. Hence substitutes are being applied in production of polycarbonates and epoxy resins. Major BPA substitutes (Figure 1.1) are BPS (2,2-bis 4-hydroxyphenol sulfone) (Figure 2.2) and BPF (2,2-bis 4-hydroxyphenol methane) (Figure 2.3) (Sidorkiewicz *et al.*, 2018). They have similar chemical structures like BPA which are currently unregulated and unrestricted.

2.6.1: Bisphenol S (BPS)

Bisphenol S (BPS) is a structure having two functional groups of phenol on both sides of a sulfonyl group (Figure 2.2), making it structurally identical to BPA (Figure 1.1). Being more heat and light resistant BPS has been adopted as a viable replacement to BPA in a variety of industrial applications. BPS is utilized in applications in the industry, like in cleaning products as it finds use like a wash fastening agent, a solvent used in electroplating, and a phenolic resin ingredient. In thermal papers, including "BPA-free" papers, BPS is used as a developer (Eladak *et al.*, 2015).

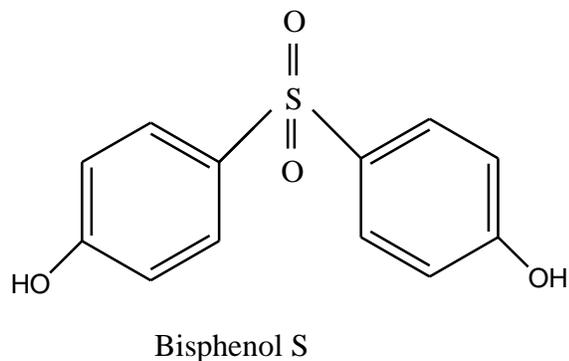


Figure 2:2 Bisphenol S chemical structure

2.6.2: Bisphenol F (BPF)

Systems requiring increased durability and thickness like pipes and tank linings, floors for industries among others use epoxy resins and coatings, are made with BPF (Fiege *et al.*, 2000). Lacquers, plastics, water pipes, dental sealants, food packaging among others are made with BPF (Figure 2.3) epoxy resins.

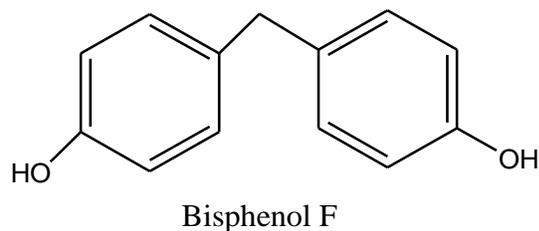


Figure 2:3 Bisphenol F chemical structure

In a research conducted in 2019 the researchers discovered a link between urine BPF concentrations, children and adolescent's abdominal obesity in the United States, particularly in males. Furthermore, substantial correlations between levels of BPA in urine and abdominal obesity were discovered in young boys (Liu *et al.*, 2019). The researchers concluded that they had strong evidence from laboratory research which supported their results that BPF (Figure 2.3) has similar obesity-promoting effects as BPA (Figure 1.1)..

2.7: BPA regulations

Studies indicate BPA is toxic to living organisms, there needs to be close monitoring with its contact with food materials being the most common human exposure route. That's why European Food Safety Authority (EFSA) had to put in place temporary tolerable daily intake (t-TDI) for

BPA of 4 µg/kg body weight/day, in its 2015 risk appraisal of BPA lowering it from 50 µg/kg body weight/day (EFSA, 2021). The Food Safety Authority expert Panel on Food Contact Materials, Enzymes, and Processing Aids (CEP) in December 2021 formulated a TDI of 0.04 nanograms per kilogram of body weight per day as it drafted re-evaluated 2021 BPA review. The decrement in the TDI is the outcome of studies assessment literary published between 2013 and 2018, in particular indicating adverse BPA effects on the immune system (EFSA, 2021). Bisphenol A was added in the European Union List of the Regulation EU No 10/2011 and the latest specific migration limit (SML) set at 0.05 mg kg⁻¹ (Dreolin *et al.*, 2019). As per EFSA in January 2011, the European Commission acquired Directive 2011/8/EU, banning use of BPA (Figure 1.1) in the manufacturing of PC infant feeding bottles (Johnson *et al.*, 2015).

Canada was the first country to classify BPA a harmful substance, its import was banned in 2008. Health Canada came up with a temporary tolerable daily intake (pTDI) for BPA of 25 µg/kg body weight/day (Cao *et al.*, 2008). In September 2012, an amendment was published by Belgium and made into a law, aiming at banning the manufacturing of products containing BPA, majorly for children within the ages of zero and three years. This law aimed to protect the health of the consumer in regards to food commodities. The law was enforced on January 1st 2013 (EFSA, 2015).

Bisphenol A use in baby feeding bottles was restricted in May 2010 by Denmark, particularly for children aged between zero and three years (EFSA, 2015). In the United States, a possible negative impact of BPA exposure on fetus, babies, children's brains and prostate glands was raised. The Administration declared in July 2011 the restriction of BPA in bottles used for feeding babies. However, the Government hasn't limited its use to other goods (Noonan, 2011).

On December 24, 2012, France passed legislation prohibiting import, manufacture, sale and export of products in contact with food containing BPA. This legislation gradually took effect, beginning on January 1, 2013, for items in contact with food especially for children aged zero to three years.

Columbia also passed legislation in March 2011 banning the production, selling or shipment of plastics manufactured using BPA that were meant to be filled with foodstuffs and liquids. As of 1st July 2011 (Noonan, 2011). Sweden resolved to ban BPA containing compounds in food

packaging coatings meant for children (Regulation SFS 2012:99113). The ban was enforced 1 July 2013 (EFSA, 2015)

However, in Kenya, there are no regulations regarding to BPA use in the production of polycarbonate plastics even in products used by children.

2.8: Spectrophotometric techniques.

2.8.1: Ultraviolet-visible spectroscopy

Ultraviolet-visible spectrophotometry finds common use in many analytical laboratories to do qualitative and quantitative analysis. Using Beer's law, sample deduction can be done from the UV-Vis absorbance data (Lam, 2004). Ultraviolet-visible spectroscopy operates based on electromagnetic radiation interaction in the ultraviolet-visible region and matter. Figure 2.4 below presents schematic diagram of the UV-Vis spectrophotometer. The ultraviolet (UV) region approximately covers 10–380 nm range in the electromagnetic spectrum. With three main sub-regions, that is: UVA between 320–380 nm; UVB between 280–320 nm; and UVC between 100–280 nm. Additionally, the 10–200 nm range named as vacuum ultraviolet (VUV), however explored only if measurements are being done in a vacuum. The visible (Vis) region covering the spectral range of 380–750 nm (Picollo *et al.*, 2019). It's easy and fast to use making it a more popular analysis technique. Due to significant ultraviolet absorption for phenolic compounds, UV-Vis spectroscopy is of special interest (Giglio *et al.*, 2023).

The UV-Vis spectroscopy operation principle is that ultraviolet light or visible light absorbed by chemical compounds produces a spectra similar to absorbed light. Basically, it operates based on the interaction between light emitted at a specific wavelength and matter. The resulting spectrum is formed as a result of absorption of light by matter undergoing excitation and de-excitation thus the spectrum. Upon absorption of ultraviolet radiation by matter, excitation is undergone by the electrons making them jump from a ground state to excited state equivalent to ultraviolet radiation amount absorbed by it. Its operation and functioning is best described by Beer-Lambert law.

Beer-Lambert law which states that when a monochromatic beam of light makes an incident on a solution containing a monochromatic light absorbing substance, the decrease in beam intensity along the solution thickness is directly proportional to the absorbing substance concentration in the solution and directly proportional to the intensity of the incident monochromatic radiation.

This thus implies that the extent of radiation absorption is greater with the number of absorbing molecules.

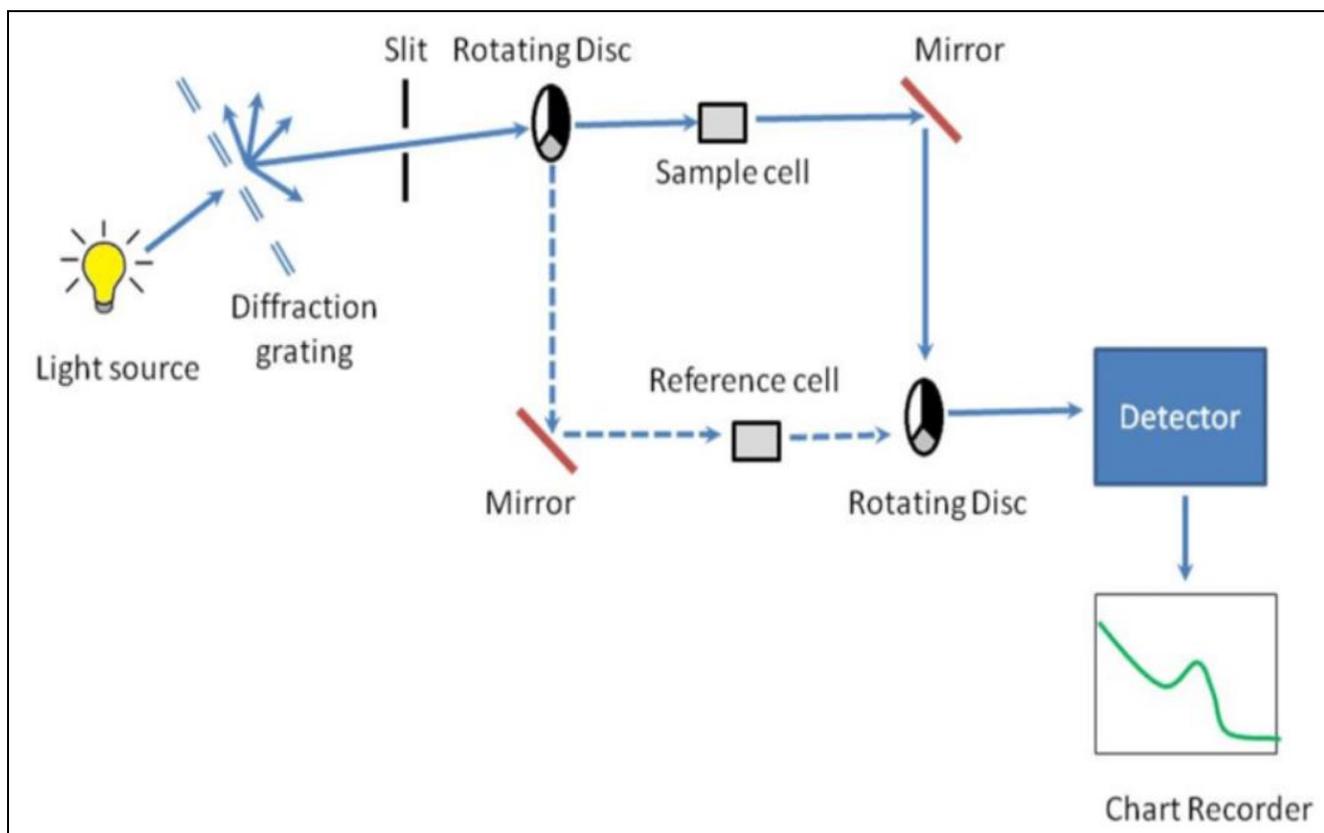


Figure 2:4 A scheme showing UV-Vis spectroscopy

Source (Perkampus, 2013)

2.8.2: Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy is a most commonly used technique in vibrational spectroscopy involving studying radiation interaction with molecular vibrations to obtain molecular structure information and substance amount in a sample. a wide variety of samples can be analyzed, among them solid, liquid, gases, be it in hot state, cold state, in bulk, microscopic particles or as surface layers.

A graph of light intensity against transmittance is called an infrared produced using an infrared spectrometer. Examining an infrared spectrum gives the concentration and type of particles present

in a sample. The spectrum is plotted in absorbance units. The multivariate and non-destructive nature of infra-red (IR) spectroscopy technology makes it a promising technique. Figure 2.5 below represents the major components of an FTIR spectrophotometer.

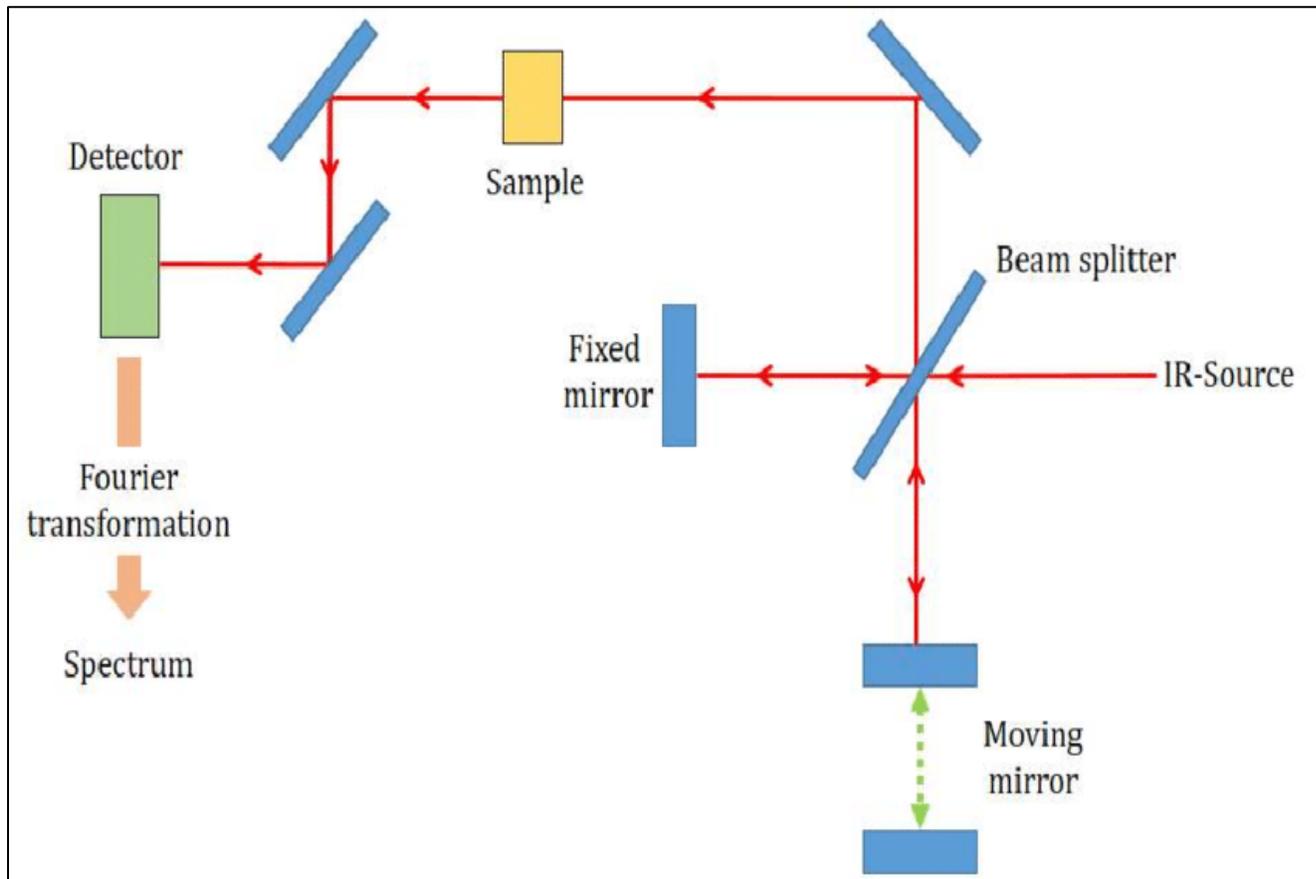


Figure 2:5 A scheme showing an FTIR spectrophotometer.

Source (Makhdoom, 2018)

2.8.3: Diazo-coupling

Diazo coupling reactions are a major class of organic reactions that are currently carried out with mineral acids as catalysts (Rahimizadeh *et al.*, 2012). The diazotization of aromatic amine is a two-step electrophilic substitution reaction (Bu *et al.*, 2022). In a typical example, the primary aromatic amines (aniline/sulphanilic acid) react with NaNO_2 in an acidic medium at a low temperature to produce diazonium salt. The diazonium salts are then coupled with strong nucleophiles (such as electron rich aromatic / heteroaromatic compounds) to form colored azo compounds (Sudhir & Nadh, 2013). The diazotization coupling reactions produce colored substances known as azo compounds (Xu *et al.*, 2017). Azo dyes are composed of azo groups linked to methine or aromatic sp^2 -hybridized C-atoms (Dabbagh *et al.*, 2007). The diazo coupling process is a proton-eliminating condensation of a positive diazonium ion with another compound containing an active hydrogen atom (Sudhir & Nadh, 2013). The figure 2.6 below shows a diazotization mechanism reaction

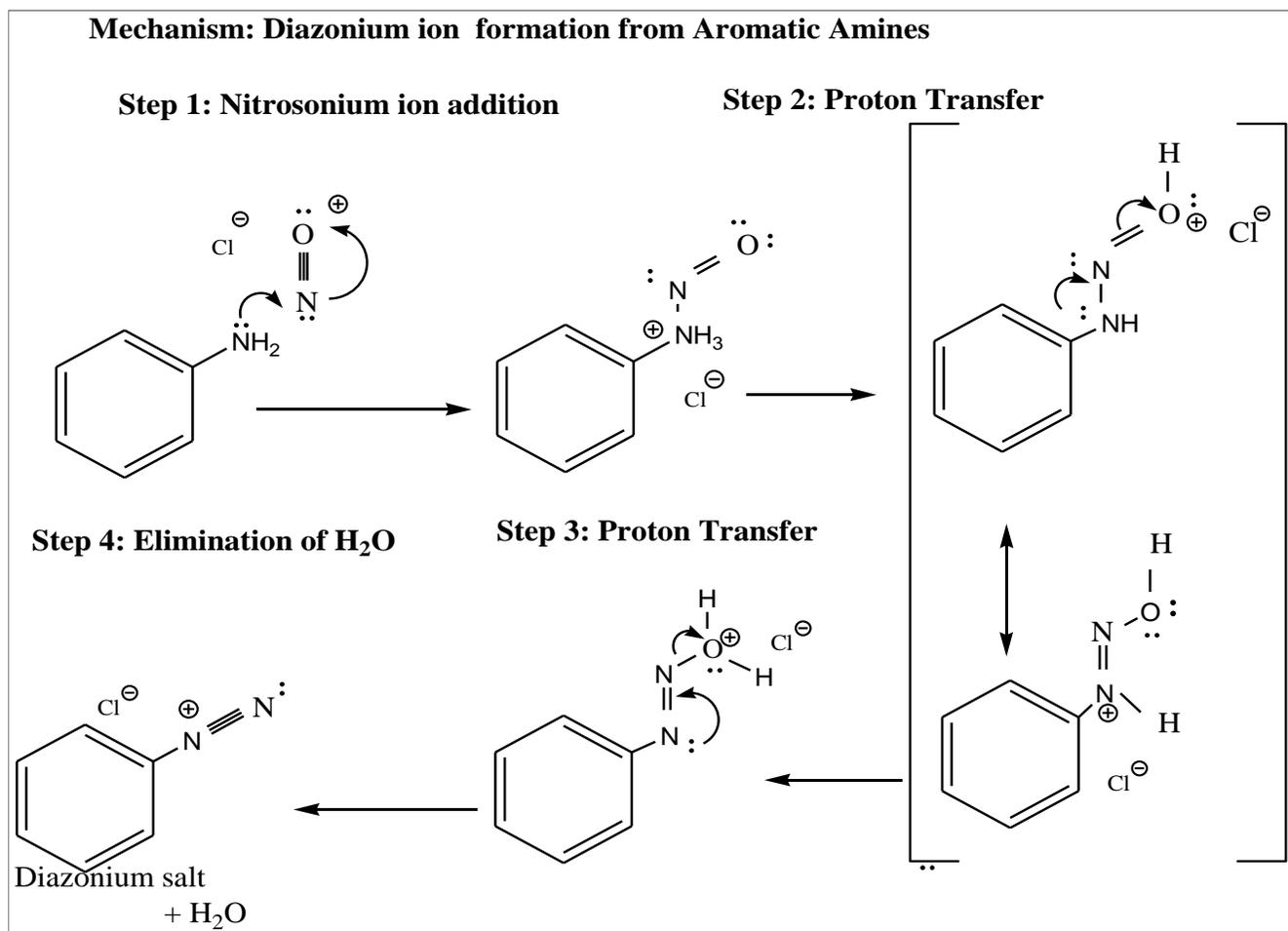


Figure 2:6 Schematic representation of a diazotization mechanism

2.9: Voltammetric techniques.

2.9.1: Cyclic voltammetry.

Cyclic voltammetry (CV) is a robust electrochemical technique used to study the reduction and oxidation of molecular processes. Cyclic voltammetry has been used to characterize electrodes, comprehend early electrochemical investigations of frontier systems, and track chemical processes. However, the analysis of the CV is not always straightforward because of the reliance on analyte conditions, like electrolyte and electrode, as they depict information on the kinetic and thermodynamic. Cyclic voltammetry is of importance in studying chemical reactions initiated by transfer of electron, like catalysis (Elgrishi *et al.*, 2018). Computation of homogeneous and heterogeneous reaction rates in conjunction with simulation software CV can be used. Varying the scan rate (along with other parameters) and evaluating how characteristic features such as change in peak currents and peak potentials in response to these changes are typical of such investigations (Bott, 1997). For instance, in reversible redox systems, the Randles-Sevcik plot, which is produced by plotting peak current against square-root of scan rate, can be used in determining the redox species' diffusion coefficient. In the solution phase, mass transport/diffusion processes determine the shape of 'reversible' voltammetric current responses (Marken *et al.*, 2010). Figure 2.7 below shows a cyclic voltammogram of a reversible process.

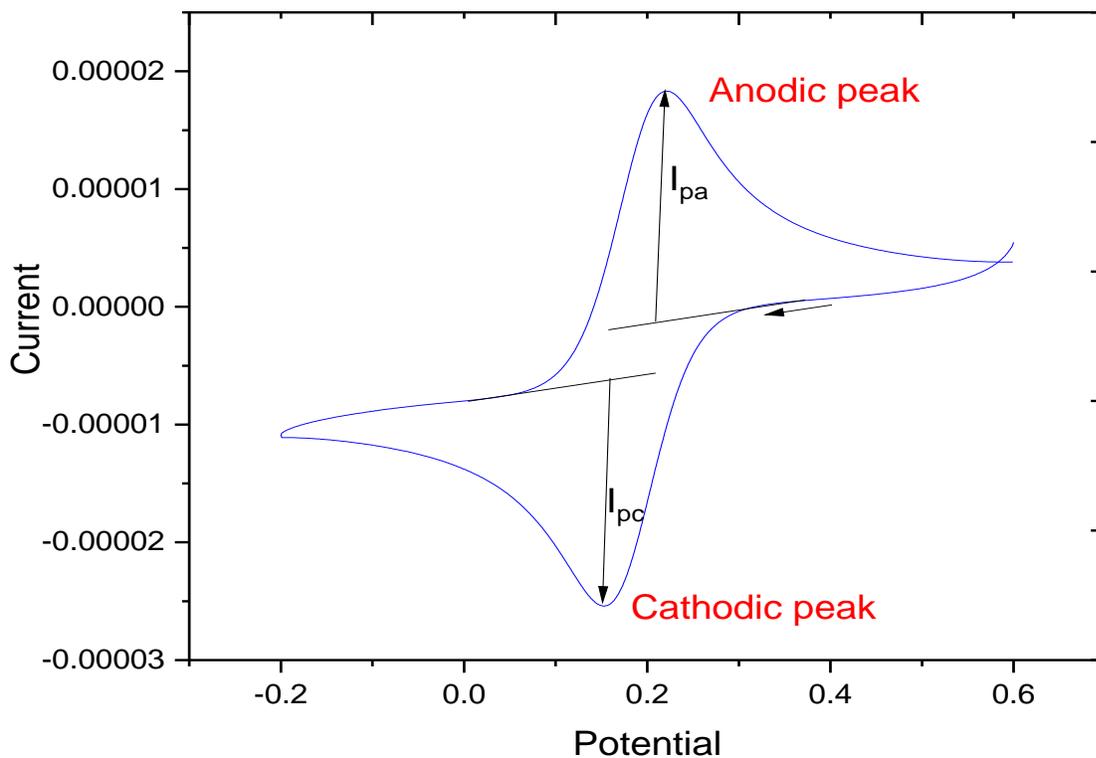


Figure: 2.7 A cyclic voltammogram of a reversible process.

2.9.1.1: Reversibility in cyclic voltammetry

Cyclic voltammetry can be used to measure a variety of parameters, including the electron transfer rate, diffusion coefficient, redox potential, and the quantity of adsorbate present in the adsorption system. The forms of the CVs can be used to assess the electrochemical reversibility in CVs, which is categorized as reversible, quasireversible, and irreversible. The degree of connection between the electron transfer rate and the mass transfer rate of diffusion has a significant impact on CVs. Electron transfer rate or mass transfer rate of diffusion are examples of reversible processes.

Change from reversible to irreversible in a quasi-reversible procedure. Irreversible process: electron transfer rate; mass transfer rate of diffusion

The mass transfer rate of diffusion, however, is dependent on the voltammetry's time scale corresponding to scan rate v (Yamada *et al.*, 2022).

2.9.1.2: Reversible electrode process

When the rate of electron transfer is significantly greater than the mass transfer rate of diffusion in reversible electrode process, potential is given by Nernst equation below:

$$E = E_O + \frac{RT}{nF} \ln \frac{C_{ox}}{C_{red}} \quad \text{Equation (2.1)}$$

where n denotes the electron number, R denotes universal gas constant ($8.314 \text{ JK}^{-1} \text{ mol}^{-1}$), T denotes the temperature in kelvins, F denotes the Faraday constant ($1F=96485.33 \text{ C mol}^{-1}$)

For a reversible electrode method, the peak currents' amplitude can be calculated using Randles-Sevcik equation given below;

$$i_p = 0.446nFAc \sqrt{\frac{nFvD}{RT}} \approx (2.69 \times 10^5)n^{3/2}AD^{1/2}cV^{1/2} \quad \text{Equation (2.2)}$$

Where,

n = Electrons number.

D = Diffusion coefficient of electro-active analyte in square centimeter per second (cm^2s^{-1}),

V = Potential scan rate in volts per second (Vs^{-1}),

A = Area of electrode in square centimeters (cm^2),

C = Electro-active species concentration in moles per cubic centimeter (mol/cm^3).

N.B: The equation holds at 25°C and may not apply at different temperatures given that the rate of diffusion is temperature dependent.

The following are the characterization features of reversible systems at 25°C ,

Square root of scan rate is directly proportional to peak current

Peak current ratio, i.e. anodic and cathodic current is one.

$$i_{pa}/i_{pc} = 1 \quad \text{Equation (2.3)}$$

The formal potential position is related to anodic potential (E_{pa}) and cathodic peak potential (E_{pc}). Peak separation for reversible one electron system is 59mV/n as given in the equation 2.4 below where n is electron number. It follows then that Equation 2.4 can be used to calculate electrons number in fast electron transfer process.

$$\Delta E_p = E_{pa} - E_{pc} = 59/n \text{ mV} \quad \text{Equation (2.4)}$$

The Nernst Equation, as written above, can be used to link the species concentrations that are experiencing reduction and oxidation. Electro-reversible system is one that fulfills the above conditions. A process is not completely reversible if neither of the aforementioned parameters are not o. These systems might be either irreversible or quasi-reversible. If the formal potentials of each redox couple are well separated out, the voltammogram for reversible multi-electron couple electron transfer systems will be having multiple discrete peaks.

2.9.2: Differential pulse voltammetry

It can provide quantitative information about electrochemical mechanisms as well as providing a basis for electroanalytical measurement (Melville & Compton, 2001). It measures current difference before and after a potential pulse. This method averts current measurement caused by charging the double layer (capacitive current).

Additionally, time delay before every pulse allows recovery of depletion zone adjacent to electrode surface (Instrumentation & Paschkewitz, 2019). Differential pulse voltammetry reduces background interferences' influences like impurities induce redox current. It therefore gains greater detection sensitivity and lower limit of detection than former electrochemical approaches, making it more quantitative detection sensitive tool. DPV is of more advantageous for its simultaneous multiple substance detection, low electrolytes demand, and less needed analyte. Thus being widely used analytical field, mostly for quantitative substance determination (Zhang *et al.*, 2018). It's a helpful technique for tracing both organic and inert compounds. In Differential Pulse

Voltammetry (DPV), (Fig. 2.8), the base potential is increased from initial potential toward final potential. A potential pulse is applied. A function of potential is as a result of the difference between the current potential before and the end of the pulse.

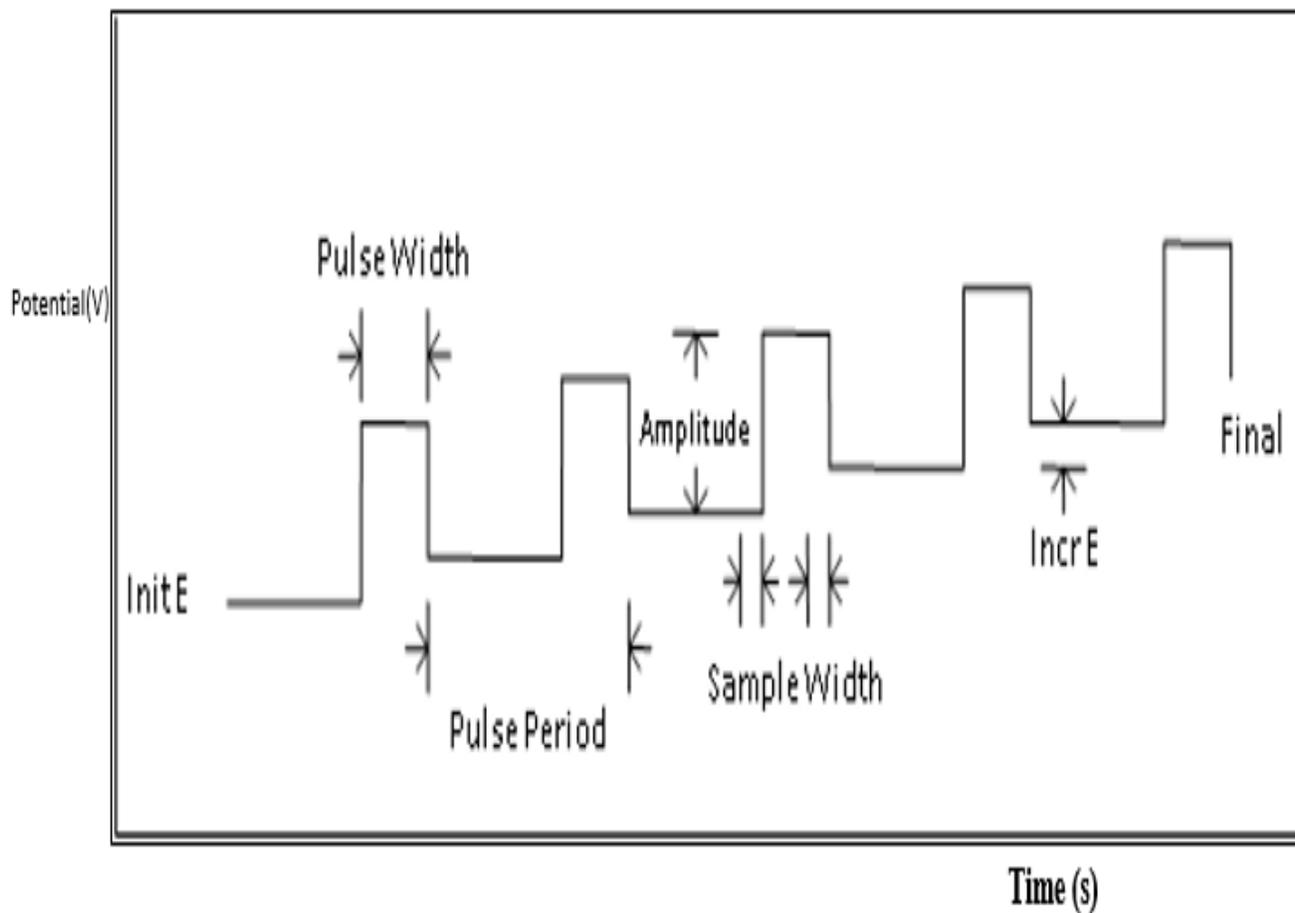


Figure: 2.8 A figure of potential waveform applied as the function of time and the current

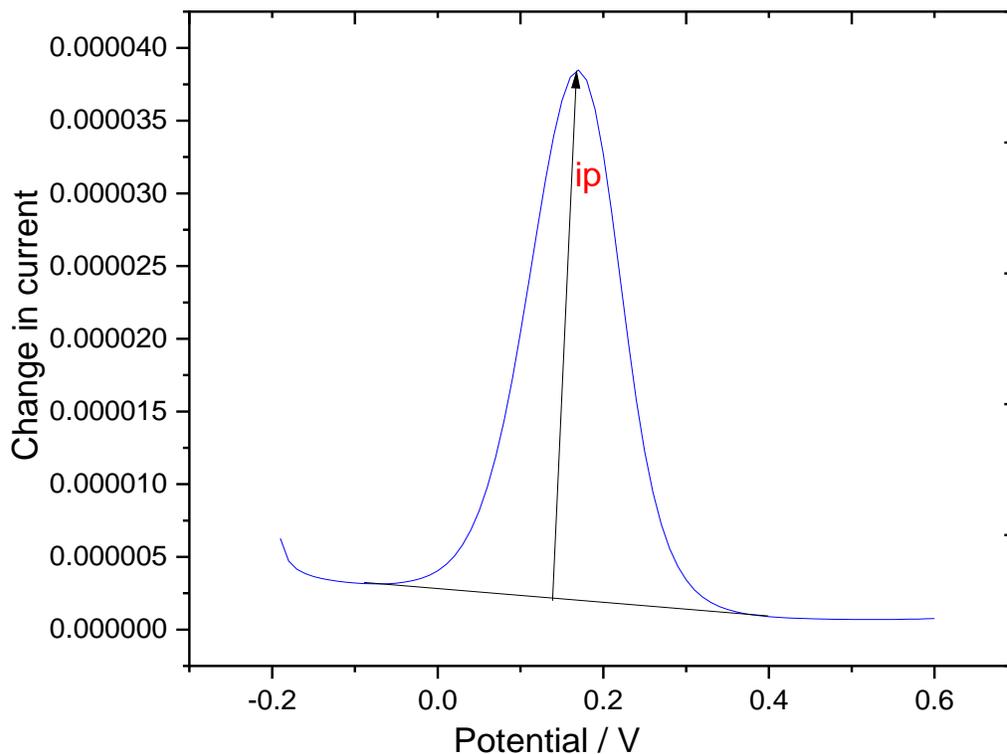


Figure 2:9 A differential pulse voltammogram of 3mM Potassium ferricyanide

The height of the peak current resulting from differential pulse voltammetry depends on the analyte's information (Fig. 2.9).

2.10: Carbon nanotubes

Carbon nanotubes (CNTs) were discovered in 1991 (Andrews et al., 2002). CNTs are nanomaterials with several uses that are mass-produced. It has unique qualities such as a high surface-to-volume ratio, high adsorptive capacity, high electrical conductivity, and chemical stability (Zou *et al.*, 2022). All of these characteristics make carbon nanotubes a unique material for use in electrochemical devices. A pictorial of some CNTs is presented in figure 2.10 below.

Carbon nanotubes can be formed as single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs) depending on the CNT synthesis methods used (Goulart *et al.*,

2016). The CNTs have also been demonstrated to exhibit very high aspect ratios (length/diameter). The SWCNTs have a cylindrical nanostructure created by rolling up a single graphene sheet containing carbon atoms in a honeycomb pattern into a tube. The MWCNTs are made up of numerous concentric cylinders of graphene sheets (Walcarius *et al.*, 2013).

The MWCNTs are usually hydrophobic, so they are not readily dispersed in water. Organic solvents (acetone, methanol, dimethyl formamide and ethanol) are commonly used (Lehman *et al.*, 2011). The extraordinary electric-current-carrying capacity throughout their length (1000 times that of copper wires) makes CNT-based novel electrode materials particularly appealing (Walcarius *et al.*, 2013).

In addition, MWCNTs' extraordinary and unique qualities provide a significant advantage in the manufacture of enhanced composites, but their utilization inside a matrix is mostly dependent on the connection between the matrix and the other material. These properties make carbon nanotubes (CNTs) extremely useful in nanofilter reinforcement, probes, energy storage, gas filters, bio applications, and numerous electrical and thermal devices (Kumar *et al.*, 2016).

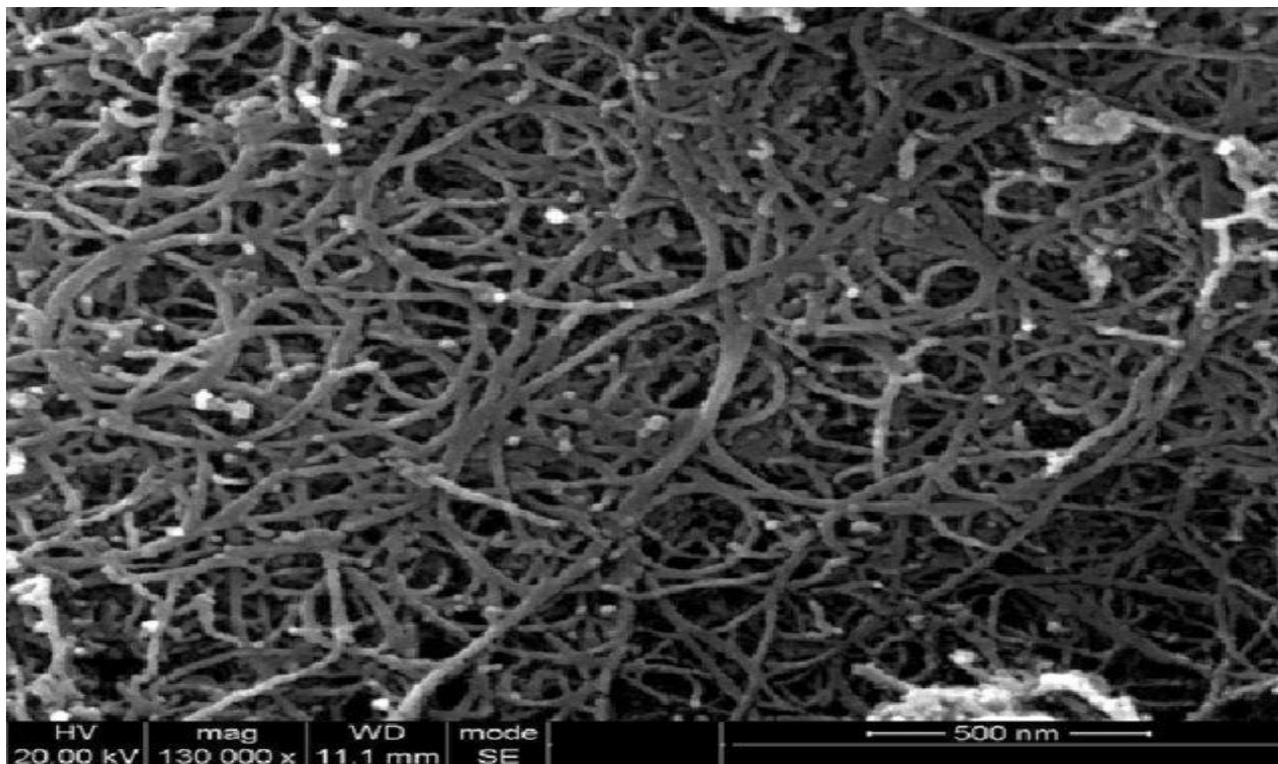


Figure 2:10 Scanning Electron Microscope image of multiwalled carbon nanotubes

Source (Amin *et al.*, 2018).

2.11: Electrochemical sensors

Electrochemical sensors are among a group of chemical sensors currently available systems for practical applications. A device transforming chemical information of a specific sample component to total composition analysis, into analytically useful signal is a chemical sensor (Thevenot *et al.*, 2001). Figure 2.11 below is a schematic presentation of the major components of an electrochemical biosensor. Due to its its advantageous characteristics, which include low detection limit, their portability, affordable instrumentation, target-oriented structure, and simplicity of use, electrochemical sensors are very well-liked as a detection system (Sanko *et al.*, 2022). Chemical sensors contain two components connected in series: a chemical (molecular) recognition system (receptor) and a physico-chemical transducer. To fabricate electrochemical sensors, single and/or multi-walled carbon nanotubes, metals and metal oxide, conductive polymers are used.

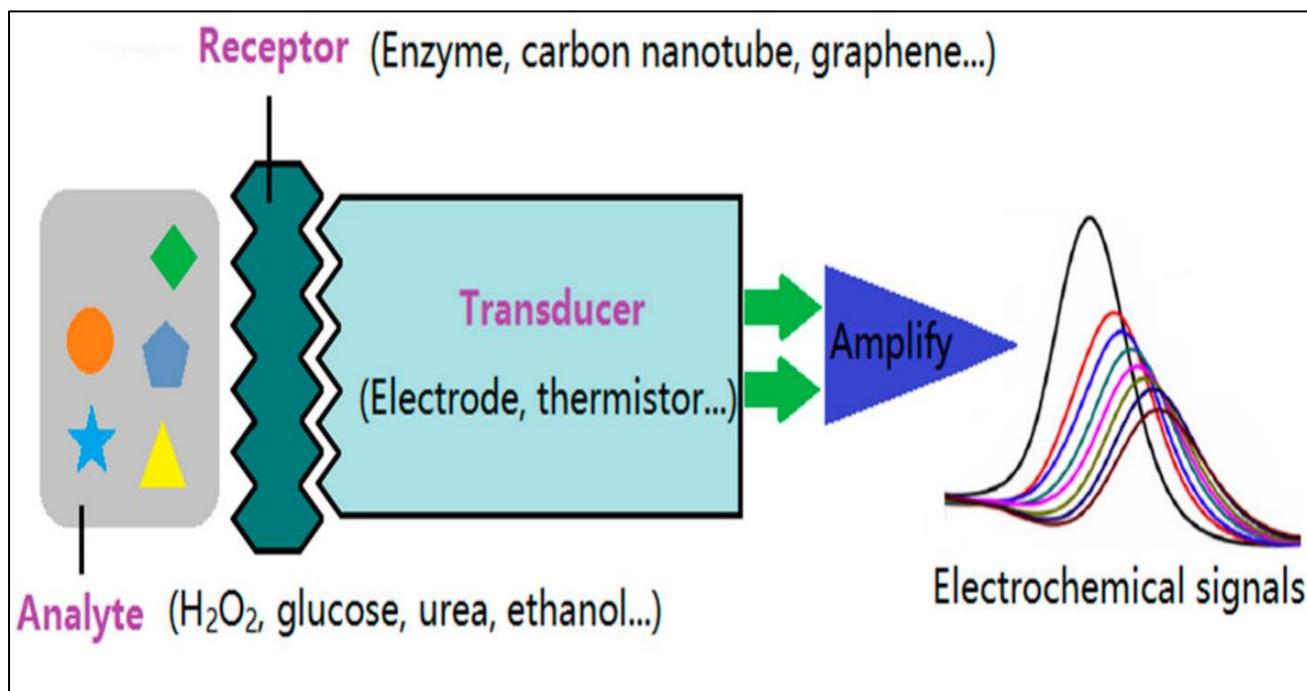


Figure 2:11 Schematic diagram of an electrochemical sensor.

Source (Chen *et al.*, 2019)

2.12: Method validation.

Method validation is defined as "the process of providing recorded evidence that the method achieves what it is supposed to do " since it ensures reliability throughout normal use. Validation mainly demonstrates that the procedure used is appropriate to the targeted results providing specific, precise and accurate analytes stated range. Among the validation characteristics include: quantification limit, accuracy, precision and detection limit (Prakash Chanda Gupta, 2015).

2.11.1: Accuracy.

The measurement accuracy defined as how near the assessed value relates to the real value. A known value sample is precisely studied, the value that is measured is the true value. Recovery studies frequently depicts and assess accuracy. Precision can be determined using one of three methods:

1. As a comparison, a reference standard is utilized.
2. Analyte recovery after being spiked into a blank matrix
3. Standard analyte addition (Ravisankar *et al.*, 2015.)

2.11.2: Precision.

Agreement degree among individual test findings when performing an analytical process on many homogeneous samples is known as precision. A set of measurements' coefficient of variation is used widely to express the precision of an analytical approach. The equation used to calculate RSD is shown in as equation 2.5 and the obtained results are shown in table 4.9 below.

$$\text{Relative Standard Deviation} = (\text{Standard Deviation} / \text{Mean}) * 100 \quad \text{Equation (2.5)}$$

(Gupta, 2015)

2.11.3: Limit of detection (LOD)

Analytes analyzed with known concentrations and determining minimal level at which the analyte may be consistently identified, under the provided experimental circumstances indicates LOD. The limit of detection refers to the lowest analyte concentration likely to be reliably detected. It is obtained by statistical data analysis through obtaining sample means, variance and consequently and consequently the standard deviation δ . Consequently, the limit of detection is obtained using

the formula $LOD = 3.3 \delta / S$. Where δ is the standard deviation of calibration curve intercepts. S being the linearity plot's slope (Ravisankar *et al.*, 2015).

2.11.4: Limit of quantification (LOQ)

The limit of quantification is defined as the least concentration in a sample that with sufficient accuracy and precision be quantified under defined experimental circumstances. LOQ is calculated using the formula $LOQ = 10 \delta / S$. Where δ = standard deviation of response. S = Mean calibration curve slopes (Ravisankar *et al.*, 2015).

2.12: Statistical Treatment of Data

To verify used method validity in the study, the data collected during method validation must be statistically analyzed. When dealing with data, an analyst can make one of two mistakes: rejecting the hypothesis of similarity of two quantities when statistically similar, or accepting the hypothesis that two quantities are similar when statistically different. To avoid such inaccuracies, statistical data treatment procedures are utilized. To determine levels of confidence data treatment methods are used, replicates number needed for believable results, if there is a significant measurement of two sets of measurements, comparing two data sets means, and either reject or keep results appearing to be an outlier in a data set, among other things.

Data processing statistical methods regularly utilized include confidence intervals, standard deviation and mean.

CHAPTER 3: MATERIAL AND METHODS

3.1: The study area

The research was carried out in Thika town, Kiambu County, with a catchment area of 839 km² and a population of 279, 429 people (The Kenya National Bureau of Statistics, 2019) as its high population and typical urban set-up means possibility of many polycarbonate users. Urban dwellers like to carry water/ juice in bottles as they go to work. They also leave behind toddlers (those who have) who feed from bottles back at home. However, the other main reason why Thika was chosen for sampling was for convenience due to proximity of sampling sites. The study area lies on the A2 road at a length of 42 kilometers, Northeast of Nairobi. It lies between longitude 37°4'9.59"E and latitude 1°1'59.74"S (Figure 3.1). The figure below shows the sampling sites for the PC bottles.

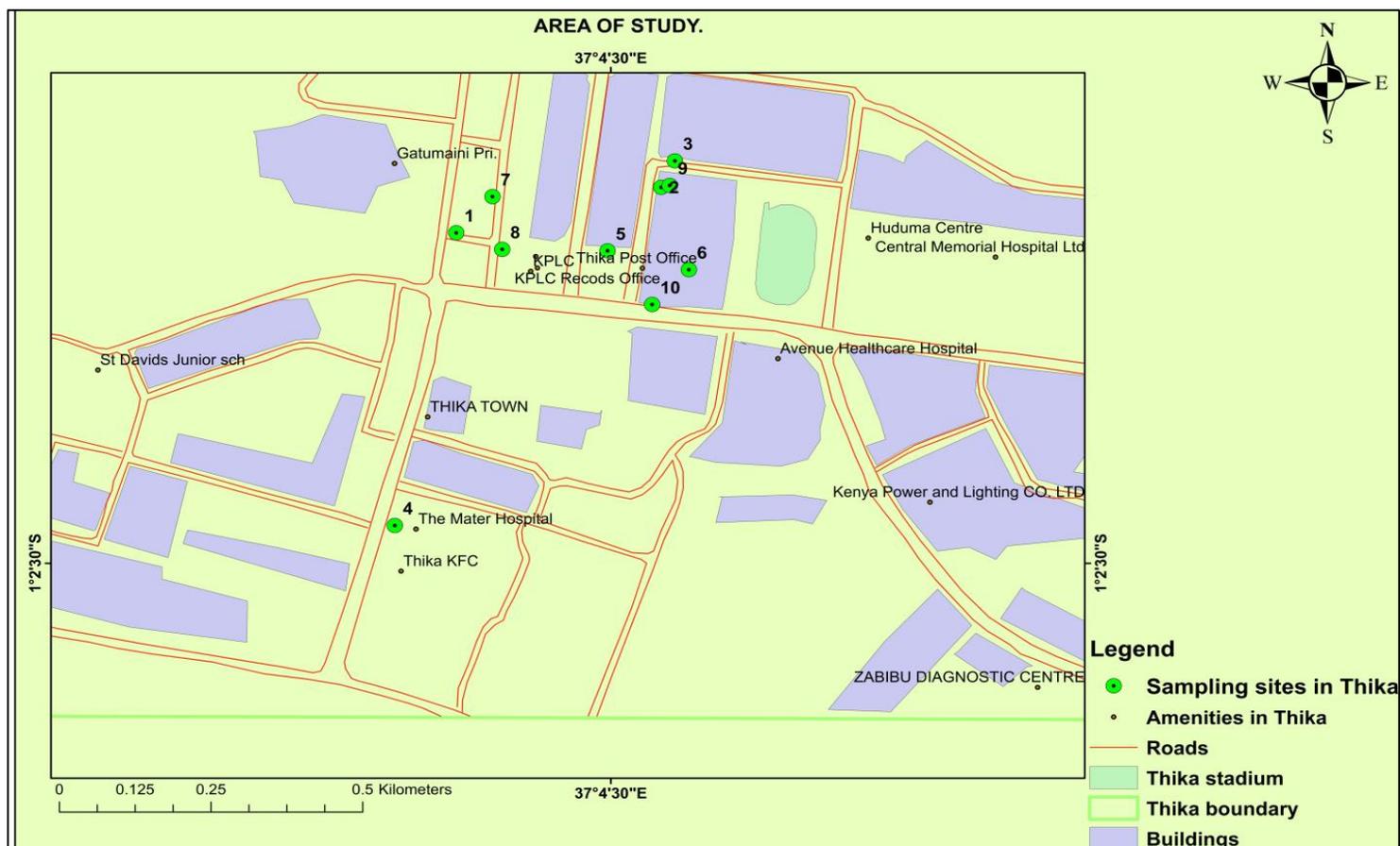


Figure 3:1: The map of Thika town showing the supermarkets sampling sites

Sampling was done in 10 randomly selected supermarkets/sampling sites hereby designated as sampling sites 1 -10. The details of the sampling site locations and the economic activities around the sites are given in Table 3.1 below.

Table 3:1 Coordinates and human activities at the sampling sites

Sampling Sites (Supermarkets)	Latitude	Longitude	Height (m)	Human activities around the sampling sites
Site 1	-1.036417°	37.072709°	1507	Public transport and banking
Site 2	-1.035694°	37.075746°	1505	Wholesaling and retailing services
Site 3	-1.035273°	37.075950°	1504	Printing and photocopy services
Site 4	-1.041067°	37.071799°	1502	Banking and teaching activities
Site 5	-1.036703°	37.074952°	1507	Wholesaling and retailing services
Site 6	-1.037003°	37.076158°	1506	Wholesaling and dental services
Site 7	-1.035842°	37.073247°	1508	Printing and hotel services
Site 8	-1.03668°	37.07339°	1507	Wholesaling and retailing services
Site 9	-1.035664°	37.075874°	1505	Public transportation and retailing services
Site 10	-1.037556°	37.075613°	1508	Banking and public transportation

3.1.1: Economic activities in Thika town

Thika is a leading trade center in Kiambu County, Kenya as it is a city which contains several industries. Agriculture, in particular coffee and pineapple (exported primarily in the US and Europe); horticulture, cooking oils and production of animal feeds are the main economic practices. Other industries include fabric, macadamia nuts, and wheat, and tannery, assemblies of

motor cars, cigarette manufacturing, bakery and consumer packaging goods. Several small businesses work in and around the city. It also contains more than 15 big factories (Table 3.1). In the service sector, there is a collection of many educational and financial institutions.

3.2: Chemicals, reagents and apparatus

3.2.1: Chemicals and reagents

The reagents utilized in spectrophotometric technique included; 99% analytical grade BPA standard and 99.9% methanol both purchased from Sigma Aldrich. Other reagents included; 99% sodium nitrite from BDH PROLABO; 37% (w/w) hydrochloric acid and 98% sulphuric acid from Merck; 98% analytical grade sodium hydroxide pellets, 99.5% aniline, Sigma Aldrich, 67% nitric acid, 99.8% acetonitrile Sigma Aldrich, and 99.5+% acetone purchased from Alfa Chemicals. Other products used included baby formula (from supermarkets) and juices.

Reagents employed in voltammetry measurements included; analytical grade Potassium ferricyanide, $K_3Fe(CN)_6$ 99.99% from Fisher Scientific; 99.8% potassium nitrate, potassium dihydrogen phosphate, sodium dihydrogen orthophosphate and disodium hydrogen phosphate from PROLABO; potassium chloride, copper (II) sulphate, lead II nitrate, calcium chloride, magnesium nitrate, sodium nitrate, manganese sulphate all purchased from Sigma Aldrich. 99.9+% dimethyl sulfoxide, 99.9+% dimethylformamide (DMF) purchased from Alfa Aesar. Distilled water was utilized in all studies. The chemicals and reagents purchased were of analytical grade thus used without much further purification.

3.2.2: Apparatus and instruments

The instruments used in this study were Shimadzu IRAffinity-1S - Fourier Transform Infrared Spectrophotometer, Shimadzu UV-1700 PharmaSpec UV-VIS Spectrophotometer, all electrochemical experiments were conducted on a Basi Epsilon Eclipse potentiostat composed of a three-electrode system. The three-electrode system comprised of a glassy carbon (working electrode), a platinum wire (auxiliary electrode) and KCl-saturated calomel electrode (reference electrode). At room temperature, a 30.0mL electrochemical cell was utilized for all analytical work. All data was analyzed using Microsoft Excel and Origin software, version 2019b 64bit.

3.3: Sampling and sample pretreatment

Thika town was chosen because it comprises of many polycarbonate users as it is an industrial town with a rapid growing population. The population rose from 136,386 in 2009 to 279,429 in 2019 (KNBS, 2009: KNBS, 2019). During sampling, ‘suspected’ polycarbonate bottles were purchased from the various supermarkets/sampling sites. Most of the bottles did not have a PC label and selection was done on generally known characteristic of PC materials such as; strength, stiffness, transparency, rigidity amongst others. In total six samples were purchased of which three were baby feeding and three were portable water carrying bottles. At the same time used bottles were also sampled from nearby homes randomly selected. For this study, used bottles were defined as those that were more than 2 months in usage. These bottles were obtained in exchange for new ones. In total twelve ‘suspected’ PC bottles were sampled of which six were new and six were ‘used’. Of the new category, six were baby feeding bottles and six were water bottles. Of the ‘used’ category, six were baby feeding bottles and six were water bottles.

Each sample purchased and collected from the mothers within Thika was stripped with labeling masks and were labelled A, B, M, C, D, J, I, F, G, L, P and H, and transported to the Chemistry Department, Physical Chemistry laboratory at the University of Nairobi for analysis. The selected purchased and labeled food substances included baby formulas, juices, milk and drinking water was allowed to stand in the characterized PC bottles for a certain period of time with regular shaking.

3.4: Sample preparation

The different makes of the selected baby feeding (BFBs) and water bottles, obtained from the local supermarkets were cut into tiny pieces with scissors, then the samples were analyzed by Fourier-transform infrared FTIR spectroscopy since the type of plastic making the baby and water bottles was not always specified. A Shimadzu IRAffinity-1S - Fourier Transform Infrared Spectrophotometer was applied using the attenuated total reflectance mode. ATR were measured within the 4000-500 cm^{-1} range. Before each measurement, ethanol was used to clean the ATR crystal, and a background spectrum was run to make sure that the solvent had completely evaporated. Polymer identification was achieved based on comparisons with reference spectra from literature. The analysis confirmed that all 12 samples were made of polycarbonate. The infrared light spectra were recorded across the 4000–500 cm^{-1} range.

3.5: Milk and Infant formula food preparation

To prepare the baby formulas and milk for protein precipitation, 100 ml of boiling water was poured to a polycarbonate baby feeding bottle, after which 3 spoons (12.0 g) of the baby formula was added as directed by the manufacturer. The mixture was well mixed and set aside for two hours. After that, the mixture was acidified with 100 μ L of concentrated hydrochloric acid. Then 5mL of the acidified solution was then combined with 10 mL of acetonitrile in 20 mL conical flask. The precipitate that formed was centrifuged for 10 minutes at 4000 rpm and filtered using Whiteman filter papers (Bashir & Audu, 2020).

3.6: Standard and working solutions

1000 mg/L BPA standard stock solution was prepared through dissolving 0.1g of BPA standard solution in 100 ml of methanol analytical grade. The subsequent working standard solutions were prepared from a 100mg/L standard solution prepared from 1000mg/L stock solution with the equation 3.1 shown below.

$$C_1V_1 = C_2V_2 \quad \text{Equation (3.1)}$$

(C_1 and V_1 are the stock standard concentration and volume respectively while C_2V_2 represent the working standard concentration and volume respectively) and kept at 4°C in an amber bottle.

3.7: Preparation of working standard solutions

Appropriate portions of 100mg/L standard solution were diluted to 10 ml mark to make 1.00, 2.00, 4.00, 6.00, 8.00 10.00 and 12.00 ppm BPA standard solutions. A 20ml methanol solution was used as a calibration blank.

3.8: Preparation of aniline

Pure aniline was obtained through triply distilling aniline then storing it under nitrogen. Aniline is hygroscopic and tends to darken due to atmospheric oxidation when exposed to light and that's why it needs to be triply distilled. To prepare aniline of 1000ppm, 0.93ml of distilled aniline was placed into a 1000 ml volumetric flask topped to the mark using distilled water.

3.9: Spectrophotometric method

3.9.1: Optimization of reaction conditions

In order to obtain the best developed method performance by maximizing the yield and selectivity, a number of experiments were done aiming at optimizing diazo-coupling reaction reagents. Optimized parameters included concentrations of aniline, sodium nitrite, sodium hydroxide, hydrochloric acid and diazotization reaction time. The optimization was carried out base on one factor at a time, other conditions remained constant during each optimization.

3.9.2: Spectrophotometric procedure for diazo-coupling reaction

1 ml of 120 μgml^{-1} aniline was added to a mixture containing 1 ml 0.1 mol L^{-1} HCl and 1 ml 100 μgml^{-1} sodium nitrite NaNO_2 to form benzene diazonium chloride (Figure 3.2). The solution was shaken thoroughly using a magnetic stirrer at room temperature operating at 3,000 revolutions per minute. Thereafter, 1 ml of BPA 120 μgml^{-1} was then added and the mixture with continual stirring for 2 minutes, this was followed by addition of 1 ml 0.1 mol L^{-1} sodium hydroxide NaOH for complete coupling reaction. Distilled water was used to fill to the mark, at 424 nm in 1.00 cm quartz cells absorbance was measured after color development (Figure 3.3) utilizing distilled water as a reference. Figure 3.3 shows a scheme showing the two steps reaction

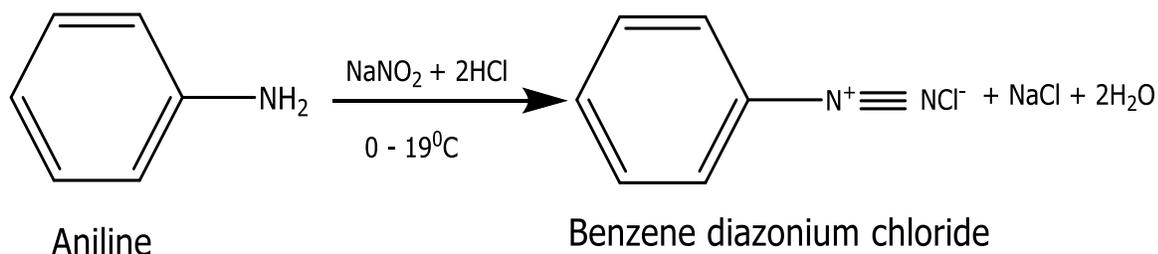


Figure 3:2 Formation of Benzene diazonium chloride

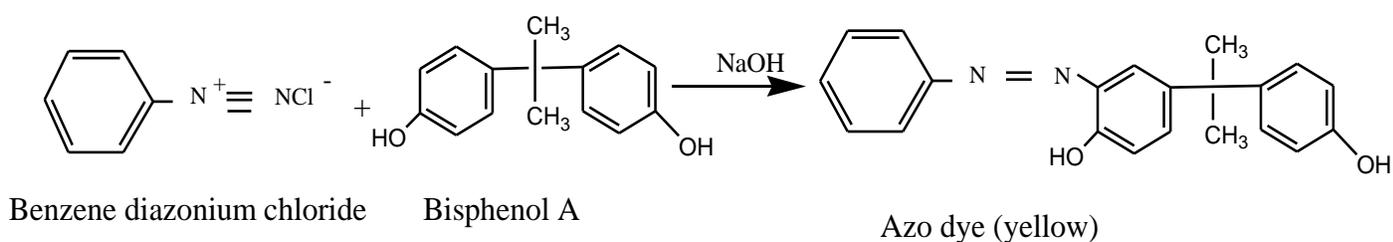


Figure 3:3 Diazo-coupling reaction with Bisphenol A

3.10: Method Validation Studies

3.10.1: Recovery studies

The Spectrophotometric method recovery was done with distilled water which has been spiked with BPA at concentration of 2, 4 and 6 µg/ml and analyzed like the unknown samples.

3.10.2: Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from calibration curve slope according to ICH guidelines. The formula for calculating LOD is $LOD = 3.3 \delta/S$ Where δ = standard deviation of intercepts of calibration curves. S = the slope of linearity plot. The formula for calculating LOQ is $LOQ = 10 \delta/S$. Where δ = standard deviation of response. S = Mean calibration curves of slopes.

3.11: Voltammetric techniques

The electrochemical behavior of BPA was investigated by a modified glassy carbon electrode (GCE) modified using multiwalled carbon nanotubes (MWCNTs) and checking the electrochemical response in the absence/presence of Bisphenol A in solution. The BPA standard stock solution was prepared, different aliquots of the BPA analyte was added into the electrochemical cell carrying the three-electrode configuration (working, reference and counter electrodes). A phosphate buffer system pH 7.0 was used as the support electrolyte. A calibration curve of the concentration of BPA versus the amperometric current response formed the basis for the BPA quantification in the analyte samples. The analyte samples consisted of baby formula, juices, water placed in polycarbonate bottles or baby feeding bottles for certain periods of time and the effects of varying temperature was also considered.

3.12: Preparation of functionalized Multiwalled Carbon Nanotubes (MWCNTs-COOH)-modified glassy carbon electrode

The electrode was prepared by drop casting method where a bare glassy carbon electrode GCE was polished with alumina powder to produce a surface that is mirror-like, rinsed using distilled water later dried at room temperature before use. 1 mg Multiwalled Carbon Nanotubes (MWCNTs) were dispersed in 1 mL of dimethylformamide DMF to make a suspension. A 5 µL

aliquot of the MWCNTs suspension was casted on the GCE surface then air dried for 1 hour at room temperature, the electrode was ready to work after solvent evaporation. The dried electrode was designated as MWCNTs/GCE and used for the electrochemical sensor measurements without further treatments.

Dimethylformamide (DMF) was selected due to its compatibility than other solvents (e.g., methanol, acetonitrile, dimethyl sulfoxide, acetone) with the hydrophobic GC surface nature.

3.13: Preparation of phosphate buffer solution

A buffer solution of Phosphate of 0.1 M was prepared by dissolving 9.453g disodium hydrogen phosphate (Na_2HPO_4) and 5.21 g of sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) separately in 800 mL distilled water, salt solutions were then mixed in accordance to the Henderson-Hasselbalch equations to attain required pH range of 7.0. A Solution of 0.1 M hydrochloric acid and 0.1 M sodium hydroxide was then used in pH adjustment to 7.0. The pH of the phosphate buffer solution was maintained by storing it in a refrigerator at 4°C.

3.14: Preparation of BPA standard for voltammetric techniques

A 500 μM BPA standard stock solution was prepared by dissolving 11.41 mg of the BPA standard in 100 ml of methanol analytical grade. The working standard solutions 10 μM , 20 μM , 30 μM , 40 μM and 50 μM were prepared from the 500 μM standard stock solution using equation 3.1

3.15: Redox marker studies

Ferricyanide studies are conducted to check whether the potentiostat was in the proper working conditions. For well calibrated potentiostat the peak separations should be $59/n$ mV.

A stock solution of 10 mM Potassium ferricyanide, $\text{K}_3\text{Fe}(\text{CN})_6$ in potassium nitrate was prepared by dissolving 164.63 mg in 50 ml 1 M potassium nitrate solution.

The working standard solutions 1 mM, 2 mM, 3 mM, 4 mM and 5 mM were prepared from the stock solution by dilution method using equation 3.1.

3.16: Cyclic voltammetry and differential pulse voltammetry for BPA analysis

Since BPA is an electroactive compound that gives an oxidation peak at 0.621 V on a GCE electrode on the cyclic voltammetry mode. The BPA leaching studies were investigated using cyclic voltammetry and differential pulse voltammetry within a potential range of -1.0 V to +1.0 V. Differential pulse (DPV) voltammetry was also used to show the relationship amidst the oxidation peak current and BPA concentration. Aliquots of 0.01M BPA stock solution were added into the phosphate buffer while increasing their concentrations and their voltammograms recorded.

By using different concentrations of BPA in the electrolytic cell, the peak current dependence on the concentration of the BPA was determined. This was done by putting the plastic fragments in the food and water stimulants at known temperature for 60 minutes then investigating the presence and concentration of BPA in the water in 0.1 M phosphate buffer at pH of 7.0. The temperature was varied at 95°C, 50°C and 25°C. The concentration of BPA in drinking water bottles and feeding baby bottles was determined

3.17: Interference Studies

The impact of numerous probable interference substances on the determination of Bisphenol A was investigated using cyclic voltammetry, 50 µl of 0.1M interferent solution (Table 3.2) prepared above were added separately into an electrochemical cell containing 10 ml phosphate buffer solution, 50 µl of BPA was then added followed by thorough stirring and the solution allowed to settle. The interferents were chosen on the basis that they could occur in the specimen matrices in nature since they are likely to be found in water (a major constituting reagent). Furthermore, they are electrochemically active and it was important to ensure their electrochemical signals did not overlap BPA detection. The resulting voltammetric currents and potentials were recorded observing any change in the electrochemical behavior.

3.18: Preparation of interferent solutions

To make interferent solutions, a specific amount of salt was weighed and dissolved in 25 ml of distilled water while being stirred. The mass of all interferent salt solutions was 0.1 M, as shown in the table 3.2 below.

Table 3.2: Preparation of interferent solutions from salts with cations or anions of interest

Type of salt	Mass of salt dissolved (grams)
Magnesium chloride	0.5000 g
Sodium nitrite	0.1725 g
Lead nitrate	0.8280 g
Potassium dihydrogen orthophosphate	0.3400 g
Calcium chloride	0.2775 g
Magnesium nitrate	0.6410 g
Zinc chloride	0.3400 g
Potassium chloride	0.1864 g
Sodium nitrate	0.2120 g
Copper sulphate	0.6242 g
Potassium nitrate	0.2528 g
Sodium dihydrogen orthophosphate	0.3900 g

These interferences were selected because most of them are found in water and also can be detected in the same potential window with Bisphenol A.

CHAPTER 4: RESULTS AND DISCUSSIONS

In this chapter, discussion is made on a number of techniques including: Fourier transform infrared spectrometry, diazo coupling spectrophotometric technique, cyclic voltammetry and differential pulse voltammetry. The aim was first of all to sample ‘suspected’ PC bottles from randomly selected supermarkets and then verify through appropriate techniques whether they were of the polycarbonate type or not because they were not so labeled. Two types of bottles were sampled namely those used for baby feeding and those used for carrying drinking water/ or juices. FTIR was used in classifying the sampled bottles (baby feeding, water carrying) to confirm if they were polycarbonates by checking out whether the functional groups characteristic of polycarbonates were present in the various specimen spectra. Library polycarbonate (PC) spectra/data were used for the purpose of verification. After the identification of the PC containing bottles/ samples, UV-vis spectroscopy, cyclic voltammetry and differential pulse voltammetry were used in determining BPA levels in from the samples under different environments.

4.1: Confirmation of materials of baby bottles and water bottles.

Figure 4.1 (a) shows the FT-IR spectrum of polycarbonate baby feeding bottle used in the study. Figure 4.1(b) shows an FT-IR spectrum obtained from the literature.

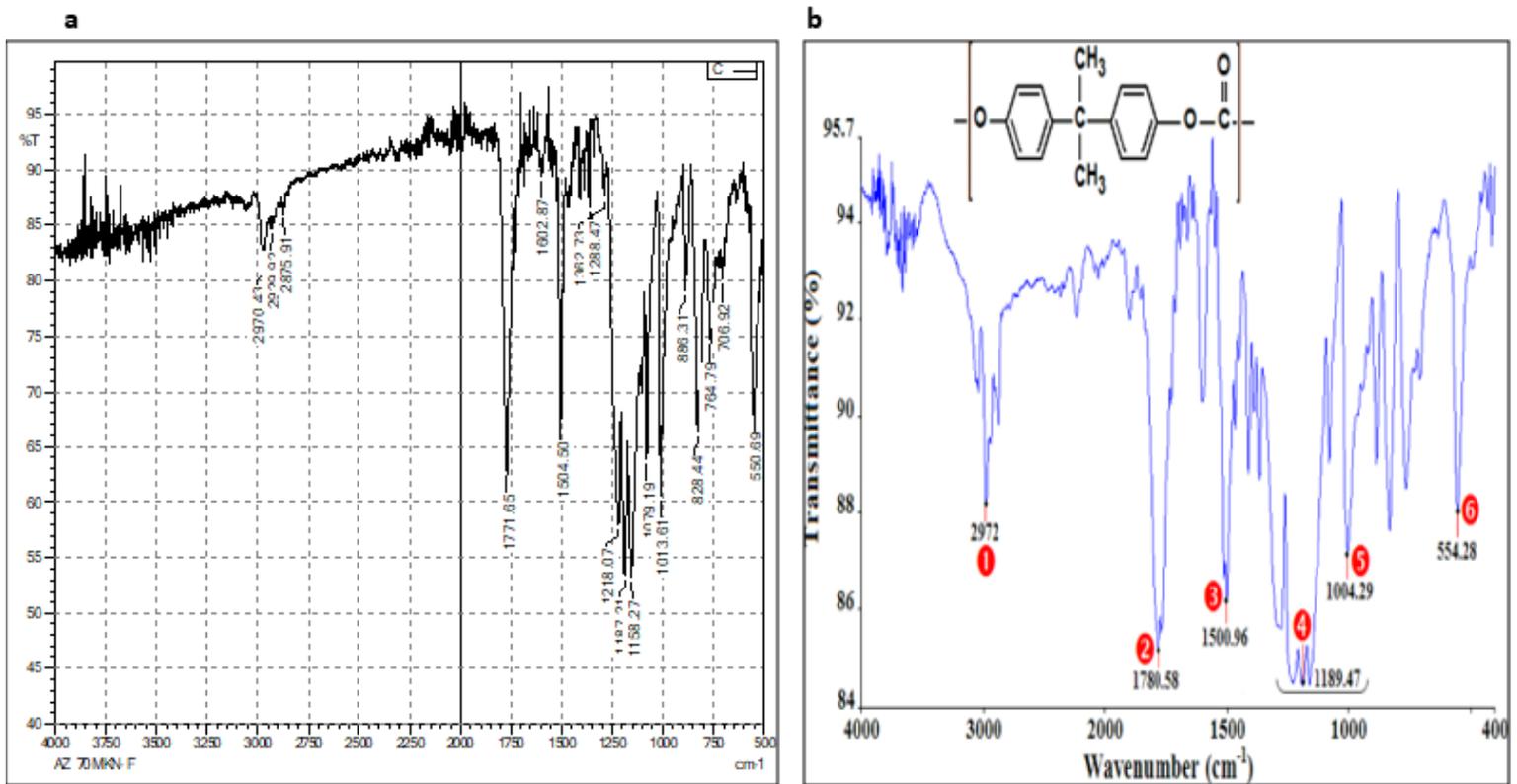


Figure 4.1: FTIR spectrum of (a) polycarbonate water bottle compared with (b) a polycarbonate bottle from the literature (Ghorbel *et al.*, 2014).

The prominent peaks in the polycarbonate IR spectrum shown in Figure 4.1(a) above are caused by the following characteristic vibrations at 2970 the vibrations are attributed to the stretching vibration of C-H aromatic ring, at 1770 they are attributed to carbonate group (C=O) vibration, at 1504 for C=C vibration rings, at 1187 and 1013 from the two phenol rings, are bands corresponding to stretching deformations of asymmetric and symmetric O-C-O carbonate group and finally at 1079 can be attributed to CH₃ vibrations. These results are similar to what is found in the literature (Ghorbel *et al.*, 2014) for polycarbonate plastics as shown in figure 4.1 (b).

4.2: Diazo-coupling reaction for Spectrophotometric Technique

4.2.1: Optimization of reaction conditions

Figures 4.2 (a-d) presents the results for optimization of diazo-coupling reagents and conditions; the chosen conditions were selected based on their ability to give maximum absorbance values and were maintained throughout the studies. These concentrations were $120 \mu\text{g mL}^{-1}$ aniline (Figure 4.2 (b)), 0.1 mol L^{-1} hydrochloric acid (Figure 4.2 (c)), $100 \mu\text{g mL}^{-1}$ sodium nitrite (Figure 4.2 (d) and 0.1 mol L^{-1} sodium hydroxide. (Figure 4.2 (a).

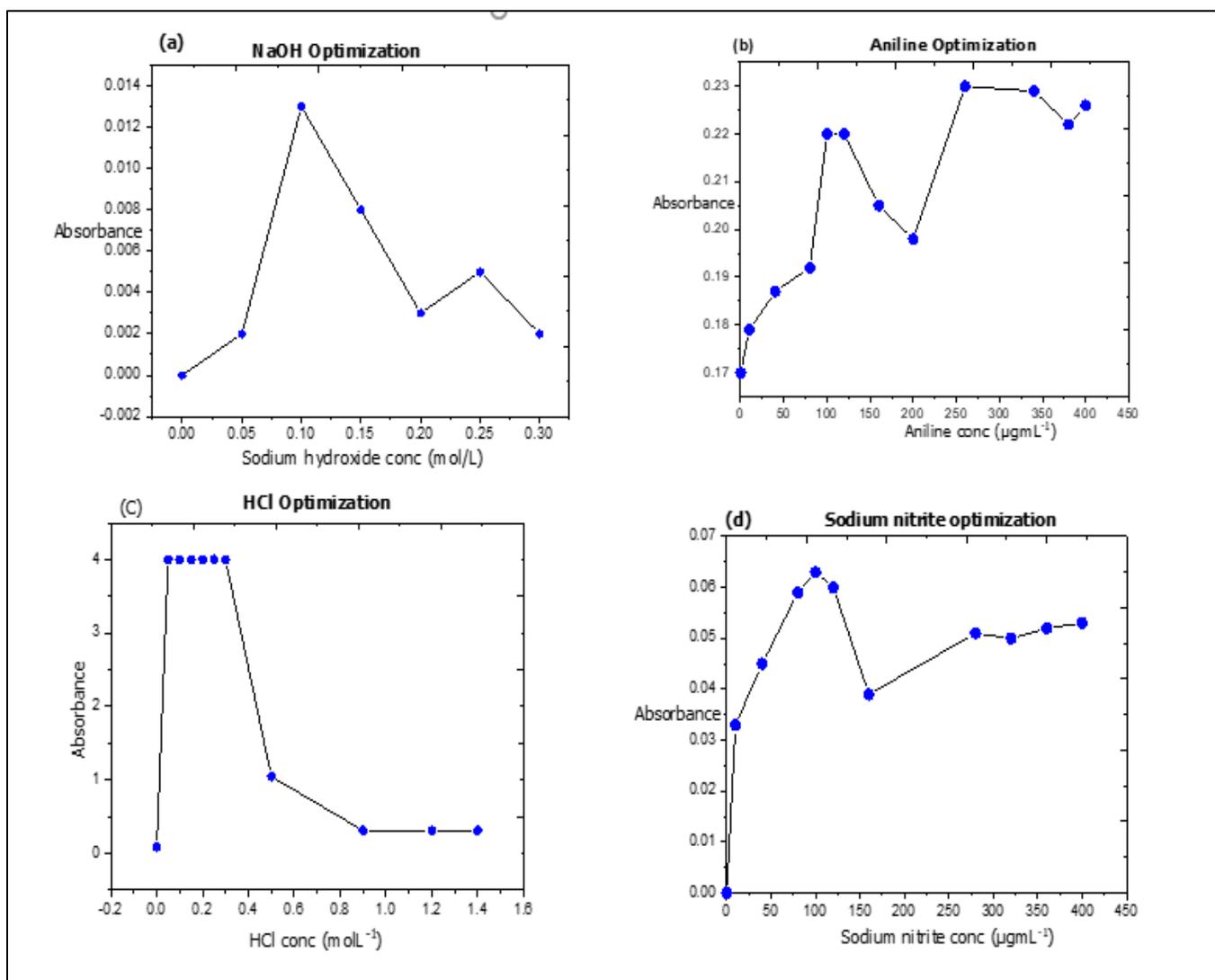


Figure 4.2: (a-d) parameters optimized for diazo-coupling reaction

4.2.1.1: Effect of sodium hydroxide concentration

Sodium hydroxide addition to the diazo coupling mixture was investigated using absorbance. Figure 4.2 (a) depicts the results. It demonstrates that the absorbance increases initially with increasing sodium hydroxide concentration up to 0.1 mol/L and then decreases as the concentration increases. To achieve a good response, 0.1 mol/L of sodium hydroxide was used in the diazotization process (Figure 3.3). A decrease in absorbance above 0.1 mol/L can be attributed to the dye partially decolorizing at higher alkali concentrations. The consideration of acid-base equilibriums of diazonium compounds can explain a decrease in color intensity of the mixture at higher alkali conditions (Sudhir & Nadh, 2013)

4.2.1.2: Effect of aniline concentration

Aniline concentration effect on sensitivity of the method was also investigated over 0.0 (blank) – 400 ppm range. Figure 4.2 (b) depicts the results. There is a gradual increase absorbance with increasing aniline concentration while it remained constant between 100 ppm and 120 ppm before decreasing. As a result, 120 ppm aniline was selected for the study as the two concentrations gave the same results when investigated further. 250 ppm was not selected even though it gave the maximum absorbance because after 120 ppm the absorbance decreased indicating that it had reached saturation.

4.2.1.3: Effect of hydrochloric acid concentration

The influence of HCl concentrations on method sensitivity was examined by diazotizing aniline at concentrations ranging from 0.0 to 1.4 mol/L. Figure 4.2 (c) depicts the findings. It reveals that absorbance increases initially with increasing HCl addition up to 0.1 mol/L before becoming constant. To achieve a satisfactory reaction, 0.1 mol/L HCl was utilized for the derivatization method as concentrations ranging from 0.1 to 0.4 mol/L gave similar results so 0.1 was maintained throughout the study.

4.2.1.4: Effect of sodium nitrite concentration

Sodium nitrite concentration's impact on method sensitivity was investigated across the range of 0.0 - 400 ppm. Figure 4.2 (d) depicts the collected findings. The absorbance progressively rose with increasing sodium nitrite concentration and began to decrease at 100 ppm. As a result, 100 ppm sodium nitrite was chosen for future investigation. Excess sodium nitrite should be avoided

since it will destabilize the diazotized salt due to the excess nitrous acid generated in the medium (Sudhir & Nadh, 2013).

4.2.1.5: Effect of temperature on the diazo coupling reaction

Diazotization at low temperatures is advantageous due to increased free nitrous acid solubility, preventing nitrous gases from escaping from acidic medium, and improved the stability of aniline. As a result, diazotization was performed at 19°C which was the room temperature during this study.

4.2.2: Calibration curve for UV-Vis analysis

Under the above optimum conditions, the observed data was plotted versus BPA concentration, a graphical calibration curve was obtained. For obtaining calibration curve for BPA solution, initially a stock solution of 100ppm was prepared and further this solution was used to prepare other BPA solutions with 0, 2, 4, 6, 8, 10, 12 $\mu\text{g mL}^{-1}$ concentration and absorbance of all solutions were recorded by UV-Vis spectrophotometer at $\lambda_{\text{max}} = 424 \text{ nm}$. The calibration curve so obtained by plotting absorbance versus concentration at λ_{max} is shown in Figure 4.3 and it allows the determination of BPA in the measuring range of 0 (blank) – 12 $\mu\text{g mL}^{-1}$.

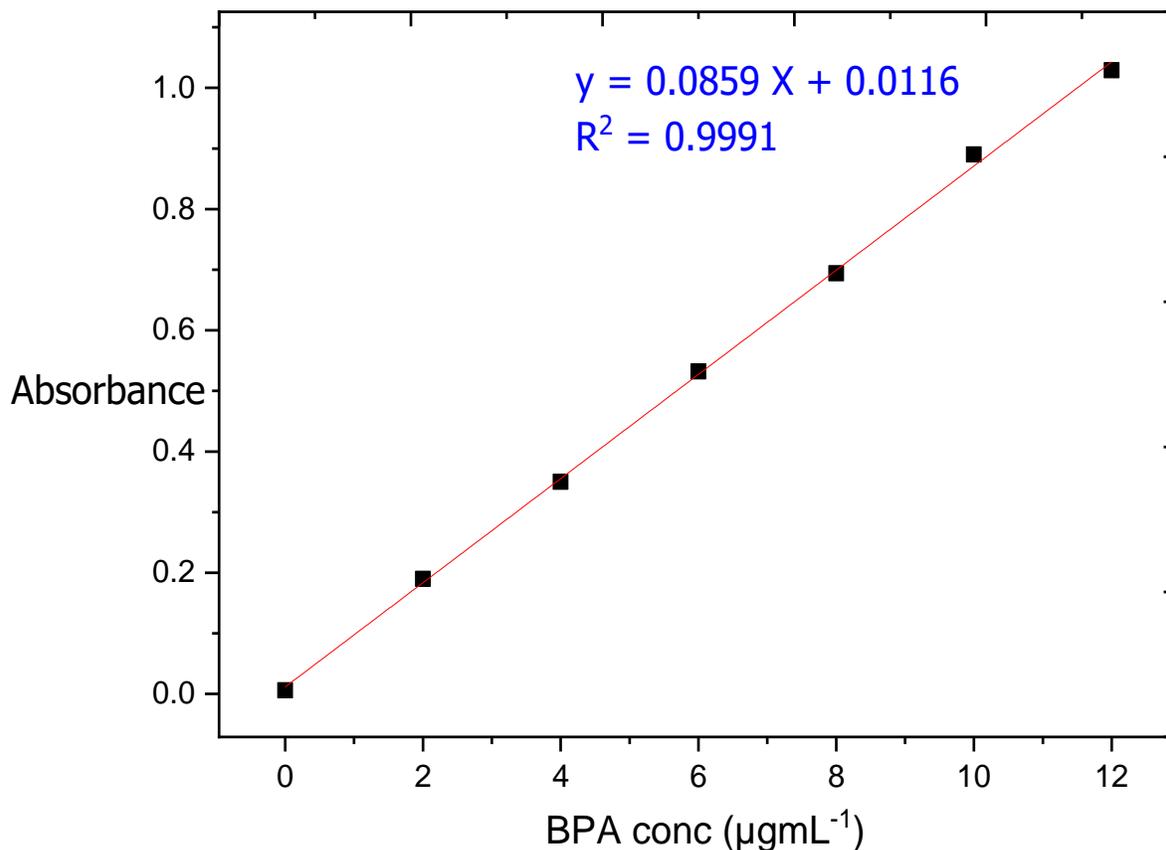


Figure 4.3 Calibration curve used for BPA analysis

A straight line calibration curve passing through the origin was obtained (data in appendix). The curve depicted that Beer's law is conformed along BPA concentration range of 0-12 µg mL⁻¹ with a linear regression equation of $Y = 0.0116 + 0.0859 X$ (X : µg mL⁻¹) having a correlation coefficient of $R^2 = 0.9991$. The detection limit, from the calibration curves' slope and quantification (LOQ) were determined. LOD and LOQ were calculated as outlined below (ICH Guideline 2005): $LOD = 3.3 \delta / S$. Where δ is the calibration curve intercepts' standard deviation. S being the linearity slope of the plot. The LOD was determined to be 0.8 µg mL⁻¹ and LOQ 2.4 µg mL⁻¹.

4.2.3: Recovery studies

The accuracy of the developed method, BPA analytical recovery experiments were done using 2, 4 and 6 µg/ml concentrations as indicated in table 4.1.

Table 4:1: Recovery test for spectroscopic method

Analyte BPA	Standard added ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery/%	RSD / %
	2.0	2.10	105.0	3.45
	4.0	4.12	103.0	2.09
	6.0	6.20	103.3	2.32

Good recovery percentages were recorded for BPA quantification method and they ranged from 103.0 ± 2.09 - 105.0 ± 3.45 %. Therefore, the method's sensitivity was analytically acceptable and being simple, quick and having the ability to determine sensitivity, this method was advantageous.

4.2.4: Determination of BPA in real samples

The analysis was done utilizing bottles that had been cut into small pieces for easier analysis. Samples of varying weights 0.5 to 1.3g were selected for analysis to represent the entire population. The results below indicate that BPA leaching was detected in all the samples.

Table 4:2: The temperature effect on BPA amount leaching in distilled water

Sample (Water bottles)	Age	Detected ($\mu\text{g mL}^{-1}$)		
		95°C	50°C	25°C
A	New	0.65 ± 0.02	0.43 ± 0.02	0.20 ± 0.03
B	New	0.54 ± 0.04	0.39 ± 0.03	0.17 ± 0.08
M	New	0.69 ± 0.09	0.47 ± 0.01	0.14 ± 0.01
C	Used	0.78 ± 0.06	0.61 ± 0.06	0.31 ± 0.06
D	Used	0.69 ± 0.05	0.59 ± 0.04	0.24 ± 0.05
J	Used	0.56 ± 0.03	0.53 ± 0.07	0.36 ± 0.04

From table 4.2 above, it was observed that there was more leaching of BPA from used bottles C, D and J at 0.78 ± 0.06 , 0.69 ± 0.05 , and 0.56 ± 0.03 respectively compared to new bottles, A, B and M at 0.65 ± 0.02 , 0.54 ± 0.04 , 0.69 ± 0.09 respectively at 95°C. This was also the trend for analysis carried out at 50°C and 25°C for distilled water. The leaching of BPA decreased as the analytical

temperature decreased from 95°C - 25°C. The leaching of BPA from used water bottles was higher may be due to the fact that the bottles had been subjected to harsh condition during washing and sterilization techniques practiced by mothers before feeding the baby. Washing, brushing and sterilizing has eventually loosened the BPA polymer bonds on the surface of the plastic material and this action has caused free BPA monomers to leach into the bottles while feeding.

Table 4:3 The effect of temperature on the mount of BPA leaching in lemon water

Sample (Water bottles)	Age	Detected(μgmL^{-1})		
		95°C	50°C	25°C
A	New	1.2±0.09	0.9±0.10	0.7±0.06
B	New	1.1±0.03	0.7±0.01	0.4±0.08
M	New	1.4±0.08	0.9±0.04	0.6±0.01
C	Used	1.6±0.01	1.4±0.03	1.1±0.12
D	Used	1.4±0.07	1.1±0.05	0.9±0.02
J	Used	1.8±0.13	1.3±0.15	1.0±0.04

From the results in table 4.3 above, it was observed that there was more leaching of BPA from used bottles C, D and J at $1.6\pm 0.01 \mu\text{gmL}^{-1}$, $1.4 \pm 0.07 \mu\text{gmL}^{-1}$, and $1.8\pm 0.13 \mu\text{gmL}^{-1}$ respectively compared to new bottles, A, B and M at $1.2 \pm 0.09 \mu\text{gmL}^{-1}$, $1.1 \pm 0.03 \mu\text{gmL}^{-1}$, $1.4 \pm 0.08 \mu\text{gmL}^{-1}$ respectively at 95°C in lemon water. This was also the trend for analysis carried out at 50°C and 25°C. The leaking of BPA in the distilled water (table 4.2) was higher than the values obtained in lemon water (table 4.3).

From table 4.4 below, it was observed that there was more leaching of BPA from used baby feeding bottles L, P and H at 2.82 ± 0.05 , 2.72 ± 0.11 , and $2.75 \pm 0.09 \mu\text{gmL}^{-1}$ respectively compared to new bottles, I, F and G at 2.40 ± 0.06 , 2.65 ± 0.08 and $2.46 \pm 0.07 \mu\text{gmL}^{-1}$ respectively at 95°C in milk. This was also the trend for analysis carried out at 50°C and 25°C. The leaching of BPA in the milk was much higher in new baby bottles when the temperature reduced from 50°C to 25°C but was lower in the used bottles at the same temperature change.

Table 4:4: The temperature effect on BPA amount of BPA leaching in milk

Sample (Baby bottles)	Age	Detected ($\mu\text{g mL}^{-1}$)		
		95°C	50°C	25°C
I	New	2.40±0.06	2.29±0.05	0.78±0.01
F	New	2.65±0.08	2.50±0.02	0.94±0.03
G	New	2.46±0.07	2.30±0.07	0.86±0.09
L	Used	2.82±0.05	2.61±0.03	2.18±0.06
P	Used	2.72±0.11	2.55±0.01	0.97±0.04
H	Used	2.75±0.09	2.63±0.03	0.88±0.02

The results in table 4.5 below shows the effect of temperature on the amount of BPA leaching in infant formula also decreased as the temperature decrease from 95°C - 25°C, in both the old and new baby bottles. The BPA leaching levels in the infant formula (table 4.5) were close levels to those in milk (table 4.4)

Table 4:5: The temperature effect on BPA amount leaching in Infant formula

Sample (Baby bottles)	Age	Detected ($\mu\text{g mL}^{-1}$)		
		95°C	50°C	25°C
I	New	2.20±0.06	2.11±0.10	0.98±0.01
F	New	2.15±0.15	2.08±0.02	0.94±0.02
G	New	2.36±0.12	2.19±0.07	0.86±0.07
L	Used	2.62±0.07	2.41±0.05	1.40±0.13
P	Used	2.42±0.11	2.25±0.03	0.97±0.04
H	Used	2.55±0.09	2.33±0.08	1.67±0.01

From table 4.6 below, the effect of contact time on the amount of BPA leaching in distilled water at 1 - 12 hours was higher for the used water bottle C, D and J at 3.23 ± 0.04 , 3.14 ± 0.09 and $3.02 \pm 0.02 \mu\text{g mL}^{-1}$ respectively than the new ones, B and M at 2.87 ± 0.10 , 3.01 ± 0.07 and $2.98 \pm 0.15 \mu\text{g mL}^{-1}$ respectively after 12 hrs. However, after 1 hour the effect of contact time of BPA leaching in distilled water were much higher than those obtained for 6 and 12 hours.

Table 4:6: The effect of contact time on the amount of BPA leaching in distilled water

Sample (Water bottles)	Age	Detected(μgmL^{-1})		
		1hr	6hrs	12hrs
A	New	0.55 \pm 0.02	2.79 \pm 0.05	2.87 \pm 0.10
B	New	0.54 \pm 0.04	2.84 \pm 0.02	3.01 \pm 0.07
M	New	0.59 \pm 0.09	2.67 \pm 0.06	2.98 \pm 0.15
C	Used	0.78 \pm 0.06	3.08 \pm 0.09	3.23 \pm 0.04
D	Used	0.69 \pm 0.05	2.97 \pm 0.01	3.14 \pm 0.09
J	Used	0.65 \pm 0.03	2.88 \pm 0.13	3.02 \pm 0.02

In milk samples BPA leaching was also high at 25°C which could be due to the solubility of BPA in fatty material, BPA is hydrophobic and hence its more soluble in fatty material. Bisphenol A leaching was higher when lemon water was used (table 4.3) compared to distilled water (table 4.2) as acidic solutions cause polymer degradation via hydrolysis, resulting in increased BPA migration.

The results also showed that leaching of BPA from polycarbonate bottles increases at higher temperatures. At elevated temperature hydrolysis of the polymer could form traces of free BPA at the surface which could then migrate during subsequent contact with food or water. The baby bottles did not show leaching of BPA at room temperature when filled with apple juice, however, leaching of BPA was observed in milk, water and infant formula samples when kept in baby bottles even at room temperature.

The results of this study is very similar to what was reported by (Zhang *et al.*, 2014) where BPA migrated in the ranges of 0.50 – 0.84 μgmL^{-1} in four different types of polycarbonate containers. The results are also comparable to results obtained by (Bashir & Audu, 2020) where BPA migration in hot water and milk stored in the bottles were 0.40 \pm 0.05, 0.80 \pm 0.11 and 0.52 \pm 0.10 μgmL^{-1} for the three water samples, 3.85 \pm 0.20 and 1.78 \pm 0.07 μgmL^{-1} for the milk samples respectively. Another study conducted by Xu and colleagues also reported BPA concentrations in water bottles and milk between the range of 0.56 – 0.68 μgmL^{-1} and 0.13 – 0.27 μgmL^{-1} respectively, BPA in milk is much lower than the present study probably due to different sources of the milk as milk powder was used in previous study, whereas fresh milk was used in the present study

The estimated safe level known as the tolerable daily intake (TDI) of Bisphenol A set up by European Food Safety Authority was given as 4 $\mu\text{g}/\text{kgbw}/\text{day}$ (EFSA, 2015). The average migration value of BPA in water and milk samples obtained in this study were 0.46 $\mu\text{g}/\text{mL}^{-1}$ and 2.07 $\mu\text{g}/\text{mL}^{-1}$ respectively. The estimated daily intake from these results assuming an infant of 5kg consuming at least 250 ml of water and milk in a day, would be 23 $\mu\text{g}/\text{kgbw}/\text{day}$ and 103.5 $\mu\text{g}/\text{kgbw}/\text{day}$ respectively. This indicates that the average daily intake of BPA in water and milk in the present study has exceeded the set tolerable daily intake of 4 $\mu\text{g}/\text{kgbw}/\text{day}$

From the findings of this research the use of polycarbonate feeding bottles and water bottles cannot be regarded as safe for the level of BPA consumed at every feeding of the infant.

4.3: Redox marker studies/calibration studies

Potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) redox probe is a red salt comprising of $[\text{Fe}(\text{CN})_6]^{3-}$ coordination compound. The redox probe is soluble in water and fluorescent. Potassium ferricyanide is used as a tool in physiological experiments. $\text{K}_3\text{Fe}(\text{CN})_6$ consisting of octahedral $[\text{Fe}(\text{CN})_6]^{3-}$ center with K^+ ions cross-linking bounded to CN ligands (KOÇ, 2021).

Potassium ferricyanide finds common use as a standard reference in calibration of a cyclic voltammogram in aqueous state. To determine the electroactive nature of the unmodified glassy carbon electrode, the use of cyclic and differential pulse voltammetry was utilized.

4.3.1: Electrochemical characterization of bare GCE

4.3.1.1: Scan Rate study

Figure 4.4 below shows a reversible cyclic voltammetry of potassium hexacyanoferrate (III) (Ferricyanide) in 1 M KNO_3 and phosphate buffer (pH 7) of which the current peaks increase with increasing scan rate. On the x-axis was applied potential which was imposed on the working electrode while the response, current appears on y-axis.

Well-defined pairs of reversible redox peaks found at the glassy carbon electrode (GCE) indicating GCE's good potential with high-quality substrate of the electrode. The separation of the peak from the expected $59/n$ mV is not so much the greatest deviation is only 6 mV which is only 10%. Further the I_{pa}/I_{pc} ratio of unity is obtained.

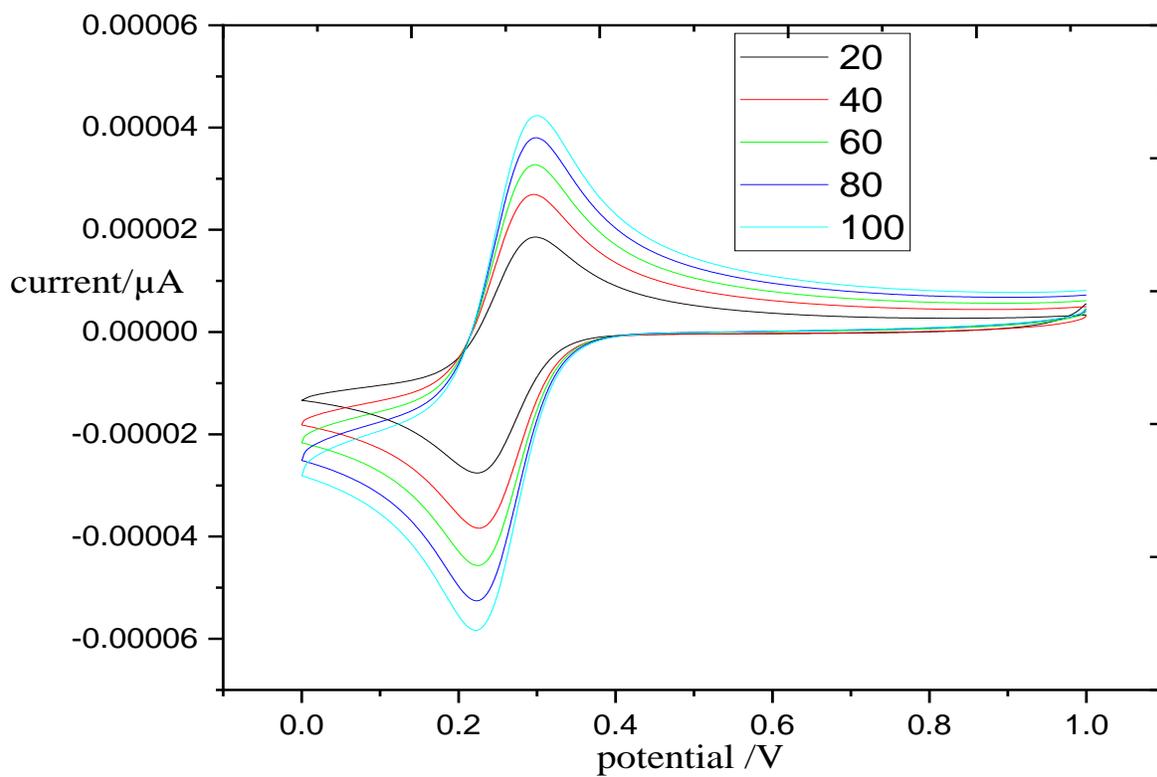


Figure 4:4 Cyclic voltammetry of potassium hexacyanoferrate (III) Ferricyanide at different scan rates scanned cathodically

Oxidation and reduction peak currents (I_{pa} and I_{pc}) from CV measurements can best be depicted by Randles Sevcik equation.

Table 4:7: Results for scan rate and peak height for 3mM $K_3Fe(CN)_6$ in 1M KNO_3

Solution Scan rate (mV/s)	$V^{1/2}$ (mV/s)	E_{pa} (V)	E_{pc} (V)	I_{pa} ($\times 10^{-5}A$)	I_{pc} ($\times 10^{-5}A$)	$ i_{pa}/i_{pc} $	$E^{0'}$ (V)	ΔE_p (V)
20	4.472	0.2878	0.2253	2.171	-2.147	1.011	0.257	0.063
40	6.324	0.2909	0.2302	3.081	-2.956	1.042	0.261	0.060
60	7.745	0.2934	0.2324	3.853	-3.769	1.022	0.263	0.061
80	8.944	0.2958	0.2324	4.349	-4.143	1.049	0.264	0.063
100	10.00	0.2983	0.2325	4.762	-4.690	1.015	0.265	0.065

Key:

E_{pa} = anodic peak

i_{pa} = peak anodic current

E_{pc} = cathodic peak

i_{pc} = peak cathodic current

i_{pa}/i_{pc} = ratio of anodic peak current and cathodic current

E° = Formal redox potential

ΔE_p = peak separation

From the studies on scan rate in table 4.7 it can be seen that peak separation values were between 0.060 to 0.065 V having a $0.062 \text{ V} \pm 0.001$ standard deviation close to $59/n$ mv the theoretical value for a reversible system, i_{pa}/i_{pc} ranging between 1.011 to 1.049 V and a mean of 1.028 with ± 0.015 standard deviation as expected for a reversible system.

The reduction potential that is applicable under a certain set of parameters, such as pH, ionic strength, and complexon concentration, is known as a formal potential. By averaging the anodic and cathodic peak potentials, formal potential (E°) for a reversible process is determined.

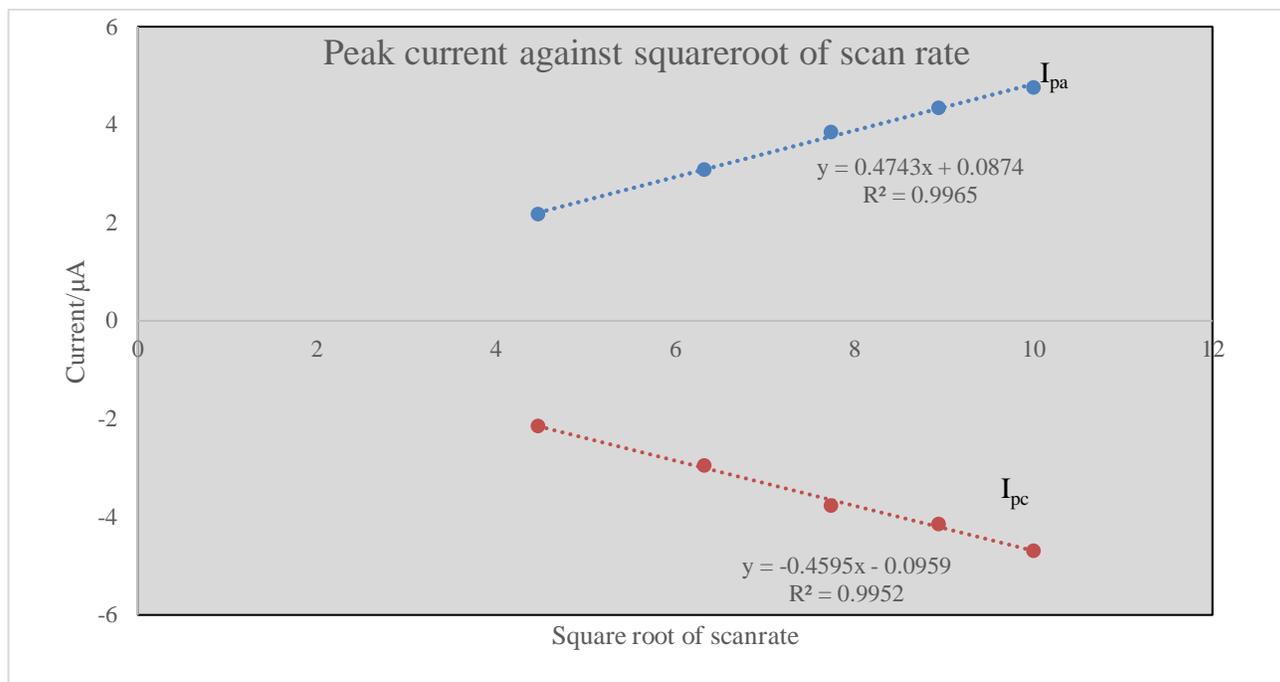


Figure 4:5 A Randles-Sevcik plot of anodic and cathodic peak currents against the square root of scan rate for the 3mM $\text{K}_3\text{Fe}(\text{CN})_6$

The relationship shown in the figure above is linear; confirming that under diffusion controlled conditions, redox couple $K_3Fe(CN)_6$ behaves reversibly. From the graph, there is a good linear regression in figure 4.5 value of beyond 0.99. This signifies a good correlation between the values. This is an indication that the potentiostat was in good working conditions well calibrated and ready for use for further analysis.

4.3.1.2: Concentration study

The effect of concentration of potassium ferricyanide on peak current was also investigated. It was observed that the peak currents increased with increase in concentration with a concentration range from 1 mM, 2 mM, 3 mM, 4 mM, and 5 mM (Fig. 4.6) below.

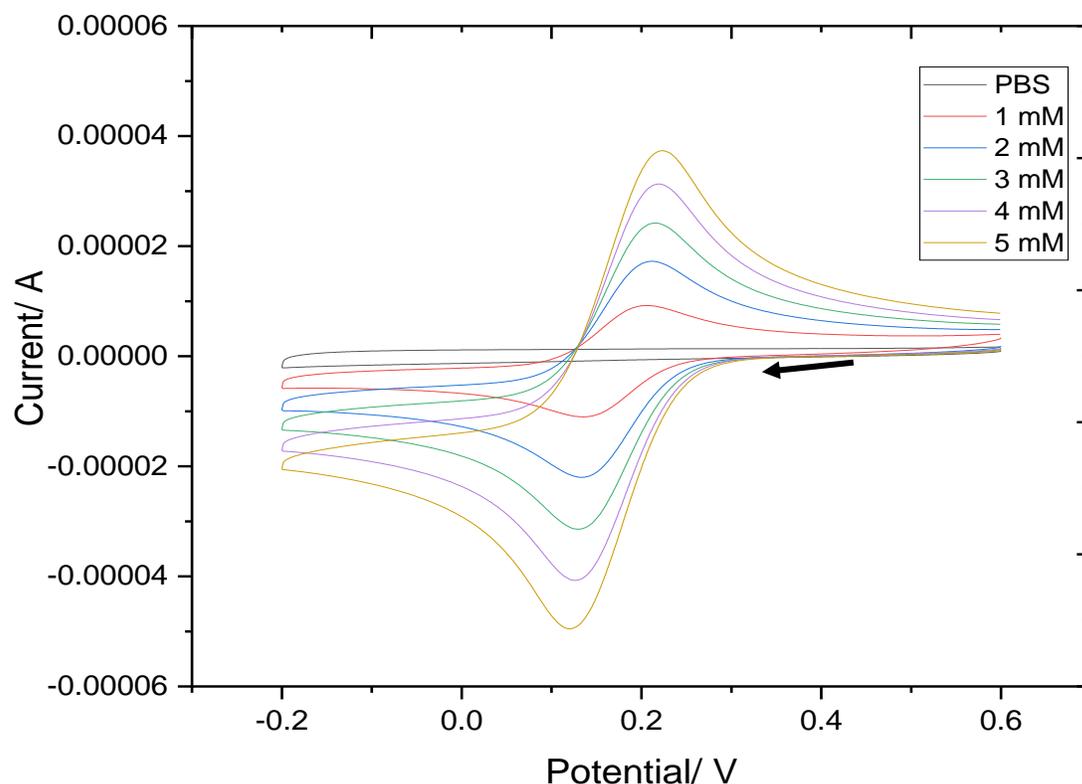


Figure 4.6 Effect of concentration of potassium ferricyanide on peak currents at a scan rate of 50mV/s

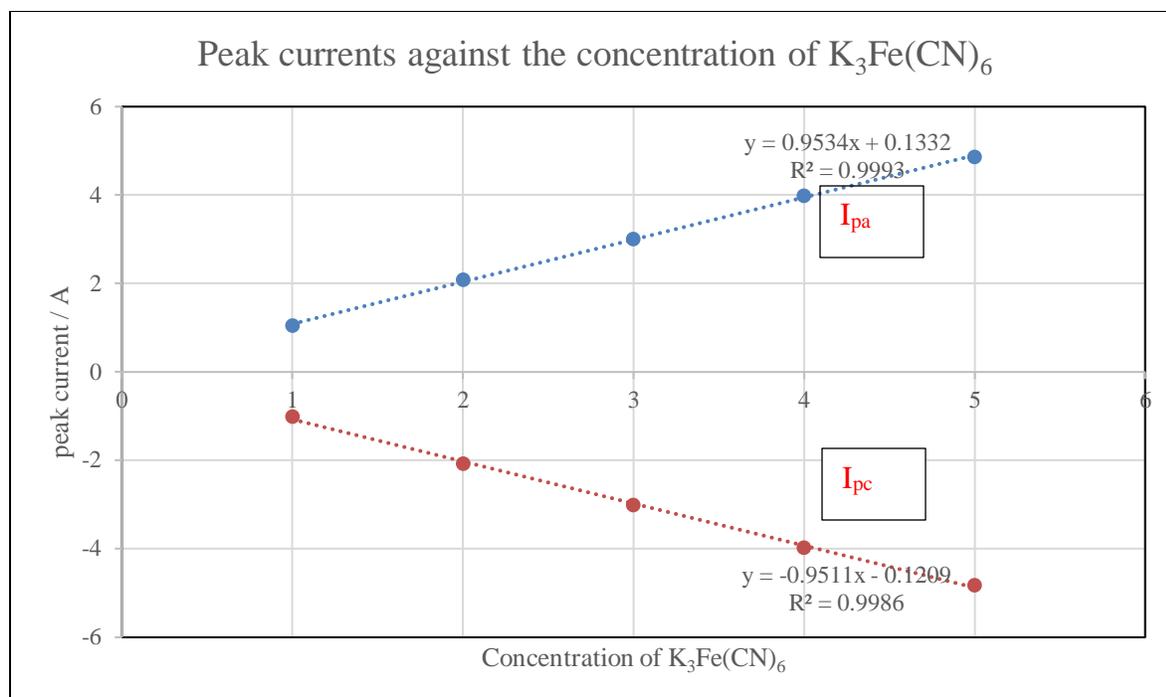


Figure 4:7 A Randles-Sevcik plot of anodic and cathodic peak currents against the concentration of $K_3Fe(CN)_6$

The R^2 for the anodic peak was 0.9993 and the R^2 for the cathodic peak was 0.9986. It was observed that the peak current (i_p) was linearly proportional to the concentration and this concentration study was also used to confirm that this was a diffusion controlled process as indicated in figure 4.7 above.

4.3.2: Electrochemical characterization of MWCNTs-modified GCE using cyclic voltammetry

Conventional bare electrodes have various limitations such as low sensitivity and low selectivity, introducing multifunctional materials such as carbon nanotubes which have high specific surface area and good electrical conductivity can be used to overcome the challenges. Thus, after surface modification of the bare GCE electrode with MWCNTs the current increases from 38.60 μA of a bare GCE up to 42.02 μA of MWCNTs-modified GCE (Figure 4.8). Indicating that multiwalled carbon nanotubes enhanced the sensitivity/conductivity of the glassy carbon electrode with supporting electrolyte of 1.0 M KNO_3 on a Scan rate of 50 mV/s. The current enhancement due MWCNTs presence however was low in the ferricyanide system and a phosphate buffer system was explored with better results.

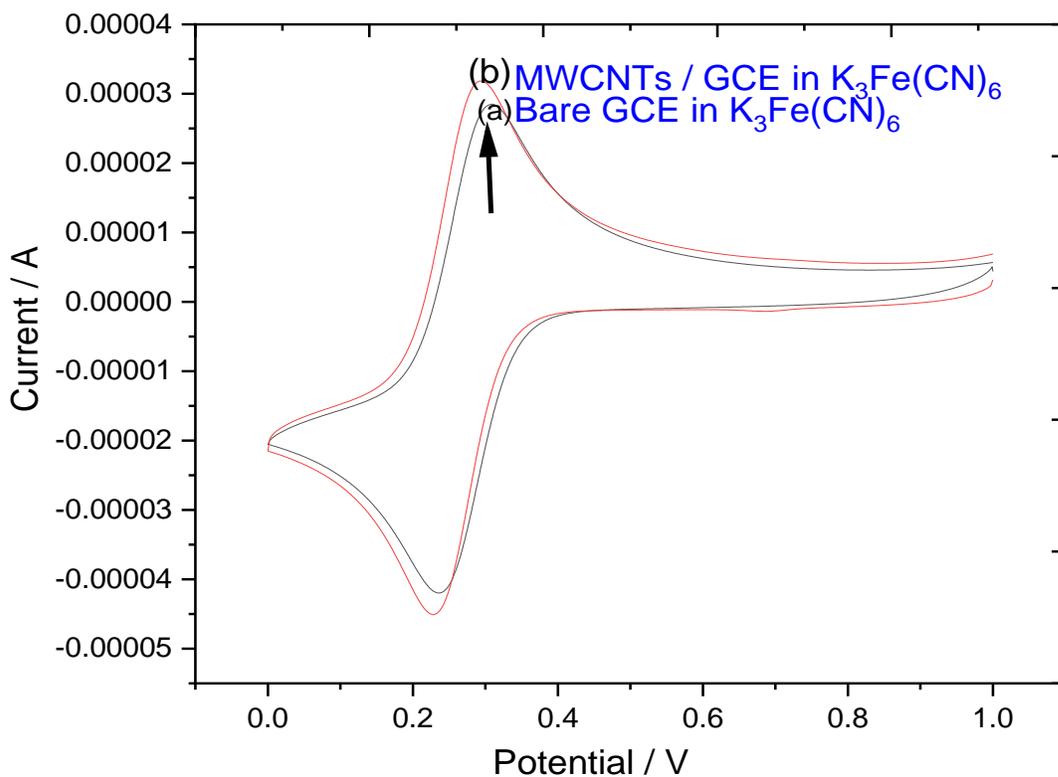


Figure 4:8 Voltammograms of bare GCE (a) and MWCNTs-modified GCE(b) in 3mM $K_3Fe(CN)_6$.

4.3.3: Electrochemical characterization of MWCNTs-modified GCE using Differential pulse voltammetry

In Figure 4.9, the bare GCE (a) depicts lower sensitivity towards $K_3Fe(CN)_6$ compared to MWCNTs-GCE (b). Increase in current from 77.0325 μA of a bare GCE up to 81.5338 μA of the MWCNTs-modified GCE is observed.

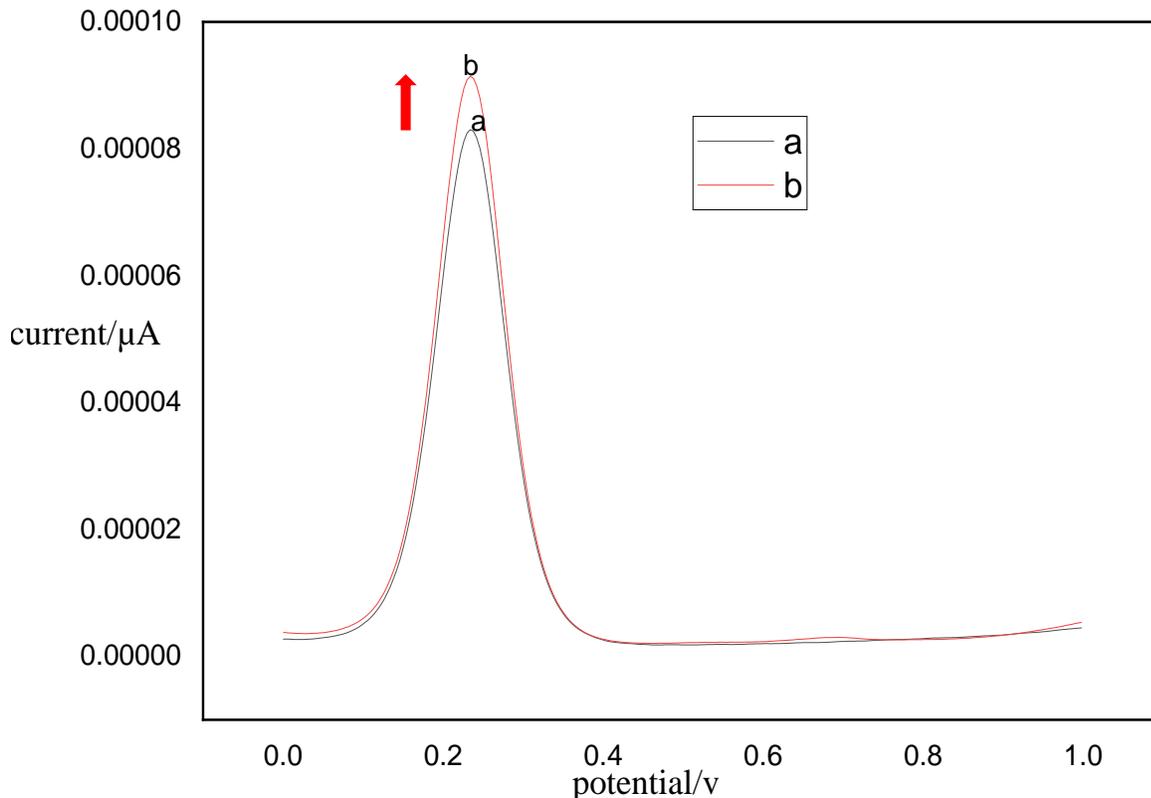


Figure 4:9 Differential pulse voltammetry of bare GCE (a) and MWCNTs-modified GCE (b) in 3mM $K_3Fe(CN)_6$.

The increase in current in both cyclic voltammetry and differential pulse voltammetry can possibly be attributed to a surface area increase coming up from the MWCNTs.

4.4: pH optimization study of phosphate buffer

The pH of phosphate buffer has a significant impact on the acid-base dissociation of BPA resulting in changes in its oxidation potential and current. Therefore, the effect of pH on the oxidation peak current and peak potentials of BPA on MWCNT-modified GCE was investigated with pH ranging from 4 to 12 as shown in Figure 4.10. The response peak currents of BPA gradually increase with increasing pH value, indicating that protons participated in the bisphenol's reaction process at MWCTs/GCE. Afterwards, the peak currents are decreased in higher pH values, which may be attributed to the electrostatic repulsion of anionic bisphenols with negative charges on the

MWCNTs/GCE sensor surface. Considering the effect of pH on the electrochemical oxidation current of BPA, the peak currents realized under various pH under the PBS system at 575 mV which corresponded to BPA oxidation ranged between $(2.5 - 6.2) \times 10^{-6}$ A. Clearly BPA oxidation at pH 7, gave rise to a peak current of 6.2×10^{-6} A. Thus the pH 7.0 was chosen as the optimal pH for the detection of BPA at 575mV.

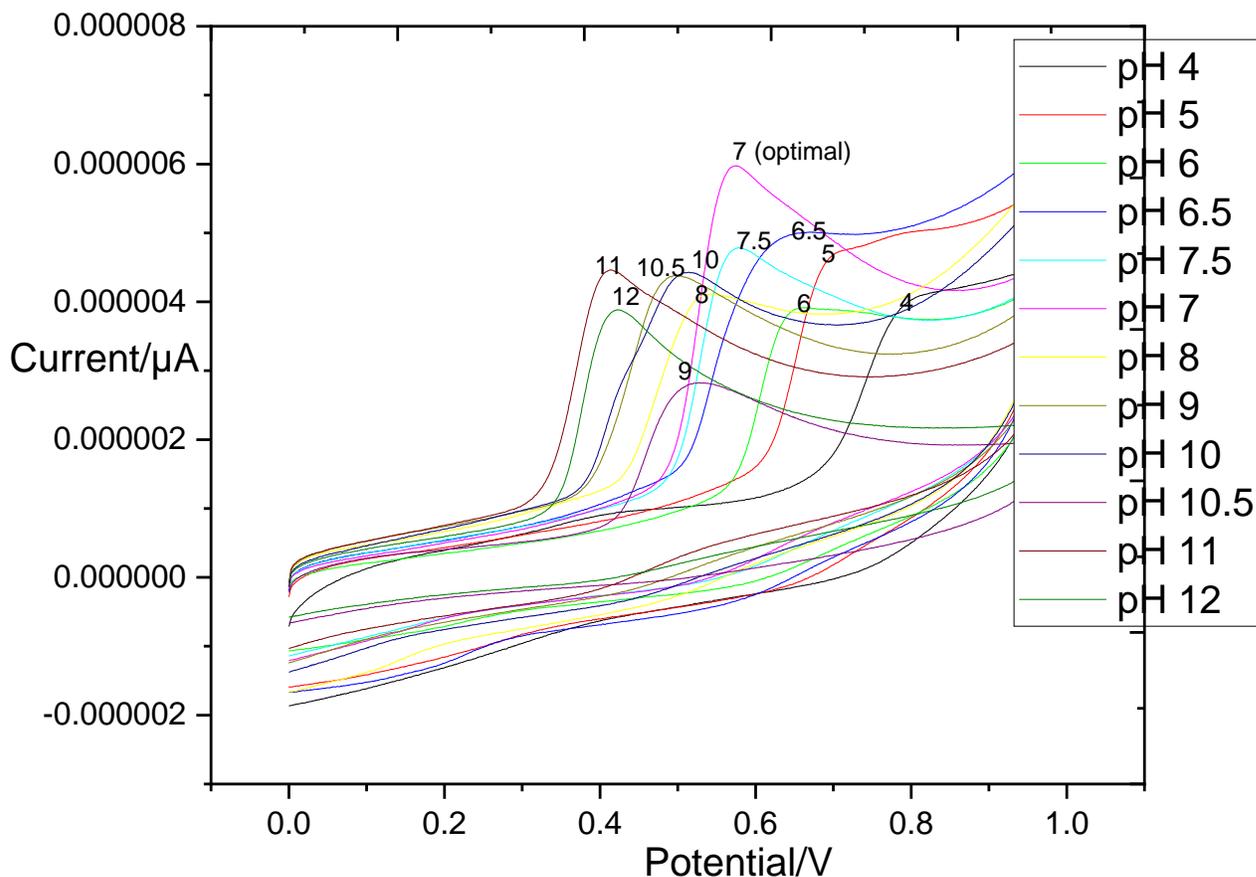


Figure 4:10 Cyclic voltammograms of pH optimization.

Figure 4.10 and 4.11 showed that pH 7.00 had the highest current and was therefore chosen as the optimal value for use.

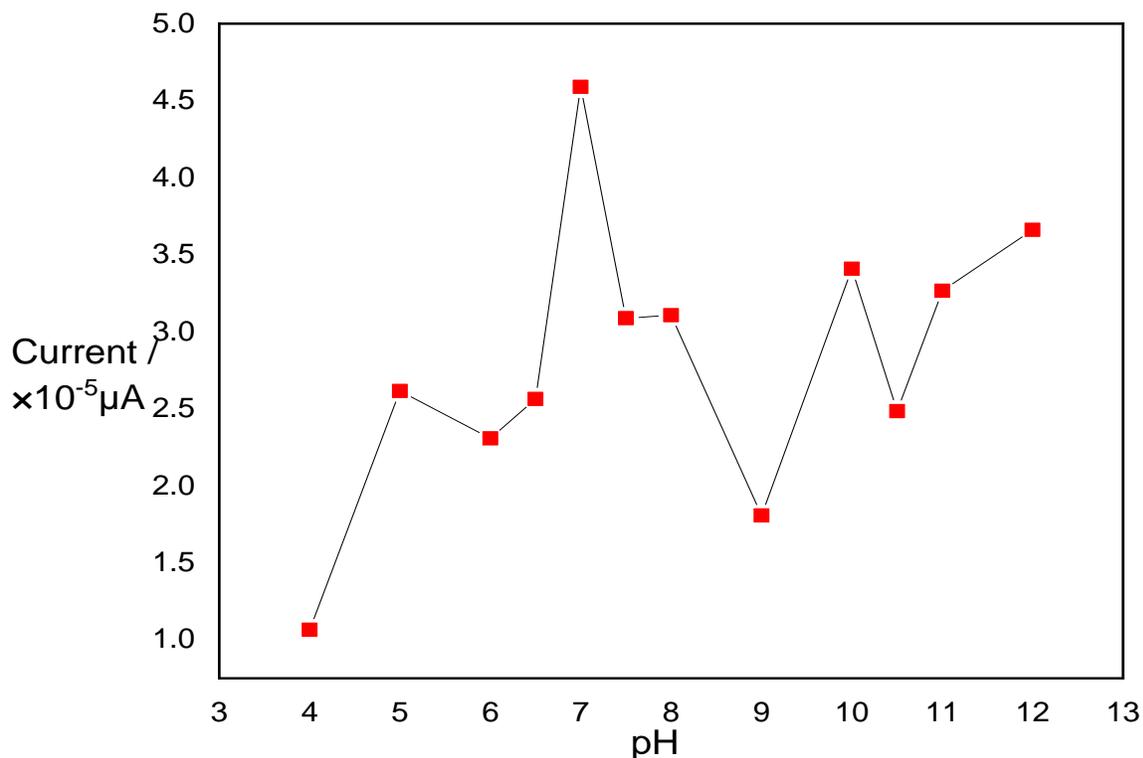


Figure 4:11 A plot of peak current against pH, pH 7 selected as optimal pH

4.5: Electrochemical oxidation of Bisphenol A.

Bisphenol A is an electroactive molecule, however because it is a phenolic structure, direct BPA electrochemical oxidation is difficult. Secondary oxidation products formation fouls the electrode system (Gugoasa, 2020).

Cyclic voltammetry (CV) was used in characterization of the unmodified and modified electrodes in BPA presence. This was by done by successive 0.01 M BPA aliquots additions into the phosphate buffer and the obtained results are as shown below in Figure 4.12. This study was conducted at potential ranges of -1.0 V to +1.0 V and at a pH of 7.0, 0.1 M phosphate buffer at scan rate of 50 mV/s. The obtained results in CV showed a centered oxidation peak at +0.5V.

BPA oxidation peak currents were determined to be 9.21 μA (curve b) and 18.89 μA (curve c) for bare and MWCNT-modified GCE respectively. Nevertheless, no peak was observed in curve a,

which indicated that peaks found in curve b and c were assigned to BPA. MWCNTs-GCE showed the highest oxidation peak current, this signifies an increased sensitivity. As observed, BPA's poor electro activity resulted to negligible and weak current peaks at bare GCE. The enhancement of oxidation peak current at MWCNTs-modified GCE shows that carbon nanotubes display electro-catalytic behavior towards the electrochemical BPA oxidation due to their large surface and electrochemical conductivity and fast electron transfer capability. BPA electrochemical oxidation leads to electrode fouling that reduces sensitivity. The use of MWCNTs leads to increase in sensitivity for BPA oxidation, enhanced peak current, and reduction or elimination of surface fouling.

Bisphenol A electrochemical behavior is typically an irreversible electrode reaction similar to other phenolic compounds (Kuramitz et al., 2001). Bisphenol A's oxidation mechanism can be linked to the hydroxyl functional group on the aromatic ring in BPA (Ławrywianiec *et al.*, 2017).

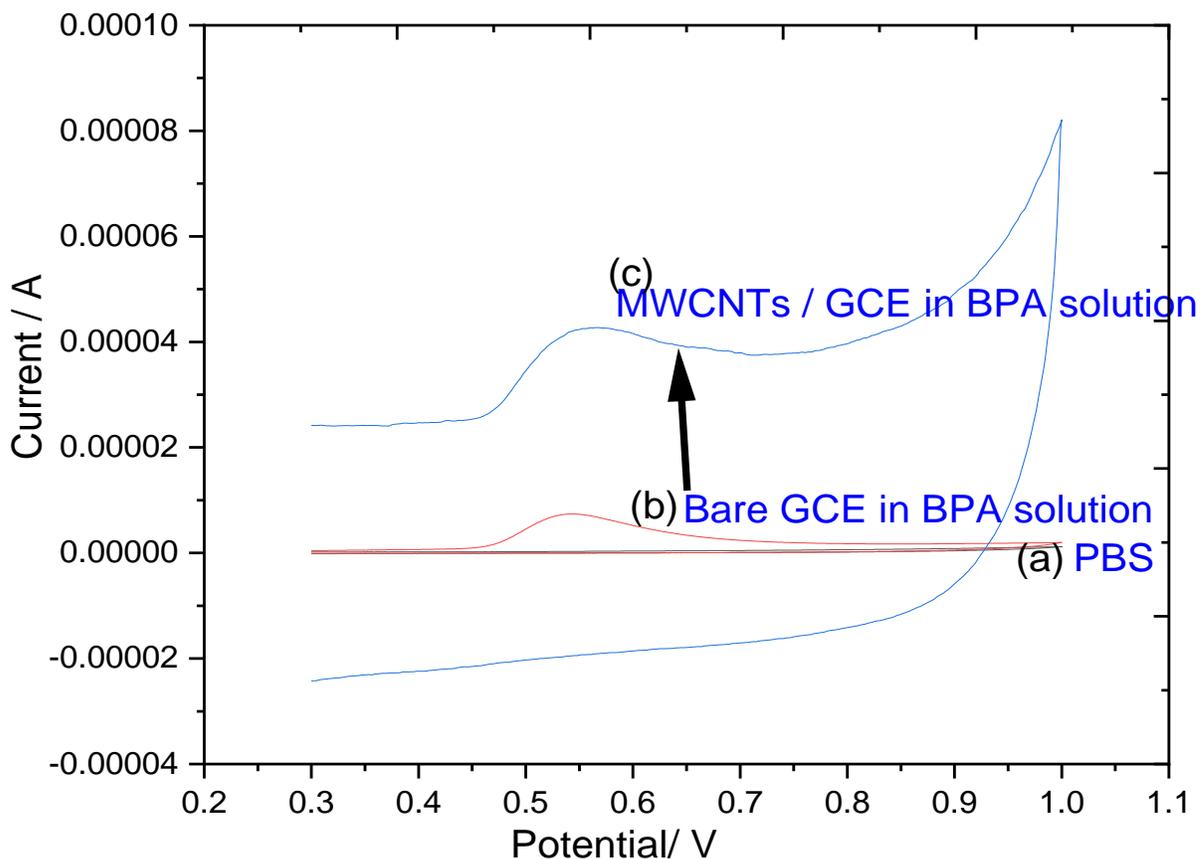


Figure 4:12 Cyclic voltammograms of (a)phosphate buffer, (b) 20 μ L BPA at bare GCE and (c) 20 μ L BPA at MWCNTs-modified GCE

The results obtained by differential pulse voltammetry in Figure 4.13 below are similar to those that were obtained using cyclic voltammetry as there is also increase in current from 9.96 μ A to 13.44 μ A showing that MWCNTs enhances the glassy carbon electrode sensitivity.

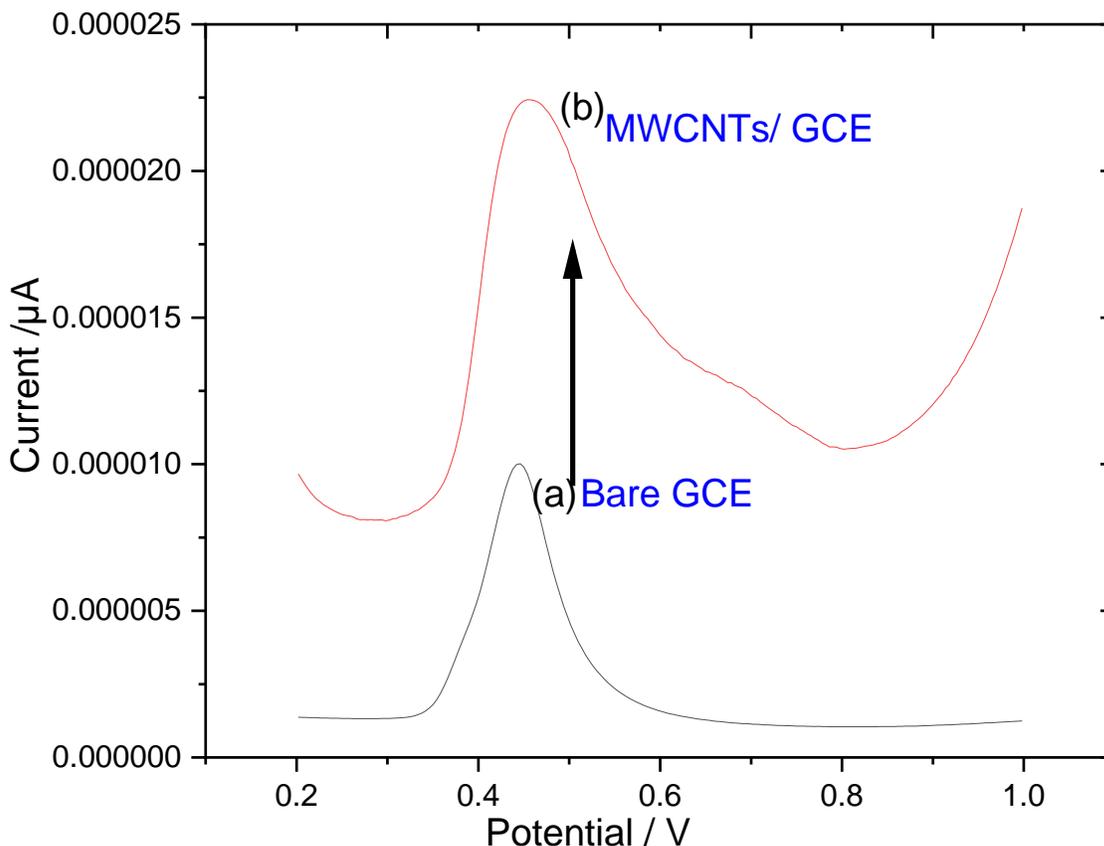


Figure 4:13 Differential pulse voltammetry of 20 μL at bare GCE (a) and 20 μL BPA at MWCNTs/GCE (b) Supporting electrolyte: 0.1M phosphate buffer.

The bare GCE and MWCNTs / GCE analytical performance was investigated by cyclic voltammetry and differential pulse voltammetry. An oxidation peak at 0.5V was observed with the bare GCE with 20 μM BPA. It was observed that the peak currents increased when the modified MWCNTs / GCE was used.

These results both from cyclic voltammetry and differential pulse voltammetry suggested that using MWCNTs contributed to improvement of electrochemical electrode response in terms of conductivity and electron-transfer rate.

4.6: Scan rate study of BPA on MWCNTs/GCE

Peak current and scan rate relationship gives valuable information involving electrochemical mechanism. Hence different scan rates between 10 to 60 mV/s were applied when studying the effect of scan rate on BPA oxidation peak current in solution using the modified electrode as represented in Figure 4.14. There is increase in peak current (I) with increase in scan rates within the range of 10 mV/s to 60 mV/s. These low range of scan rate was selected as it allowed more time for the BPA analyte to diffuse to the electrode surface for detection.

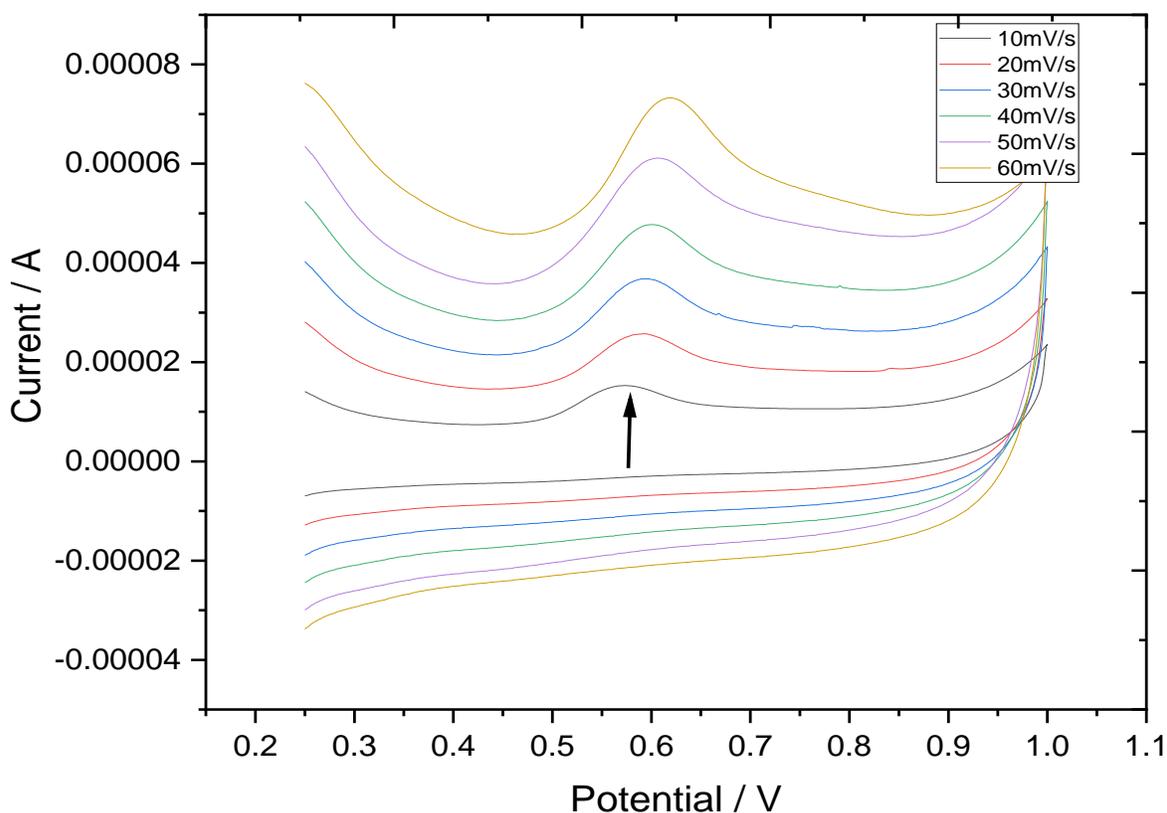


Figure 4:14 The scan rates influence on the peak current of 20 μ L BPA

The emanating results were used to explore scan rate dependence/behavior of the BPA peaks through construction of a Randles Sevcik plot, figure 4.15 below.

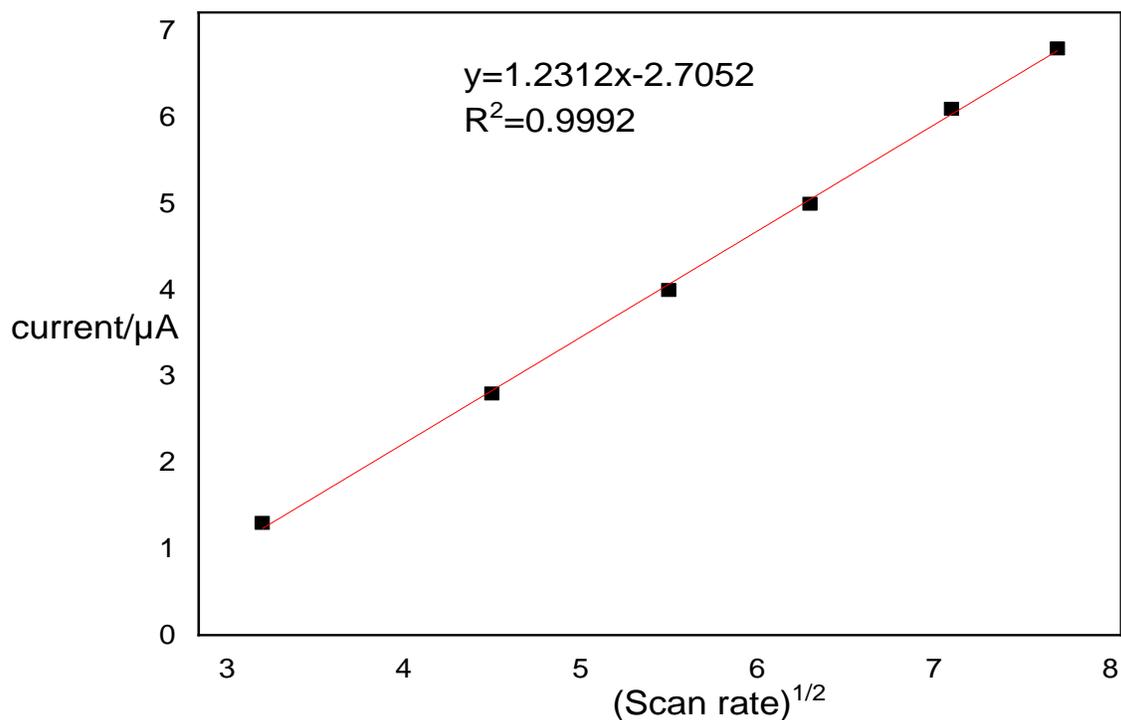


Figure 4:15 Peak currents as a function of square root of scan rate

Clearly, the peak current was linear with the square root of the scan rate, and $R^2 = 0.999$, indicating that the electrochemical behavior of BPA on MWCNTs/GCE was a typical diffusion-controlled process.

4.7: Precision studies in determination of Bisphenol A in phosphate buffer

This work employed repeatability which involved carrying out analysis of six samples using the same analytical machine (Basi Epsilon Eclipse potentiostat), a three-electrode system and analysis done by the same analyst.

In evaluating the proposed voltammetric method precision, six phosphate buffer samples solutions were spiked with 10 μL of 0.01 BPA. The oxidation potential and voltammetric currents were monitored using differential pulse voltammetry. The results were recorded as shown in table 4.8 below.

The obtained voltammetric currents from the data above were analyzed in determination of precision of the measurements. Bisphenol A's relative standard deviation, average value, and standard deviation were calculated.

Table 4:8: Potentials and currents of Bisphenol A using differential pulse voltammetry

Trial	Potential (V)	Current (μA) $\times 10^{-5}$
1	0.55	5.94
2	0.56	5.97
3	0.55	6.00
4	0.54	6.04
5	0.55	6.07
6	0.55	6.11

Table 4:9 Summary of statistical values obtained from cyclic voltammetry currents of Bisphenol A

Calculated statistical parameter	Bisphenol A
1. Number of replicate Sample	6
2. Average Value	6.02×10^{-5}
3. Standard Deviation (SD)	0.063
4. RSD%	1.06%

The standard deviation and relative standard deviation was calculated and the obtained values were 0.0637 and 1.06% respectively, (Table 4.9) above. Since the standard deviation was within the allowed limit of at most 10%, a conclusion was made that voltammetric currents were of the required precision, a further denotation that the voltammetric method developed was of good precision as seen in figure 4.16 below.

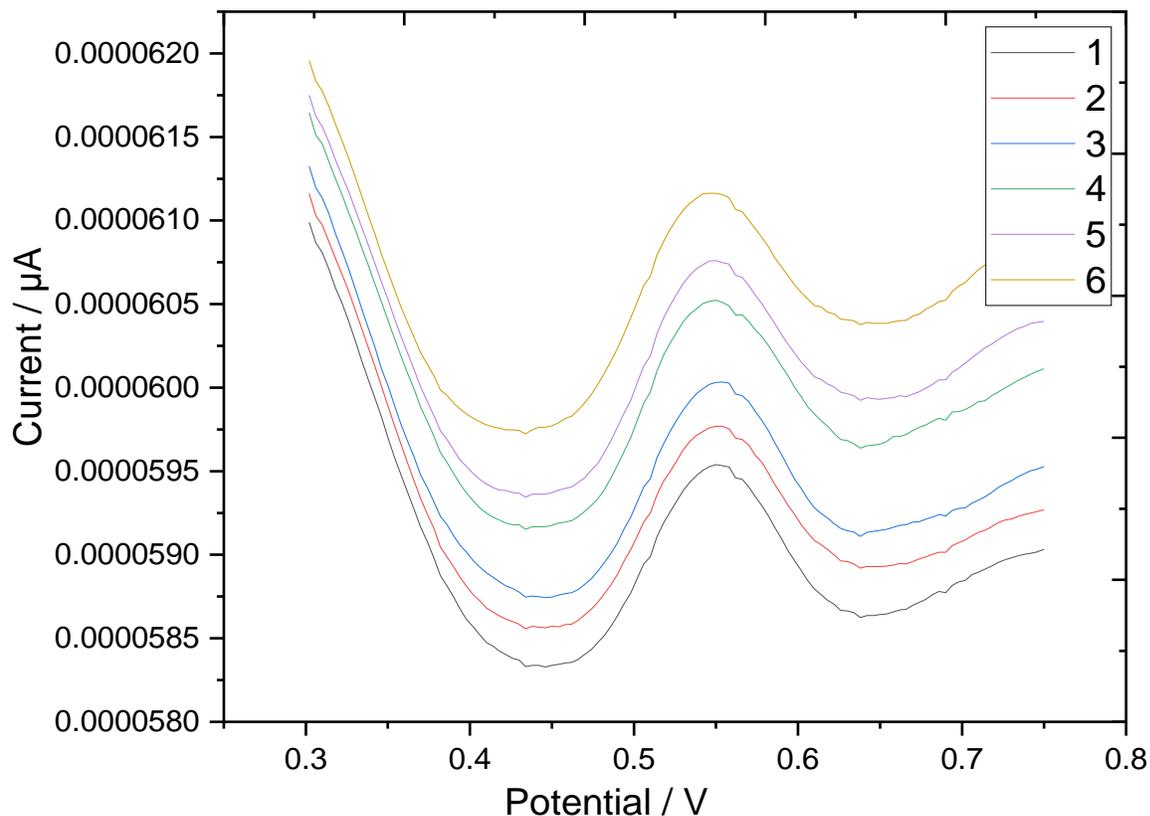


Figure 4:16 Differential pulse voltammometry voltammograms obtained for the precision study

4.8: Interference study

Any compound that has same reduction/oxidation potential as that of the analyte of interest is a potential interference. The selectivity of the modified electrode for simultaneous BPA determination was assessed in the presence of some interfering substances using cyclic voltammetry by scanning potential from -1.0 V to +1.0 V at a scan rate of 50mV/s and the resulting voltammetric currents and potentials were recorded (Table 4.10). The MWCNTs/GCE shows negligible interference in the 50-fold concentration concentrations of ions with deviations The results indicated that Ca^{2+} , Mn^{2+} , Pb^{2+} , Mg^{2+} , Zn^{2+} , Cu^{2+} , SO_4^{2-} , K^+ , Na^+ , Cl^- , NO_3^- , and PO_4^{3-} did not affect the response with deviations below 5.0% for detection of BPA. The indication from the results depict that the developed method satisfied selectivity and anti-interference ability in detecting BPA.

Table 4:10 The effect of addition of interferents on BPA in phosphate buffer

Interferent	Potential(V)	Current(μA) ($\times 10^{-5}$)
Control	0.687	5.371
Magnesium chloride	0.688	5.337
Sodium nitrite	0.689	5.957
Lead nitrate	0.689	5.168
Potassium dihydrogen orthophosphate	0.680	5.167
Calcium chloride	0.685	5.421
Magnesium nitrate	0.685	5.535
Zinc chloride	0.688	6.597
Potassium chloride	0.676	6.407
Sodium nitrate	0.688	5.167
Copper sulphate	0.689	7.274
Potassium nitrate	0.684	5.535
Sodium dihydrogen orthophosphate	0.684	6.265

From the above graph, there is no significant change in Bisphenol A oxidation potential, as the mean was 0.6855 and a standard deviation of 0.0039 from a mean and relative standard deviation of 0.57% were reported.

There was no significant effect on most of these substances both on voltammetric and oxidation potential of Bisphenol A. In real samples, the trace levels of these interferents are less, their effect of interference does not pose any major analysis effect (Figure 4.17).

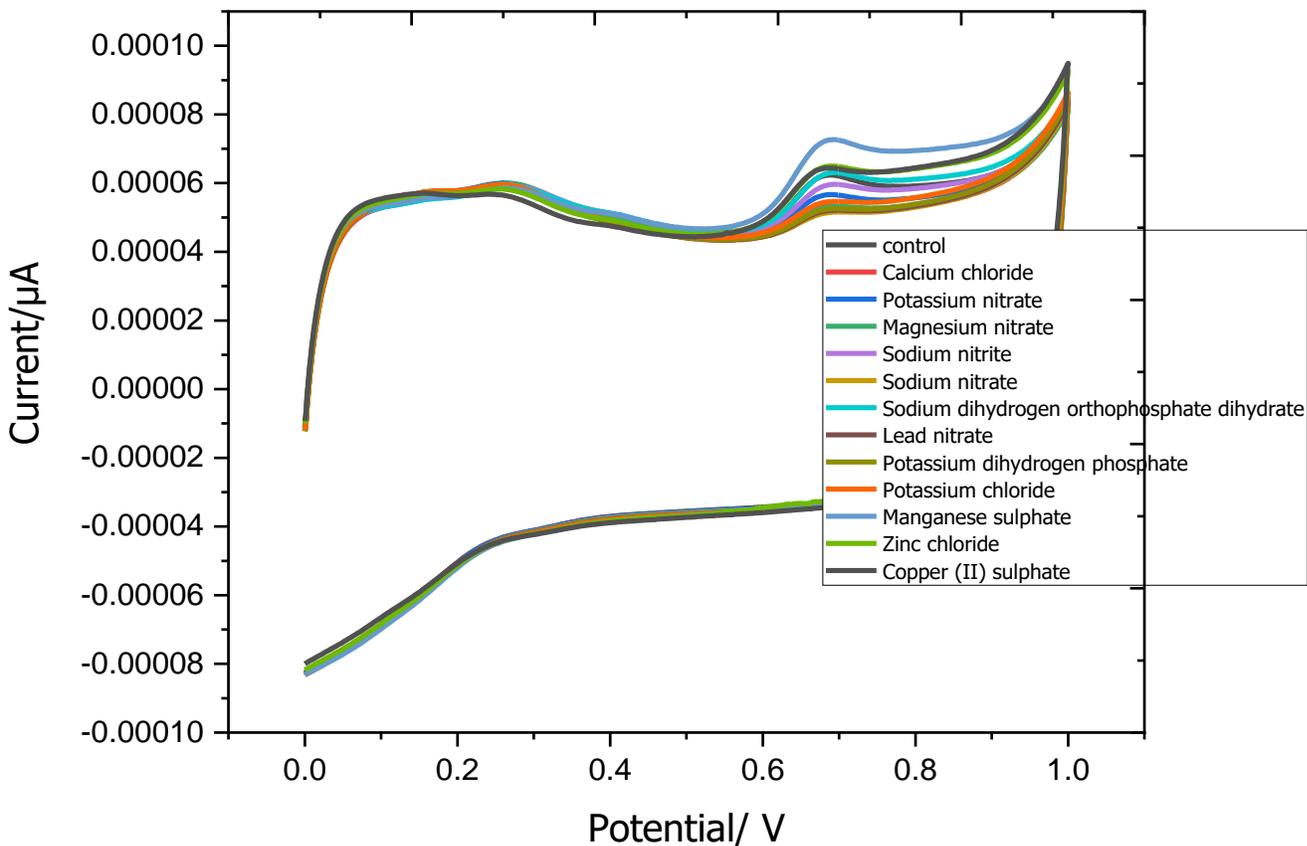


Figure 4:17 Cyclic voltammograms of the effect of interferences on BPA

4.9: Electrochemical detection of BPA using modified glassy carbon electrode.

BPA's electrochemical behavior on glassy carbon electrode modified with MWCNTs was studied using cyclic voltammetry by adding successive 0.01M BPA aliquots to phosphate buffer. Carbon nanotubes were deposited onto the bare glassy carbon surface by drop coating method and the obtained CV results indicated a centered oxidation peak at +0.5 V. The oxidation peaks were seen to increase with increasing concentrations of BPA from 10 μM up to 50.0 μM . The limit of detection was calculated to be 6.45 μM

4.9.1: Determination of BPA in plastic samples

Having no distinguishable BPA peak probably maybe because of its low concentration in real samples, the samples were thereby spiked using a known BPA standard concentration. The same

BPA concentration was used for every sample, and the results obtained are indicated in the tables below.

4.9.1.1: Determination of BPA in the samples using cyclic voltammetry.

In studying the modified GCE electrode's ability for determining Bisphenol A in real samples, firstly a calibration BPA curve was constructed through spiking BPA concentrations in the range 0 - 50 μM on to the MWCNTs/GCE in 0.1 pH 7.0 phosphate buffer. The resultant voltammetric response after addition of each aliquot of the BPA standard were recorded (Fig. 4.18) and used to construct the calibration curve given in Fig. 4.19 below. Consequently, the calibration curve was then used to obtain BPA amounts in the analytes/ specimen i.e. distilled water, juices, milk and infant formula specimens. Each experiment for each different specimen was done separately. The practicability in application of MWCNTs/GCE for BPA analysis was studied both in baby and water bottles.

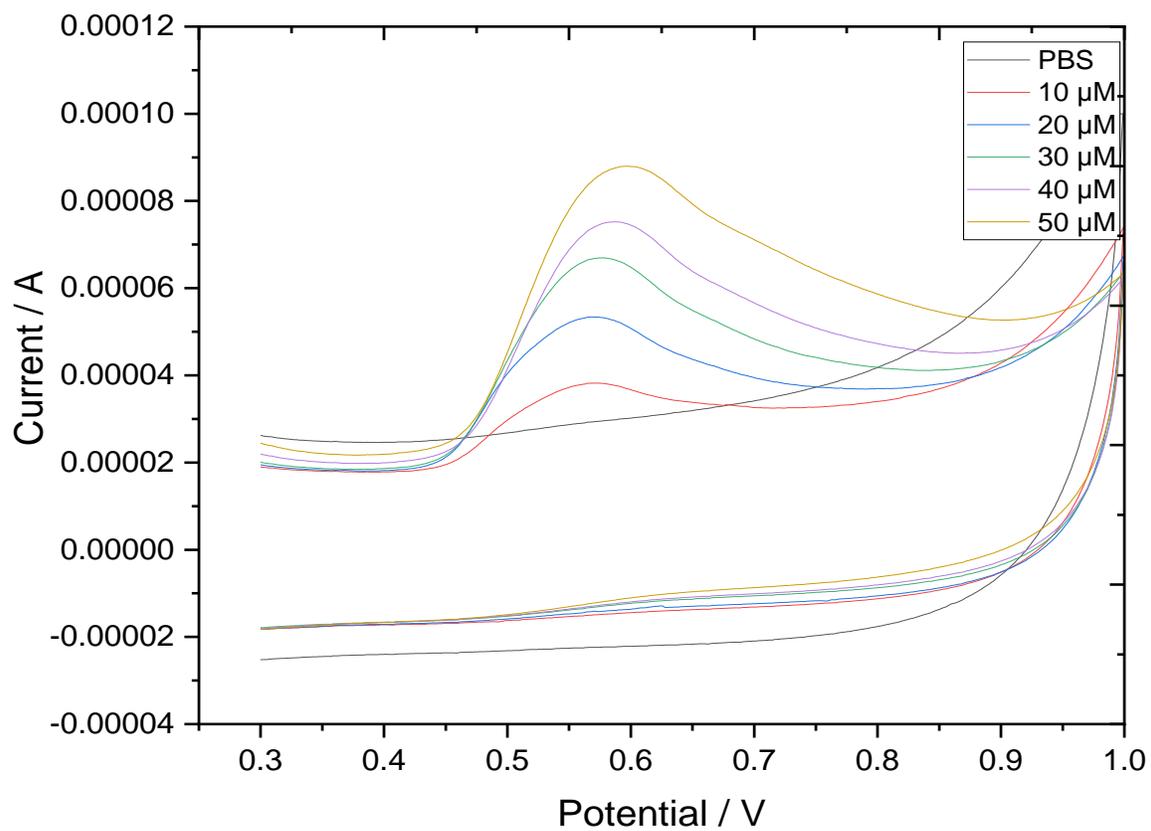


Figure 4:18 Cyclic voltammograms of BPA at different concentrations by MWCNTs/GCE

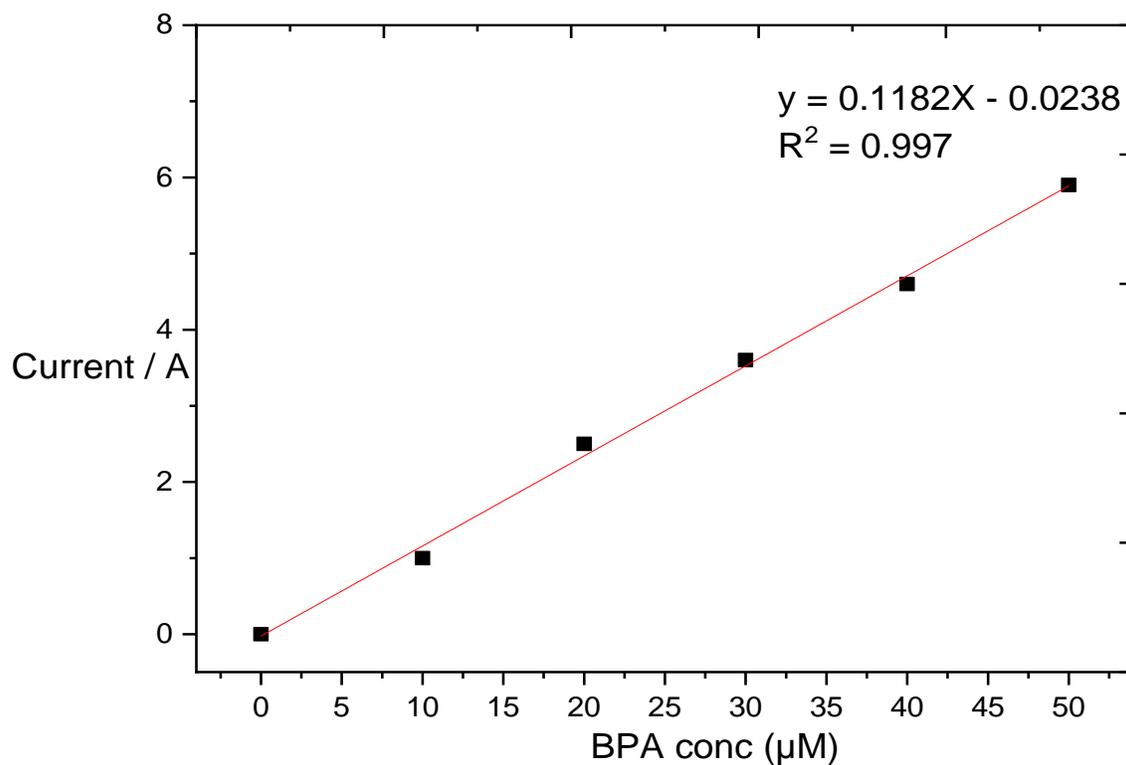


Figure 4:19 Peak of current versus BPA concentration

Table 4:11: The effect of temperature on the amount of BPA leaching in distilled water by CV

Sample (Water bottles)	Age	Added (µL)	Detected (µM)		
			95°C	50°C	25°C
A	New	10 µL	10.6±0.02	10.5±0.04	10.2±0.06
B	New	10 µL	10.7±0.07	10.4±0.08	10.1±0.05
M	New	10 µL	10.5±0.09	10.3±0.03	10.2±0.08
C	Used	10 µL	10.7±0.01	10.5±0.04	10.4±0.05
D	Used	10 µL	10.9±0.05	10.6±0.07	10.3±0.02
J	Used	10 µL	10.8±0.03	10.4±0.01	10.5±0.06

Table 4:12: The temperature effect on BPA leaching in milk by CV

Sample (Baby bottles)	Age	Added (μL)	Detected ($\mu\text{g mL}^{-1}$)		
			95°C	50°C	25°C
I	New	10 μL	12.5 \pm 0.14	12.2 \pm 0.05	11.9 \pm 0.07
F	New	10 μL	12.7 \pm 0.09	12.2 \pm 0.12	11.4 \pm 0.03
G	New	10 μL	12.4 \pm 0.05	11.9 \pm 0.07	11.6 \pm 0.10
L	Used	10 μL	12.9 \pm 0.15	12.6 \pm 0.13	12.1 \pm 0.06
P	Used	10 μL	13.1 \pm 0.11	12.8 \pm 0.01	12.5 \pm 0.04
H	Used	10 μL	13.5 \pm 0.09	12.9 \pm 0.03	12.7 \pm 0.02

Table 4:13: The effect of contact time on BPA leaching in distilled water by CV

Sample (Water bottles)	Age	Added (μL)	Detected (μM)		
			1hr	6hrs	12hrs
A	New	10 μL	10.6 \pm 0.02	11.5 \pm 0.01	12.4 \pm 0.03
B	New	10 μL	10.7 \pm 0.07	11.4 \pm 0.10	12.3 \pm 0.15
M	New	10 μL	10.5 \pm 0.09	11.3 \pm 0.02	12.2 \pm 0.09
C	Used	10 μL	10.7 \pm 0.01	11.5 \pm 0.09	12.4 \pm 0.07
D	Used	10 μL	10.9 \pm 0.05	11.8 \pm 0.08	12.7 \pm 0.05
J	Used	10 μL	10.8 \pm 0.03	11.6 \pm 0.04	12.9 \pm 0.13

4.9.1.2: Determination of BPA in plastic samples using Differential pulse voltammetry.

Differential pulse voltammetry (DPV) was used for BPA determination in the samples under optimized conditions as shown in figure 4.20. The peak currents of BPA at MWCNT/GCE increased as BPA was added. The linear range was 0 - 40 μM using 0.1M pH 7.0 phosphate buffer (Figure 4.21). A linear relationship was observed between the peak current and BPA concentration with a linear regression equation of $I_{pa} (\mu\text{A}) = 0.0127 \text{ BPA}(\mu\text{M}) + 1.195$ with $R^2=0.9996$ (Figure 4.21). The calculated limit of detection (LOD) was 2.75 μM .

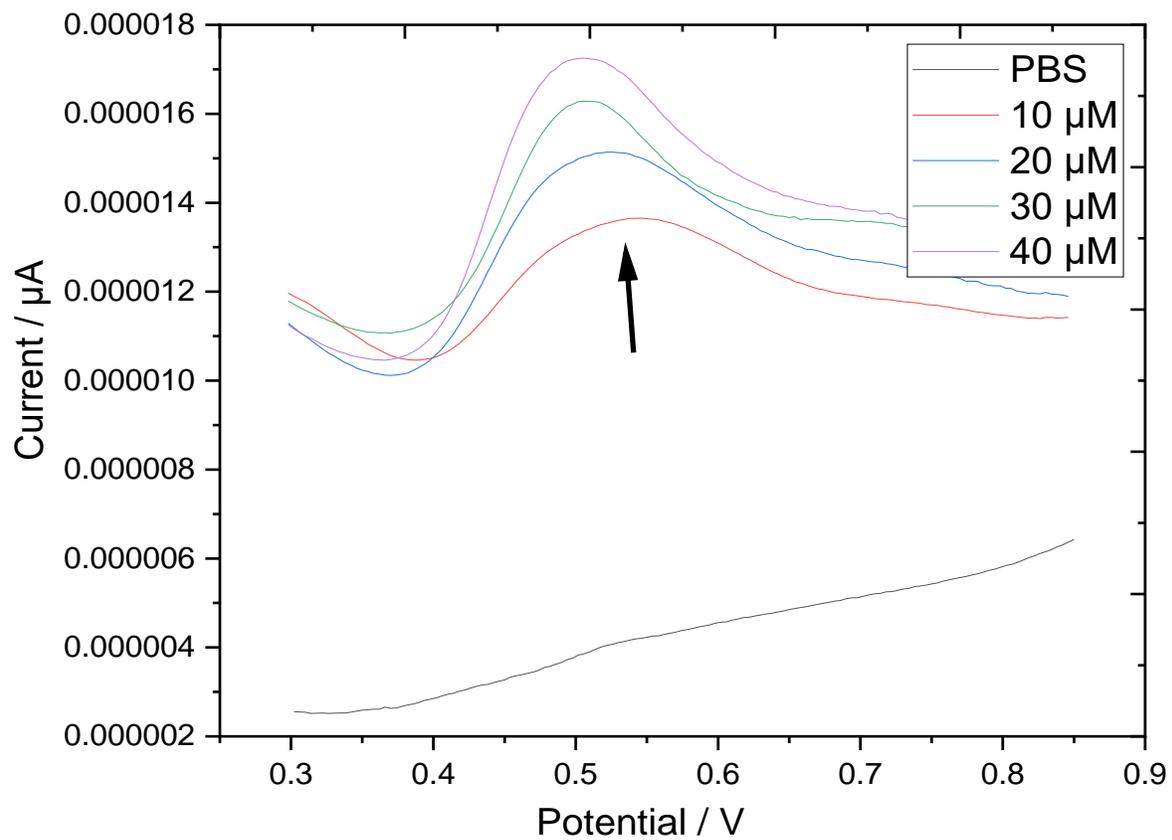


Figure 4:20 Differential pulse voltammograms of BPA at different concentrations using MWCNTs/GCE

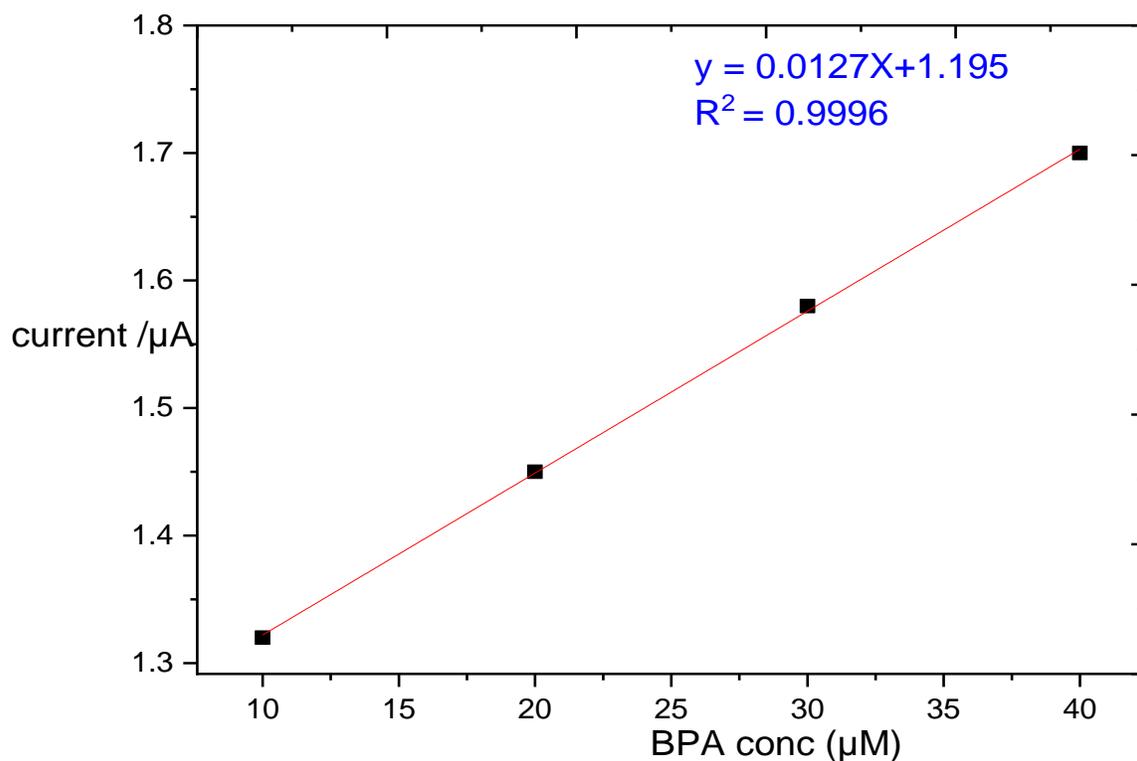


Figure 4:21 Peak of current versus BPA concentration

The results from both cyclic and differential pulse voltammetry confirmed that high temperature highly increased migration of BPA from polycarbonates. There was higher BPA leaching in samples when the temperatures were high at 95°C compared to 25°C indicating that the temperature is a main factor affecting the diffusion rate, that is the higher the temperature, the larger the atomic thermal activation energy, the more BPA is prone to migrate. The effect of temperature, contact time on BPA leachability in both water and milk is given in tables 4.14-4.16 below.

Table 4:14: The effect of temperature on the amount of BPA leaching in distilled water by DPV

Sample (Water bottles)	Age	Added (μL)	Detected (μM)		
			95°C	50°C	25°C
A	New	10 μL	11.2 \pm 0.01	10.8 \pm 0.02	10.4 \pm 0.08
B	New	10 μL	10.9 \pm 0.09	10.7 \pm 0.05	10.2 \pm 0.12
M	New	10 μL	11.5 \pm 0.13	10.9 \pm 0.02	10.5 \pm 0.09
C	Used	10 μL	11.8 \pm 0.08	11.5 \pm 0.11	10.4 \pm 0.03
D	Used	10 μL	11.4 \pm 0.03	11.0 \pm 0.06	10.3 \pm 0.04
J	Used	10 μL	11.6 \pm 0.04	10.9 \pm 0.09	10.5 \pm 0.07

Table 4:15: The temperature effect on the amount of BPA leaching in milk using DPV

Sample (Baby bottles)	Age	Added (μL)	Detected ($\mu\text{g mL}^{-1}$)		
			95°C	50°C	25°C
I	New	10 μL	13.0 \pm 0.10	12.8 \pm 0.03	12.2 \pm 0.04
F	New	10 μL	12.8 \pm 0.07	12.5 \pm 0.14	11.9 \pm 0.08
G	New	10 μL	13.4 \pm 0.05	12.9 \pm 0.09	12.6 \pm 0.11
L	Used	10 μL	13.7 \pm 0.03	13.2 \pm 0.04	12.9 \pm 0.05
P	Used	10 μL	13.5 \pm 0.10	13.0 \pm 0.02	12.6 \pm 0.01
H	Used	10 μL	13.9 \pm 0.07	13.4 \pm 0.13	12.8 \pm 0.09

Table 4:16: Contact time effect on BPA leaching in distilled water using DPV

Sample (Water bottles)	Age	Added (μL)	Detected (μM)		
			1hr	6hrs	12hrs
A	New	10 μL	11.2 \pm 0.01	12.4 \pm 0.09	13.0 \pm 0.05
B	New	10 μL	10.9 \pm 0.09	12.2 \pm 0.07	12.9 \pm 0.02
M	New	10 μL	11.5 \pm 0.13	12.6 \pm 0.13	13.3 \pm 0.10
C	Used	10 μL	11.8 \pm 0.08	12.9 \pm 0.02	13.9 \pm 0.04
D	Used	10 μL	11.4 \pm 0.03	12.7 \pm 0.06	13.4 \pm 0.01
J	Used	10 μL	11.6 \pm 0.04	12.8 \pm 0.01	13.2 \pm 0.09

Results from this study show a substantial migration of BPA occurrence with longer contact time or higher temperature. High BPA leaching at high temperatures maybe caused by hydrolysis of carbonate linkage (Vilarinho *et al.*, 2019).

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1: Conclusion

All the sampled bottles from the various sampling sites after subjection to FTIR analysis and comparison of results to library data turned out to be polycarbonates. Characteristic peaks associated with polycarbonates were observed which were; 2970 cm^{-1} due to C-H stretching of aromatic rings; C=O stretching vibrations at 1770 cm^{-1} ; C=C stretch vibrations at 1504 for the benzene rings, O-C-O vibrations at 1187 cm^{-1} and CH₃ stretching vibrations at 1079 cm^{-1} amongst others.

The confirmed PC bottles were subjected to a diazotization process and the BPA levels for all the samples ranged between 0.17 – 0.78 $\mu\text{g mL}^{-1}$ in distilled water, 0.78 – 2.82 $\mu\text{g mL}^{-1}$ in milk and 0.86 – 2.62 $\mu\text{g mL}^{-1}$ in the infant formulas.

In the voltammetric studies a bare and modified glassy carbon electrode were used. Modification was done by incorporating multiwalled carbon nanotubes (MWCNTs) onto the electrode. Results showed a 5.8% signal amplification as a result of GCE modification with MWCNTs in the potassium ferricyanide system with the increase being over 50% in the phosphate buffer system.

The BPA leachability monitoring under different environments for all the sampled PC bottles using voltammetric techniques were between 0.1- 2.3 $\mu\text{g/mL}$. All the samples surpassed the BPA tolerable daily intake (TDI) of 5 $\mu\text{g/kgbw/day}$ advocated by EFSA. From the results obtained in this study we can conclude that polycarbonate bottles use cannot be regarded as safe.

5.2: Recommendations.

From the results obtained in this study, elimination of BPA containing products and regulation policies can be effected especially in plastics that are in contact with food, majorly those targeting population under developmental stage

Additionally, there is also urgent need to bring public awareness and sensitization on the dangers of using BPA related products and advising them to use BPA free products.

From the results obtained, sensitization to industries could be done through the government on BPA usage in food contact plastic materials.

More research should be conducted on BPA substitutes as the few studies present show that these alternatives have similar effects as Bisphenol A and thus cannot be regarded as safe. Also additional research should be conducted to determine their toxicity and potential adverse health effects.

More studies should be conducted on BPA leaching in other matrices to have more data in order to educate the public on the dangers of BPA exposure.

From this study's findings, conclusion can also be made that human life, wildlife and Planet earth need to be protected from BPA exposure and especially in countries such as Kenya where the public have little information regarding Bisphenol A.

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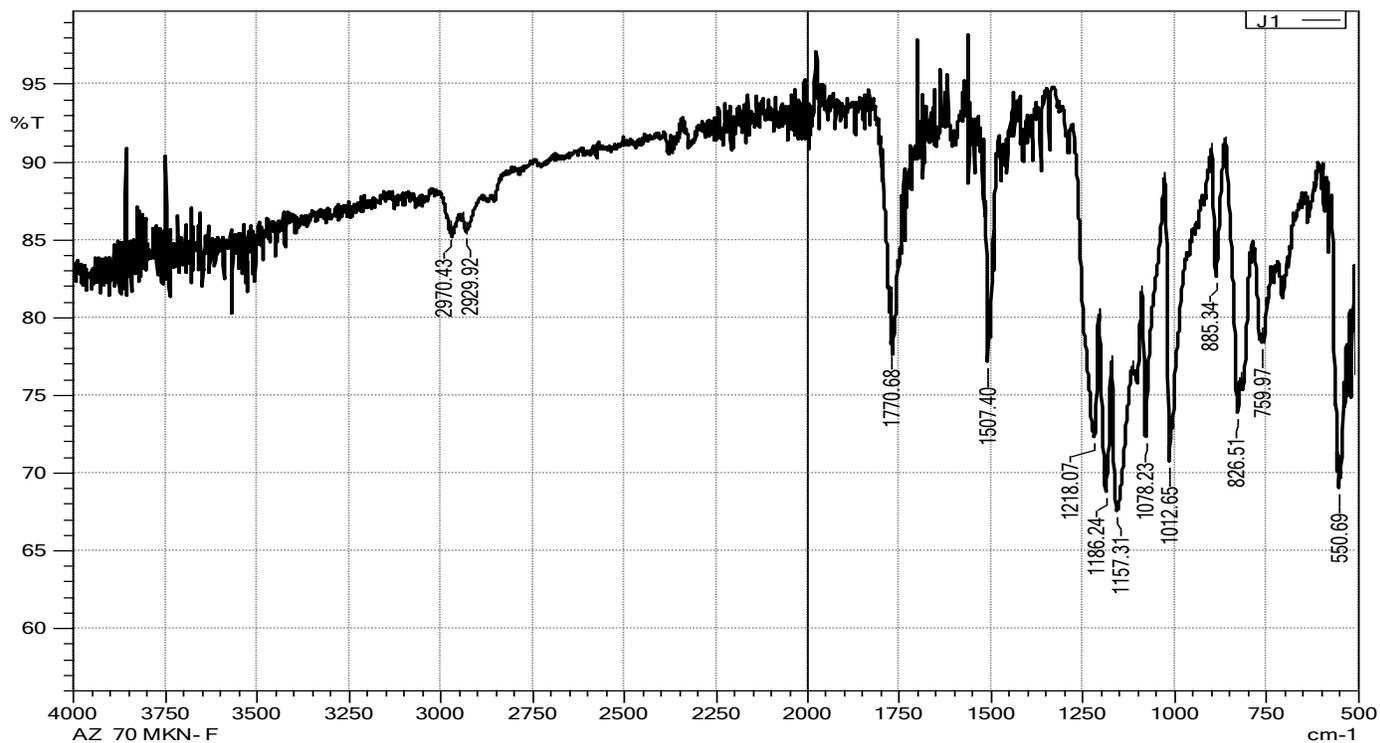
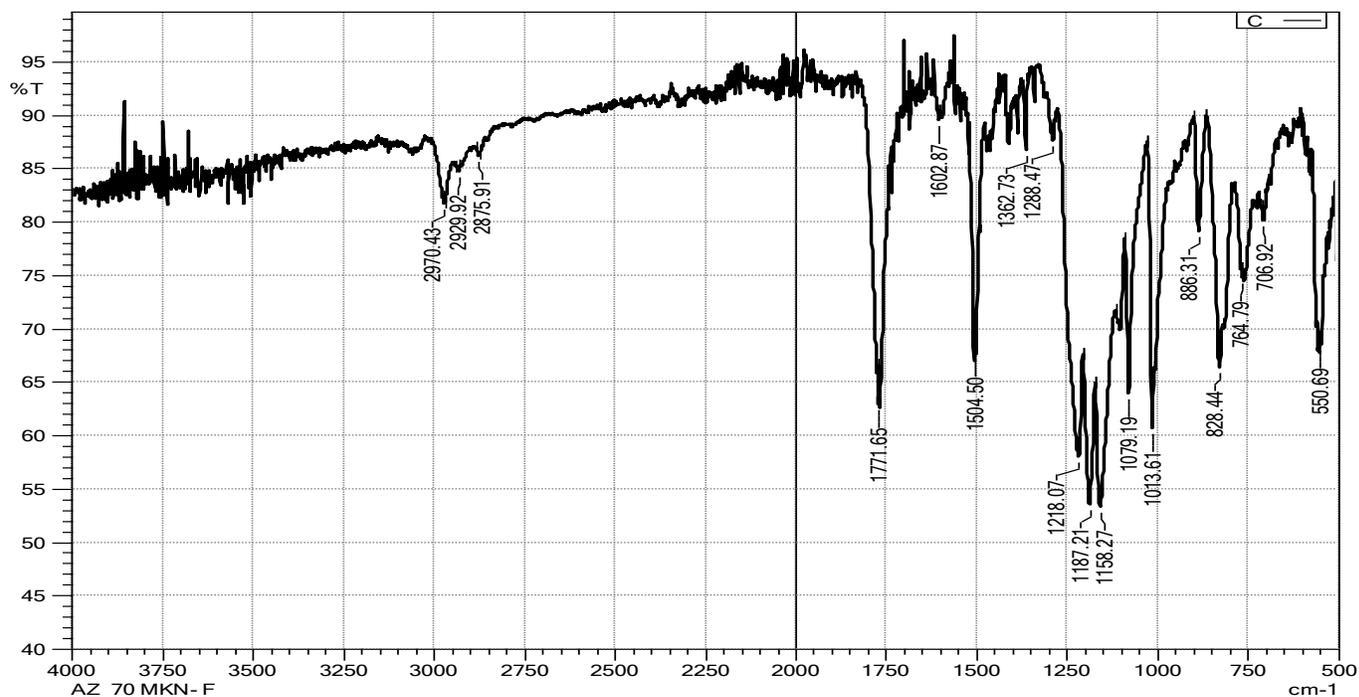
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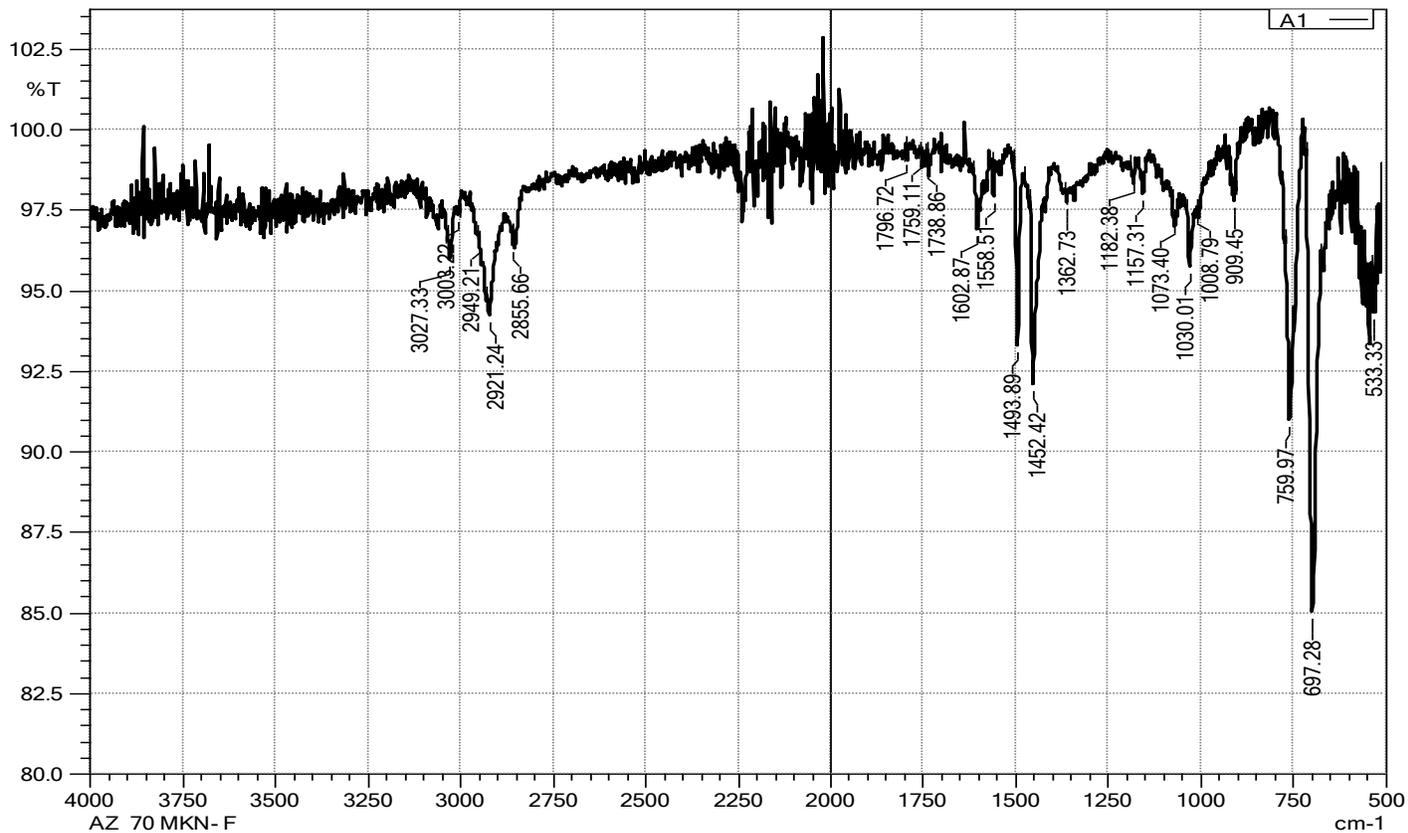
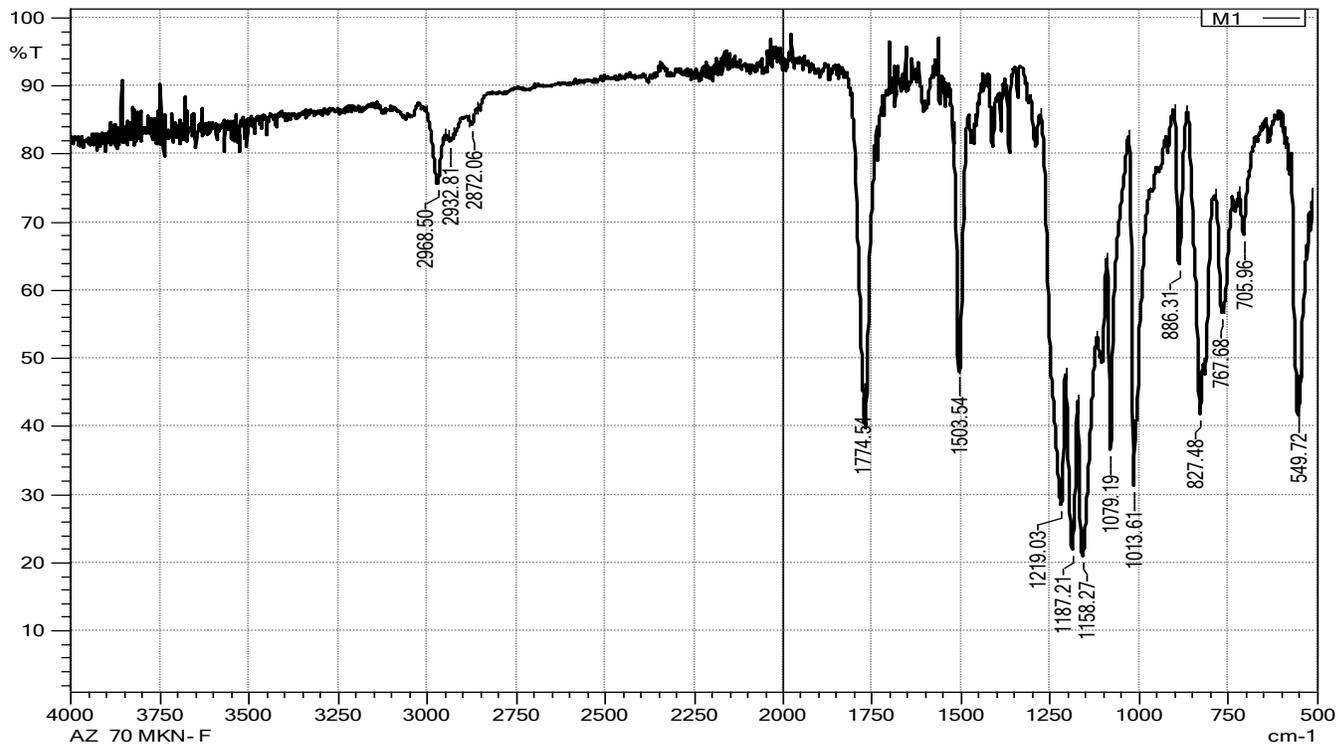
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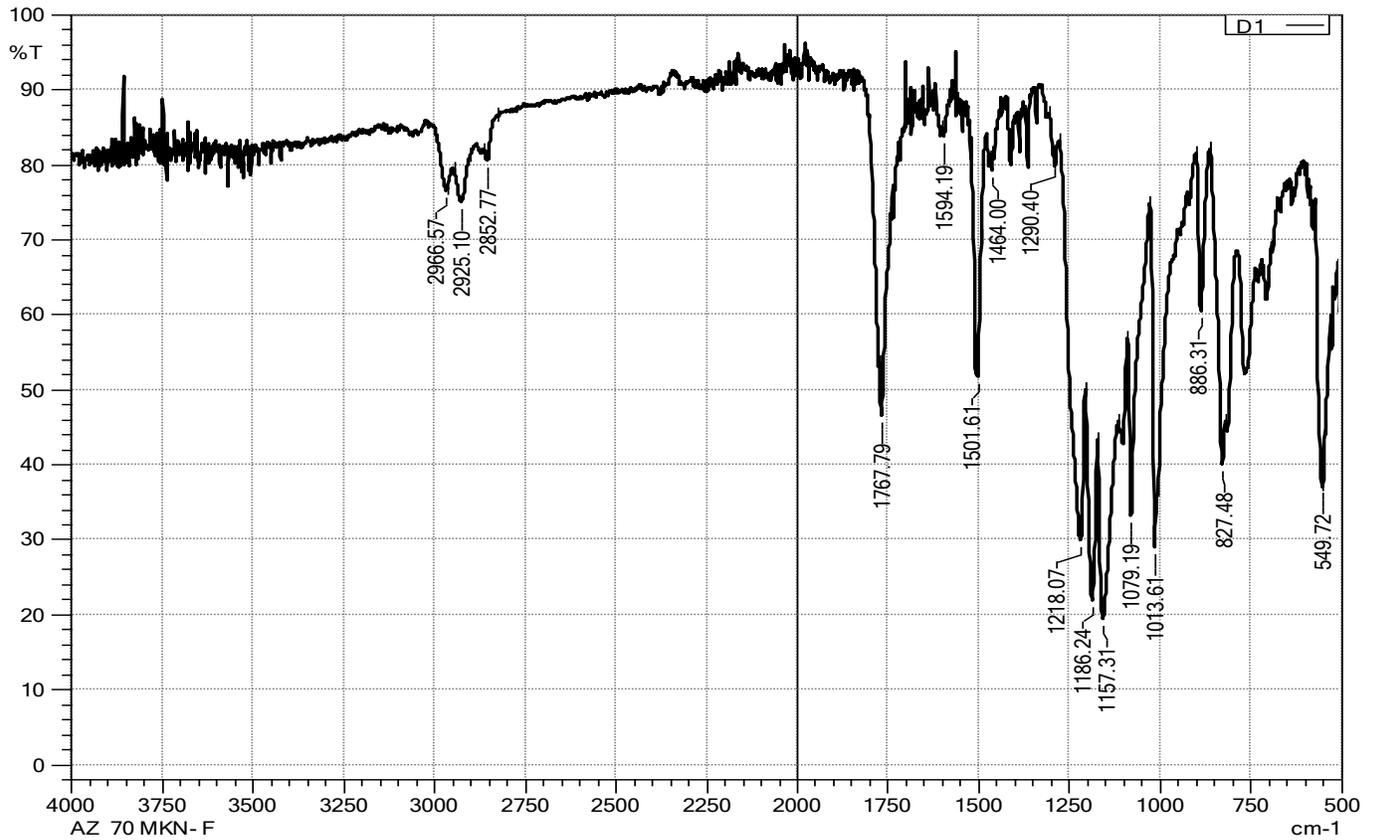
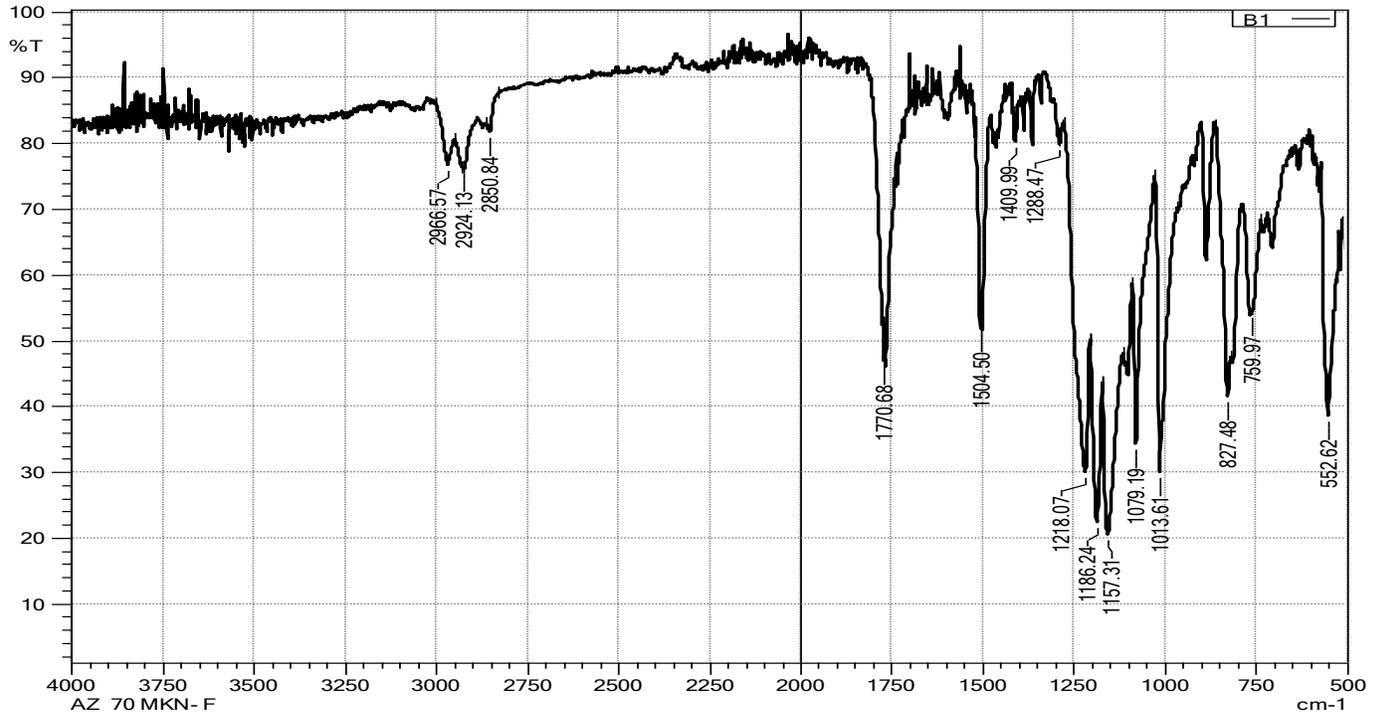
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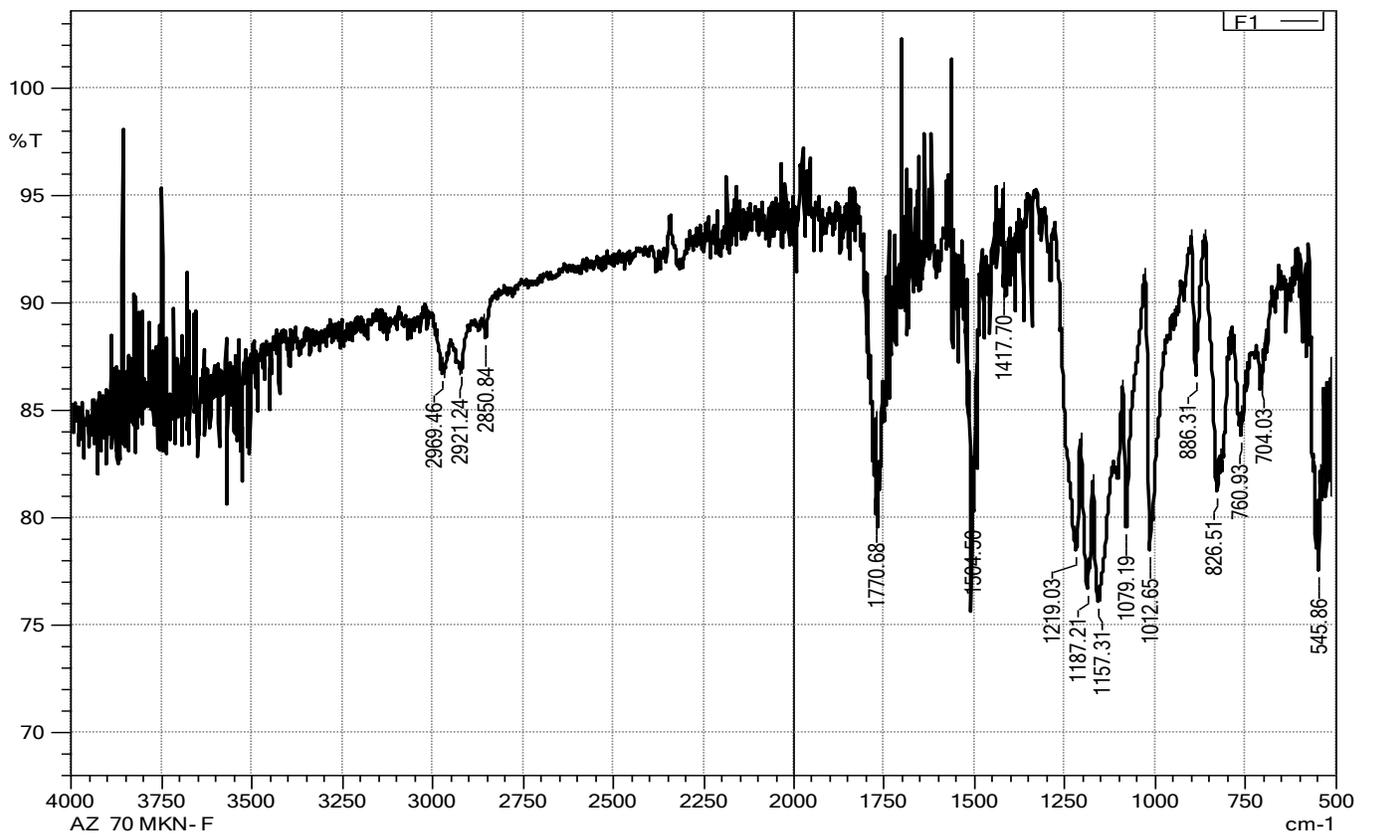
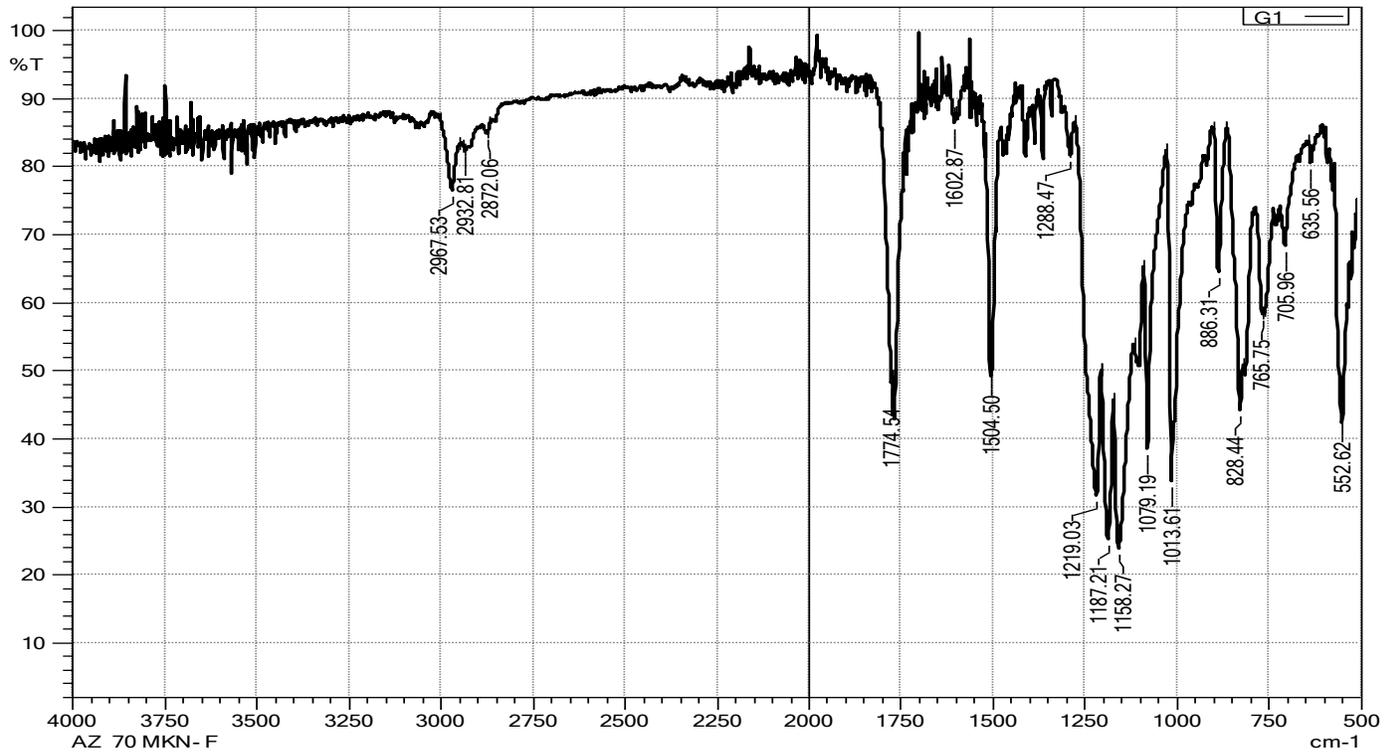
APPENDICES

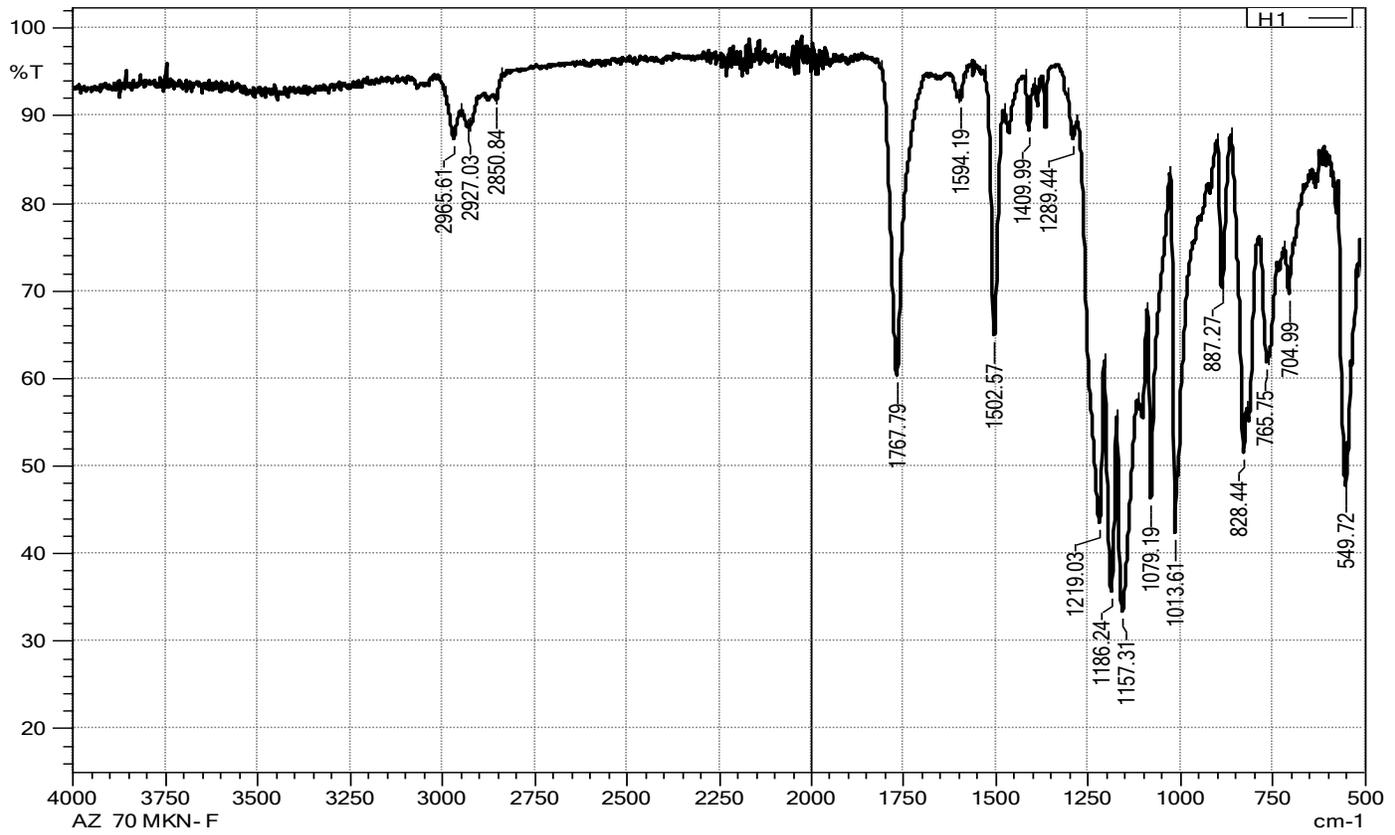
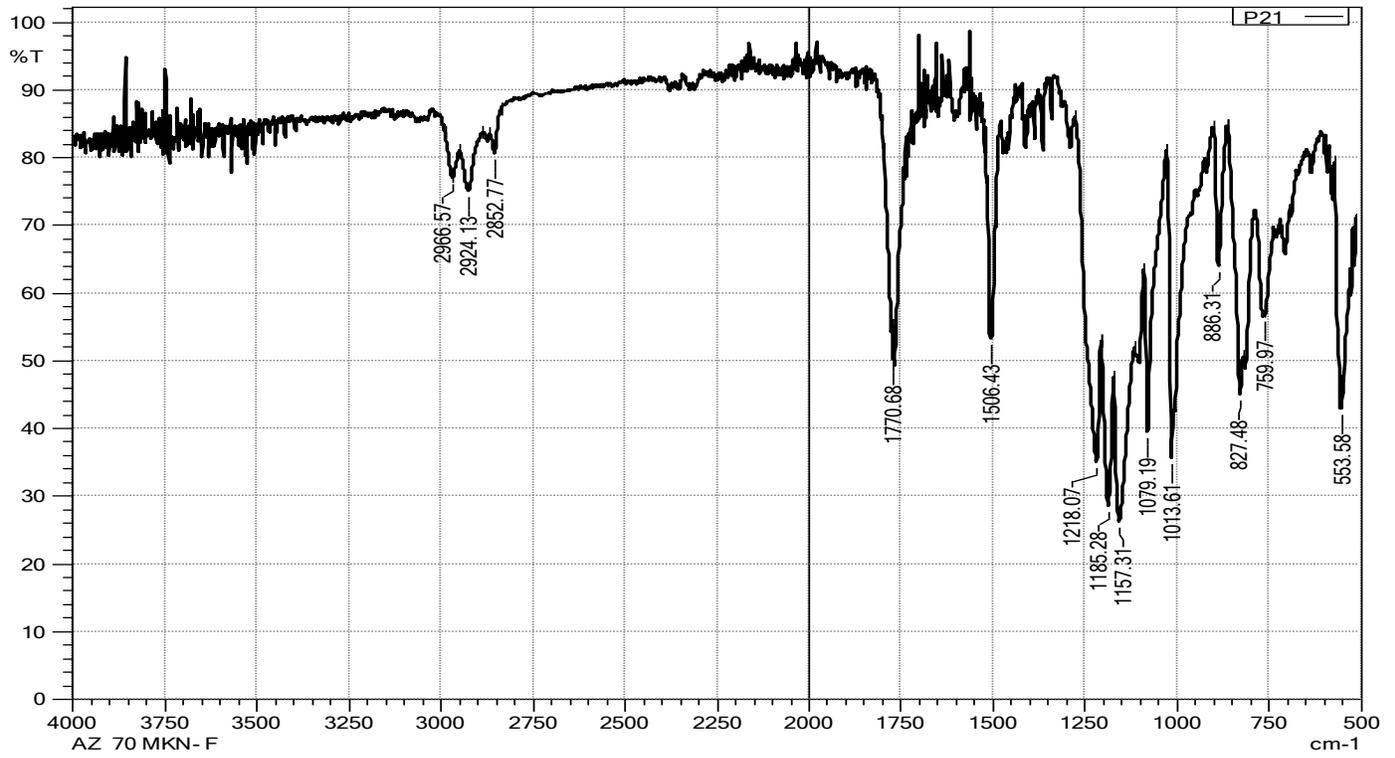
Appendix 1: FTIR spectrums of the 12 samples

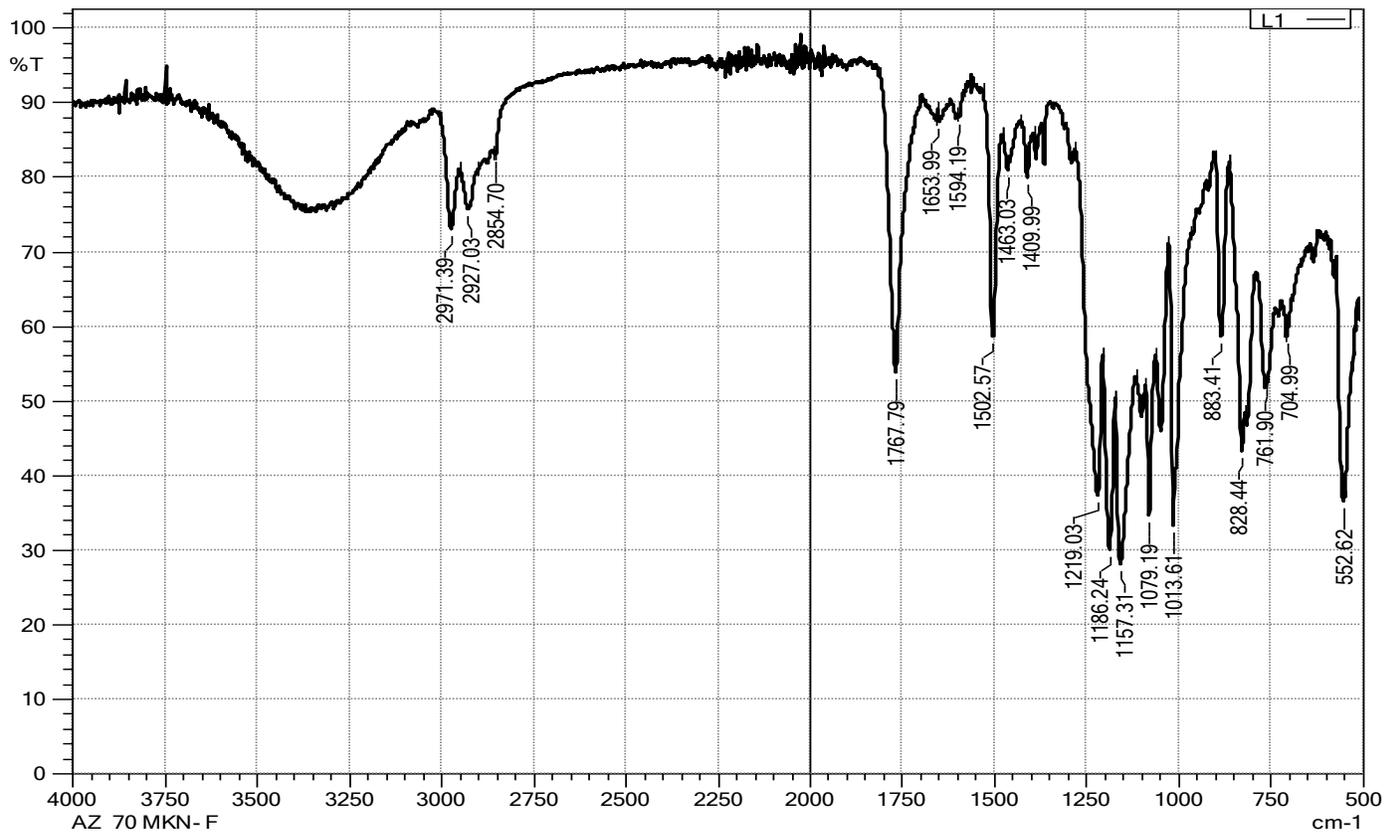
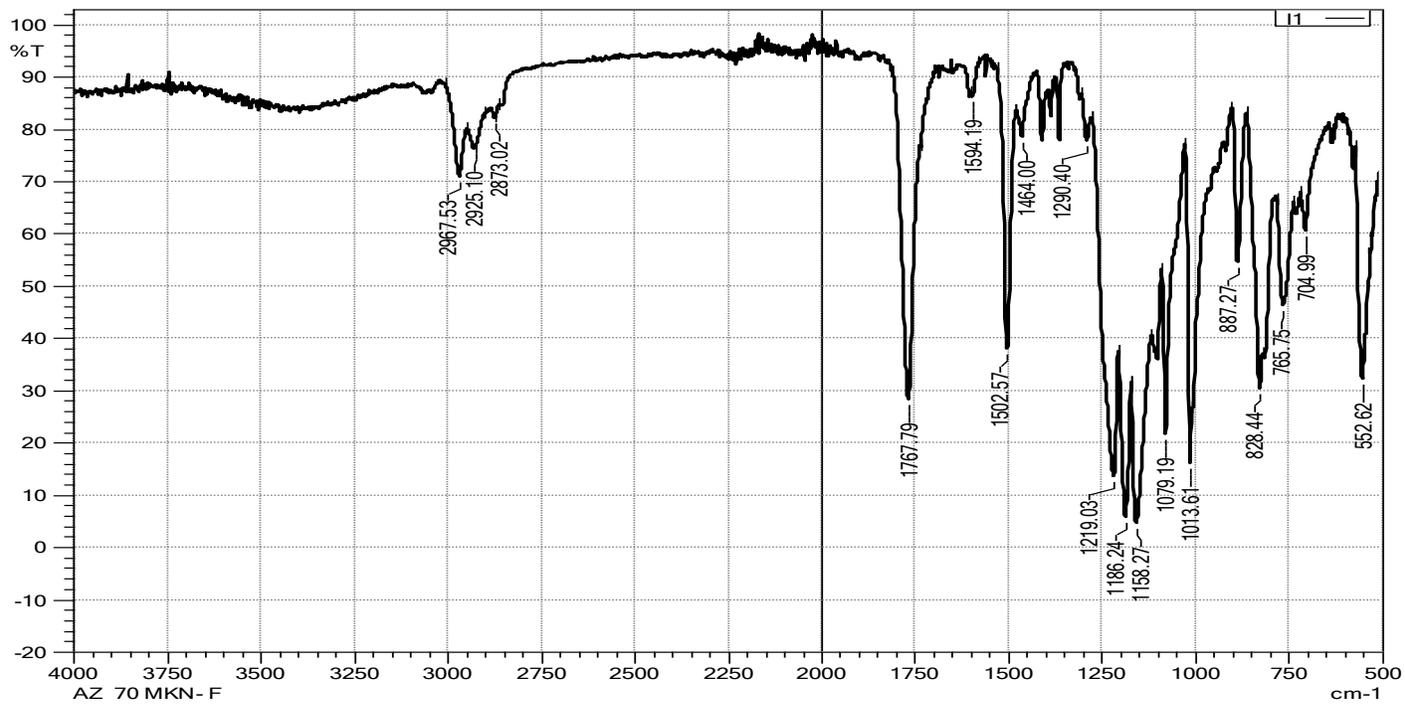












Appendix 2: Data for the calibration curves

Ultraviolet-visible spectrophotometry calibration curve data

Concentration ($\mu\text{g mL}^{-1}$)	Absorbance
0	0.01
2	0.19
4	0.35
6	0.53
8	0.69
10	0.89
12	1.03

Differential pulse voltammetry calibration curve data

Concentration (μM)	Peak current (μA)
10	1.32
20	1.45
30	1.58
40	1.70

Cyclic voltammetry calibration curve data

Concentration (μM)	Peak current (A)
10	1.02
20	2.50
30	3.60
40	4.60
50	5.90

Appendix 3: Definition of Significant Terms

TDI: (Tolerable Daily Intake) is an estimate of the amount of a substance, expressed in milligrams on a body weight that can be ingested daily over a lifetime without appreciable risk. The TDI has been set to protect all human populations for lifetime exposure, including the most vulnerable groups such as pregnant and lactating women, infants and young children (Vilarinho *et al.*, 2019).

t-TDI: (temporary Tolerable Daily Intake) is allocated if there are uncertainties in the data that may be resolved by further studies and it is known that significant new data will be available in the near future (Vilarinho *et al.*, 2019).

Migration Limit: The limit specifies the amount of BPA that is permitted to migrate into foodstuffs contacted. The European Commission Scientific Committee on Food has established a specific migration limit (SML) in food of 3 mg/kg (i.e., 3ppm), meaning that a maximum of 3 mg of BPA is permitted to transfer from the contact plastic into 1 kg of food (Tsai, 2006).