IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 12, Issue 2 Ser. I (February. 2019), PP 01-10
www.iosrjournals.org

Fabrication of a Biosensor for analysis of Bisphenol A from Modified Glassy Carbon Electrode modified using Kenyan Bentonite, Polyaniline and Tyrosinase

Kiio, L*[a], Mbui, D[a]. Michira I[a], Njenga, H[a].
[a]*University of Nairobi, Department of Chemistry, P.O. Box 00100-30197, Nairobi, Kenya*
** Corresponding author; Kiio, L*

***Abstract**: In this work, a biosensor for determination of Bisphenol A, hereafter referred to as BPA, was developed from bentonite, polyaniline, SLS surfactant and tyrosinase immobilized on the polished glassy carbon electrode, with the aim of analyzing levels of BPA in the environment. The biosensor obtained was used to quantitatively and qualitatively determine Bisphenol A. Electrochemical methods used in characterization of the biosensor were cyclic voltammetry(CV), Differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The pH of the experiments was maintained using a phosphate buffer, and the optimal pH for the operation of the biosensor was obtained as 7.2. Tyrosinase was extracted from button mushrooms and purified. Use of Kenyan bentonite in the sensor was observed to enhance the signal of the biosensor, a factor that was attributed to pre-concentration.An elaborate peak signaling the presence of BPA was found at +0.5V for reduction potential and +0.2V oxidation potential. Under optimized conditions, the detection limit for BPA using the developed biosensor was found to be 0.02 mM within a concentration range of 0.02-0.2 mM where the response time reached 95% within 15 seconds. When the concentration of BPA was altered from 0.4 µM to 18 µM the current was observed to be increasing with the increasing potential, hence a calibration curve was obtained.*

***Keywords:** Bisphenol A, biosensor, endocrine disruptor, glassy carbon electrode, bentonite and tyrosinase.*

Date of Submission: 26-01-2019 Date of acceptance:09-02-2019

I. Introduction

Bisphenol A (BPA) is a high production volume chemical used in a variety of common consumer products. Most notably, BPA is present in polycarbonate plastics, epoxy resin liners of aluminum cans, and thermal receipts (Fiege & Heinz-Werner, 2002). It has also been used as an inert ingredient in pesticides, as a fungicide, antioxidant, flame retardant, rubber chemical and polyvinyl chloride stabilizer (Rodriguez-Mozaz *et al.,* 2005).Plastics and polythene bags areadvantageous as they are not heavy,are portable, and can fit into any shape required. They are therefore widely used by many people. However, concern has been raised about some disadvantages of the plastics, for example their lack of biodegradability and the incorporation of toxic materials duringtheir production. Bisphenol A, a toxic endocrine disruptor, is one such additive. According to He'lie's-Toussaint et al., (2014) bisphenol A is a xenoestrogen, with adverse effects on the human being.Some of its well recorded effects include: neurological blastoma especially in children,(Braun *et al.,* 2009) breast and prostate cancer, sexual dysfunction (Li. *et al*., 2010(a)), early maturity,(Fatoki *et al*., 2009), obesity(Yin *et al.,* 2011), DNA methylation and disruption of the dopaminergic system(Braun *et al*., 2009). Other effects include an increase in hyperactivity and aggression in two-year-old girls due to prenatal exposure (Braun *et al*., 2009). BPA is also associated with oxidative stress, repeated miscarriages (Sui *et al*., 2012), diabetes, high levels of liver enzymes (Yin *et al.,* 2011). In vivo, increased BPA levels due to environmental and occupational exposure contributes to low sperm count (Meeker *et al*., 2010), decreased testosterone levels in men (Galloway *et al*., 2010), changes in estrogenic gene expression in adult males (Melzer *et al*., 2011) and increased incidences of coronary heart disease (Melzer *et al*., 2012).

Information regarding use and effects of Bisphenol A in Kenya is lacking, mainly due to lack of awareness, and also of appropriate methods of its analysis. The analytical methods which have been used commonly for the separation and detection of BPA include: high performance liquid chromatography (HPLC) (Katayama *et al.,* 2001), gas chromatography (GC),or gas chromatography coupled with mass spectrometry (GC-MS) (Pulgar *et al.,* 2000), Liquid Chromatography coupled with mass spectrometry (LC-MS) (Jiménez-Díaz *et al.,* 2010) and capillary electrophoresis (Mei *et al.,* 2011).While as these are highly accurate techniques, they are expensive and often require skilled manpower to operate. Electrochemical techniques provide a cheaper

alternative towards phenolic compounds detection. They provide a fast response, good reliability, low energy consumption, simple operation, and high sensitivity (Yina *et al.*, 2010). A major hurdle however in dealing with BPA is that its oxidation at bare electrodes results in dimmerization which fouls the electrode with consequences of response deactivation.

In this work, we study the possible enhancement of electrode sensitivity, by modifying the working electrode (WE) with materials that exhibit electro-catalytic properties. Cyclic voltammetry is a versatile electro analytical technique for the study of electro- active species and it has been labelledelectrochemical spectroscopy (Orata and Segor, 1999). Modified electrodes, that is the electrodes on whose surfaces chemical species have been deliberately immobilized to facilitate electron transfer from analyte to electrode (Heinze and Muller, 1998) ; have been used as sensors (Guo *et al*., 2007), catalyst (Nada *et al*., 2007; Kuralay *et al*., 2013; Oukil *et al*., 2007). Modified electrode surfaces can act as pre-concentrating surfaces in which the analyte species are collected and concentrated on the electrode(Mbui *et al.*, 2014). The collected analyte is subsequently measured by the electrochemical response to a potential sweep. Bentonite is clay where the principle exchangeable cation is sodium. It was used together with tyrosinase enzyme and surfactant (SLS). Cyclic voltammetry (CV), Square wave voltammetry (SWV) and differential pulse voltammetry (DPV) methods were used to monitor BPA using modified electrode with poly aniline, Kenyan bentonite, tyrosinase enzyme and surfactant, Sodium lauryl sulphate (SLS). The aim of the research was to develop novel analytical method for monitoring Bisphenol A using biosensor technology where by enzyme based phenolic biosensor was fabricated.

II. Experimental

All chemical reagents were of analytical grade and were used as received without further purification, except the monomer liquid Aniline (Aldrich 99%) which was triply distilled until colorless liquid was obtained. It was then stored under nitrogen. All solutions were prepared using de-ionizedwater. The clay montmorillonite, bentonite (sourced from Athi River Mining Company Ltd., Kenya) was purified as described by Bard*et al.*,(1986) and Bard *et al.*(1990).

In generating the cyclic potential scans, Auto Lab. PGSTAT 12 potentiostat with a three-electrode system consisting of platinum wire as the counter electrode, Ag/AgCl (saturated 4.0 M KCl) as the reference electrode and glassy carbon electrode (bare glassy carbon electrode, Try/ Polyaniline-bentonite/GCE and Try/ Polyaniline-BTN/ SLS/GCE were used as the working electrode.

In preparation of bentonite clay, a thick slurry was prepared by placing 0.05ml of electrochemically inert adhesive onto a watch glass and adding 0. 01g of bentonite clay. Using a glass rod, the two were mixed to form a homogeneous thick slurry which was applied to a bare glass carbon electrode by drop coating method. The mixture was spread on the surface of a polished carbon electrode up to a thickness of about 0.6mm and left to air-dry for 12 hours while covered with cotton cloth to avoid contamination. The surface area of the bentonite modified electrode was approximately 0.60 $mm^{2.}$

Biosensor construction was achieved by modification of the surface of working electrode with polyaniline- surfactant (SLS) and slurry (0.002ml) of bentonite clay. The slurry was then drop coated on the electrode then, thin film of polyaniline was electrodeposited on bentonite modified electrode. Thereafter, 5 µL of tyrosinase enzyme was mixed with 0.5mL of 0.1 M phosphate buffer solution in a vial then 5 µL of this mixture was then mixed with an electrochemically inert adhesive and drop-coated on the surface of GCE modified with PANI/ SLS- BTN. The electrode was allowed to dry at room temperature overnight in a dust-free environment. After drying, the tyrosinase, polyaniline- bentonite modified electrode, was transferred to a solution containing 0.001M BPA in 0.1M phosphate buffer.

Determination of the optimum activation pH of tyrosinase was done by immobilizing tyrosinase enzyme on the GCE / PANI-BTN surface in 0.1 M phosphate buffer at a pH range of 4.2 to 10.2 and 0.001M BPA. Cyclic, differential pulse and linear sweep voltammetric techniques were employed at a potential range of -1.0 V to + 1.0 V.

The electrochemical behavior of BPA was done on bare glassy carbon electrode and it was investigated by cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) by successive additions of aliquots of 0.001 M BPA into the 0.1M phosphate buffer p H 7.2 which was used as the supporting electrolyte.

III. Results and Discussion

Bentonite clay

It has a mesh size ranging from 150 to 200 um, cation exchange capacity (CEC) 1.18-1.22 mM/g and a pH range of 8.4-9.6. The density of the bentonite is 1.25g/cm^3 which are comparable to other clay minerals from different parts of the world (Bard *et al* 1986)

Table 1: Composition of bentonite

Solid Matrix	SiO_2	Al_2O_3	CaO	MgO	Na_2O	K_2O	TiO_2	MnO	Fe_2O_3	LOI
Bentonite clay	47.21	14.4	3.27	0.29	1.71	1.00	0.62	0.03	10.93	2.40

Electrochemical synthesis of polyaniline on bare and modified GCE

Electrochemical polymerization of the anilinemonomer on bare GCE surface, bentonite modified GCE , sodium lauryl sulphate (SLS) on bare GCE surface and aniline with SLS using bentonite modified GCE was achieved by cycling the potential repeatedly between -0.1V and +0.1 V at a scan rate of 40 mVs^{-1}. The resultant cyclic voltammetric response is shown in Figure 1 (a), (b), (c) and (d).

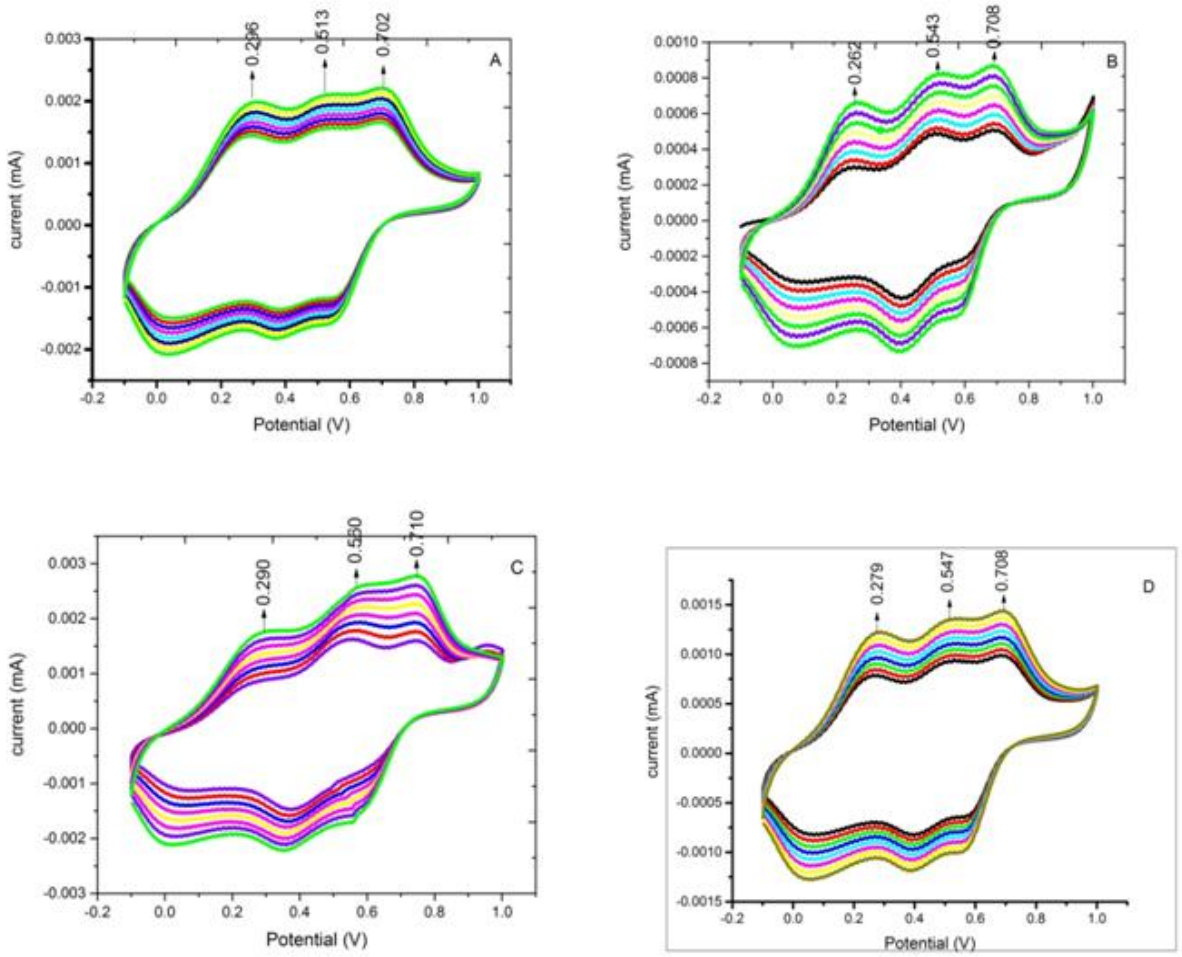


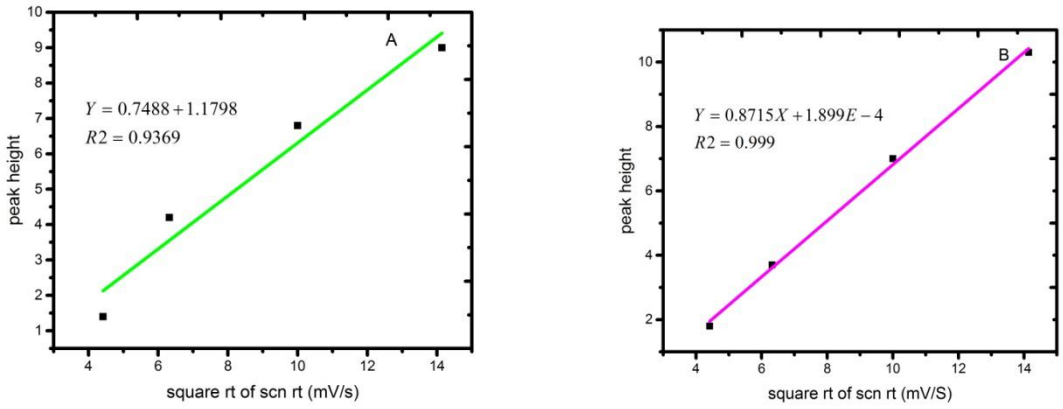
Figure 1: Electrochemical synthesis of PANI using 0.1M aniline in 0.1 M H2SO4 at scan rate of 40 mVs-1 for 8 cyclesusing : (a) bare GCE, (b) bentonite modified electrode (c)presence of0.005mM surfactant(d) 0.005mM surfactantusing bentonite modified electrode.

In all the cases,theredox process was found to be quasi reversible with the reduction peaks occurring at +0.2 V and + 0.5V. This corresponded to the transition between the oxidation states of polyaniline namely the leucoemeraldine to emeraldine and emeraldine to pernigraniline states of aniline respectively. The appearance of another pair of redox peaks at approximately +0.7 V for all the voltammograms is much more complex and can be attributed to many different intermediates and degradation products (cross-linked polymer, benzoquinone) formed during the electrochemical preparation of the polymers (Songa *et al.*, 2009).

Bentonite clay was used in this experiment to study the effect of the host matrix on the redox behavior of PANI. The electrolyte media contained 0.1M aniline in 0.1M sulphuric acid solution figure 1 (a) and (b) while for figure 1 (c) and (d) the electrolyte media contained 0.1M aniline in 0.1M sulphuric acid solution and 0.005M SLS . The sulphuric acid was used as supporting electrolyte and also as doping agent for the resulting polymer. In the cases where bentonite was used, figure 1(b) and (d), the cyclic voltammograms had better defined peaks than when bare GCE was used. The reduction and oxidation peaks occurred at +0.27V/ +0.01V, +0.54V/ +0.41V and +0.7V/ +0.6V respectively for 0.005mM surfactantusing bentonite modified electrode, figure1(d) and for bentonite modified electrode, figure 1 (b) the reduction and oxidation peaks occurred at

+0.26V/ +0.15V, +0.54V/ +0.41V and +0.70V/ +0.61V respectively. The highest reduction peak current for Pani when bare GCE was used was 2.5A, figure 1 (a) for bentonite modified electrode figure 1 (b) was 9A, while when Pani was mixed with surfactant on bare GCE the peak current was 3A figure 1 (c) and finally when Pani was mixed with surfactant on bentonite modified electrode the peak current was 13A figure 1 (d).
The improved redox response of aniline when bentonite modified electrode was used can be attributed to the pre-concentration of the aniline molecules as a result of being trapped in the octahedral layers in the bentonite (Mbui *et al.,* 2014).Similar results were obtained when characterization was done using 0.1M sulphuric acid solution only, this was a clear indication that polymerization of aniline on the electrode was successful.

To assess the effect of scan rate on the peak height, and therefore to determine the nature of the reaction, scan rate dependence studies were conducted. A plot of anodic peak height versus square root of scan rate for polyaniline on GCE and on bentonite modified electrode in 0.1 M sulphuric acid yielded a linear plot (fig. 2 (a and b). This observationshowed that the reaction is diffusion-controlled. From the linear calibration graphs obtained in figure 2 (b), bentonite modified electrode had a higher correlation coefficient (R^2) of 0.99 while bare GCE had R^2 value of 0.93.



(a) (b)

Fig 2: Peak height vs. square root of scan rate (200mV, 100mV, 40 mV, 20 mV) using (a) bare GCE (b) bentonite modified carbon.

Biosensor construction, characterization and application

The cyclic voltammogram obtained when PANI was electrodeposited on bentonite modified electrode had peak current of 0.0008 mA and it was used as the host matrix. The resultant voltammogram is shown in figure 3 below. Thereafter, 5 µL of tyrosinase enzyme was mixed with 0.5mL of 0.1 M phosphate buffer solution in a vial then 5 µL of this mixture was then mixed with an electrochemically inert adhesive and drop-coated on the surface of GCE modified with PANI/ SLS- BTN.

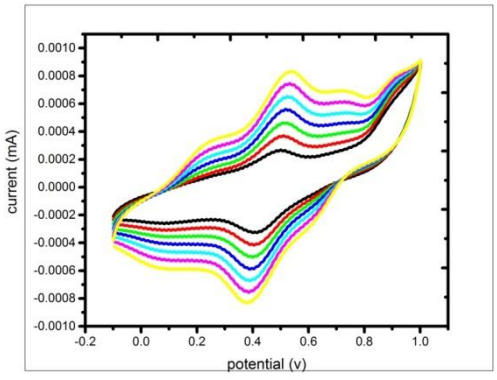
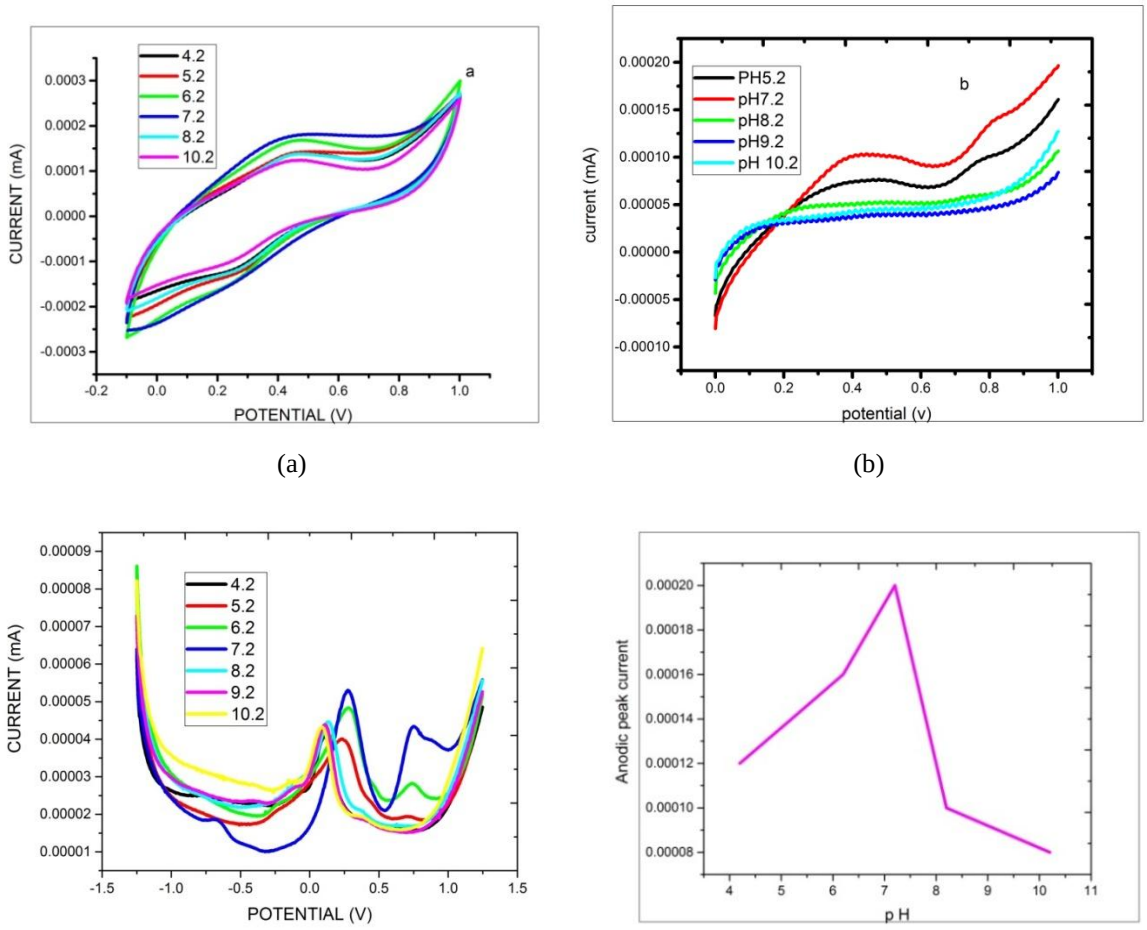


Figure 3. Cyclic voltammogram of GCE/ PANI- BTN scan rate 40mV

Tyrosinase based (Tyr/PANI-BTN /GCE) biosensor constructed was then evaluated and optimized for detection of BPA using cyclic voltammetry, SWV and DPV. The response of the immobilized tyrosinase enzyme to its substrate BPA was taken to be the measure of its activity. The detection of BPA was then carried using a fixed concentration of BPA.

Optimization pH study of tyrosinase enzyme

Figure 4 below shows results for tyrosinase enzyme pH optimization experiments.



(a) (b)

(c) (d)

Figure 4: Tyrosinase enzyme pH optimization inTyr/PANI- BTN/SLS/GCE using (a) cyclic voltammetry (b) linear sweep voltammetry (c) differential pulse voltammetry (d) linear calibration curve.

The cathodic peak current was observed to increase with increasing pH ofsolution from 4.2 until it reached 7.2 but when the pH value of the solution exceeded 7.2, the cathodic peak current decreased rapidly with each increment of pH solution until 10.2 most probably because the enzyme got denatured at high pH values. Therefore the optimized pH measurement for tyrosinase under the experimental conditions was found to be 7.2, which is similar to the value reported by Akyilmaz *et al.,* (2010) for the free tyrosinase enzyme and Khan *et al.,* (2007) who also reported the pH range of tyrosinase as being between 4.5 and 7.0. This proves that tyrosinase enzyme sustains its activation in neutral medium. This may also indicate that the optimum pH value of tyrosinase enzyme was not affected by the immobilization procedure used in the preparation of the biosensor aprerequisite towards fabrication of a stable biosensor.

It was also observed that the potential shifted with increase in pH, which was an indication that protons take part in the redox process of BPA on Tyr/PANI-BTN- SLS /GCE modified electrode(Matyholo, 2011). The reason for this behavior could be the reduction of *o*-quinone that requires H^+ in acidic medium. The H^+ concentration is higher than that of the nitrogen atoms of the polymer and phenol hydroxyl of BPA are protonated in the form of $–NH_3^+$ and $–OH_2^+$ (Kuramitz *et al.,* 2001). In theory, the optimum pH of tyrosinase is approximately pH 6.50 and *Li et al (2005)* reported that this value is even higher when tyrosinase is immobilized on modified surface.

Electrochemical detection of BPA using bare glassy carbon electrode surface.
The results obtained are shown in Figure 5.

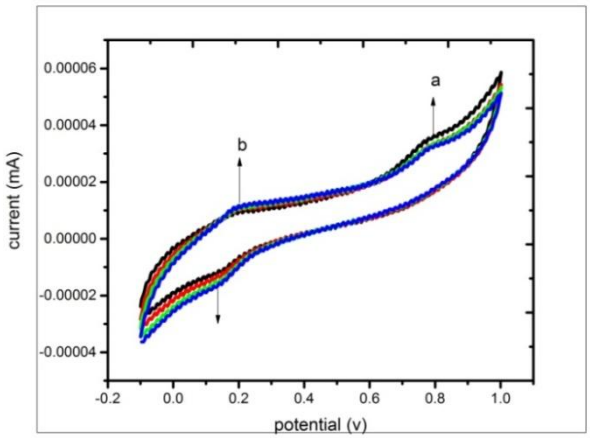
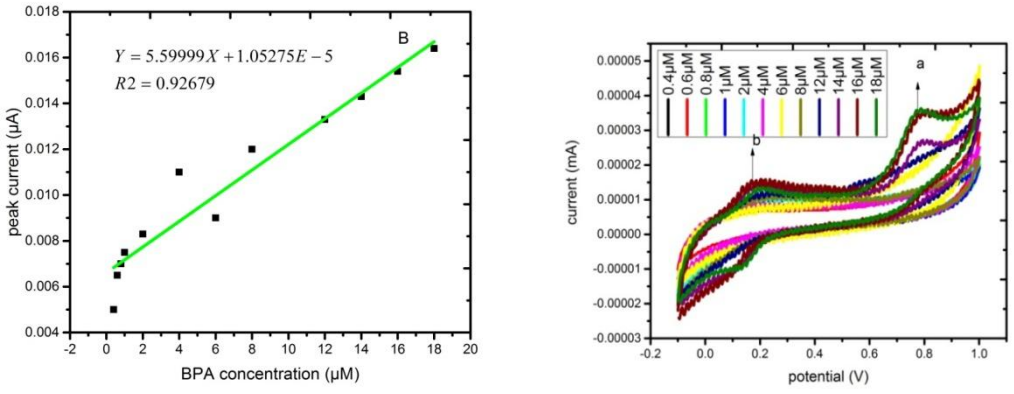


Figure 5: Electropolymerization of 120 µL of 0.001 M BPA in 80 mL phosphate buffer pH 7.2 on bare glassy carbon electrode using cyclic voltammetry**.**

The BPA was used as a substrate to establish the capability of bare glassy carbon electrode, the optimal conditions, detection limit, dynamic range, and stability. In this study, the response was monitored by peak current signal, which is proportional to the BPA concentration (Mita, 2007). The electrochemical polymerization of BPA on glassy carbon electrode was investigated by addition of 120 µL (1.2 x 10^{-5} M) of 0.001 M BPA in 80 mL phosphate buffer pH 4.2 to 10.2. The potential was cycled five times between -0. 1V and +1.0 V at a scan rate of 40 mV s^{-1}. Only one oxidation peak was observed at potential +0.2 V and two reduction peaks at + 0.13V (b) and + 0.8V (a).

The peak current was observed to decrease as the number of cycles increased. This behavior confirmed findings by Kuramitz *et al*., (2001) that bare GCE had a potential for detection of BPA but the problem was fouling caused by oxidative polymerization of BPA on GCE surface. A conducting polymer is required to intervene as it is known to work as a mediator for shuttling of electrons from the GCE surface to substrate binder and biological element which can be enzyme, DNA or antibody. The biological element used in this study is an enzyme which is incorporated within the construction of the biosensor.

The above experiment was repeated for different BPA concentrations of 0.4 *µM* - 18 *µM.*Phosphate buffer pH 7.2 was the supporting electrolyte. As in the experiment above, only one oxidation peak was observed at a potential of +0. 19 V and two reduction peaks at potential of +0.23 V and +0.80V, figure 6.



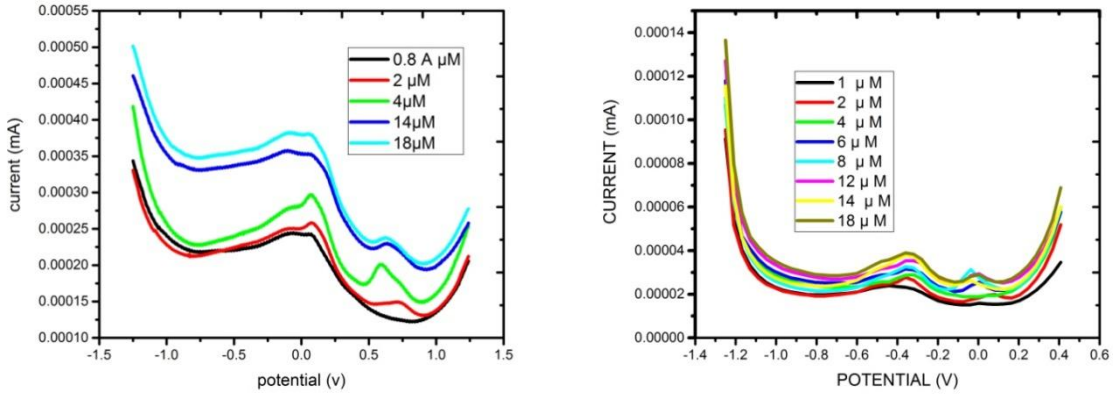
(a) (b)

Figure 6: Electropolymerization of 120 µL BPA in 80 mL phosphate buffer pH 7.2 on bare glassy carbon electrode using different concentrations ,Voltammetry of 0.001 BPA on bare GCE using (a) cyclic voltammetry (b) Linear calibration curve of anodic peak current verses BPA concentration.

The two oxidation peaks observed in the cyclic voltammogram in figure 6 (a) above are as a result of oxidative polymerization of phenolic compounds (BPA). The +0.1V (b) peak corresponds to oxidation of monophenol to diphenol while +0.71V (a) peak corresponds to further oxidation of diphenol to quinone, figure 6. The formed quinone on electrode surface gets reduced at appropriate potential and converted into signal (Kartsonaki, 2012).

Electrochemical polymerization of BPA on glassy carbon electrode was also achieved by cycling the potential repeatedly between -1.5V and +1.5V at a scan rate of 4 mV s^{-1} using square wave voltammetry and potential between -1.4V to + 0.6V using differential pulse voltammetry.

Figure 7 (a) Illustrates the SWV results for the electrochemical behavior of BPA at the bare glassy carbon electrode as a result of the successive additions of aliquots of BPA into 0.1 M phosphate buffer, the reduction peaks were centered at +0.55V and + 0.05V.The DPV results showed two reduction peaks centered at +0.05V and -0.55V(Figure 7 (a)). The reduction peak currents were observed to increase with increase in BPA concentrations. The reduction peak centered at +0.5V and the peak current was observed to be increasing with increasing concentrations up to 18.0 µM. The peak current also increased with increase in BPA concentrations up to 18.0 µM (Figure 7 a and b). DPV and SWV modes of measurements were used because they gave more defined peaks compared to cyclic voltammetry as shown in figure 7 (a) and (b) below.



(a) **(b)**

Figure 7: Electropolymerization of 120 µL BPA in 80 mL phosphate buffer pH 7.2 on bare glassy carbon electrode using different concentrations ,Voltammetry of 0.001 BPA on bare GCE using (a) square wave voltammetry and (b) Differential pause voltammetry

The DPV results obtained were found to be similar to those obtained by CV. In conclusion, the inactivation of electrode surface depends on the adsorptivity of the species of bisphenol A to the electrode, which is stronger in neutral medium than in alkaline medium (Krishnan *et al.*, 1993).

Electrochemical detection of BPA using the biosensor with bentonite (Tyr/PANI-BTN/SLS/GCE)

Electrochemical detection of BPA was done using SWV, DPV and CV. Figure 8 (a) below displays SWV plots for the biosensor (Tyr/PANI-BTN/SLS/GCE) response to different concentrations of BPA into 0.1 M phosphate buffer pH 7.2. The SWV results showed one reduction peak centered at +0.5V and the peak slightly moved to the negative potential by approximately 0.1V figure 8 (a) with increase in concentration. The slight shift in peak potentials with change in concentration could be explained by Nernst's equationwhich points to the fact that the e.m.f of a cell changes with the activity of the species being oxidized and the species being reduced. The DPV results showed one reduction peak at -0.1V. The reduction peak at -0.1 V showed increase in peak currents with increase in BPA concentrations which is supported by the increase shown in the DPV and SWV results presented in the Figure 8 below.

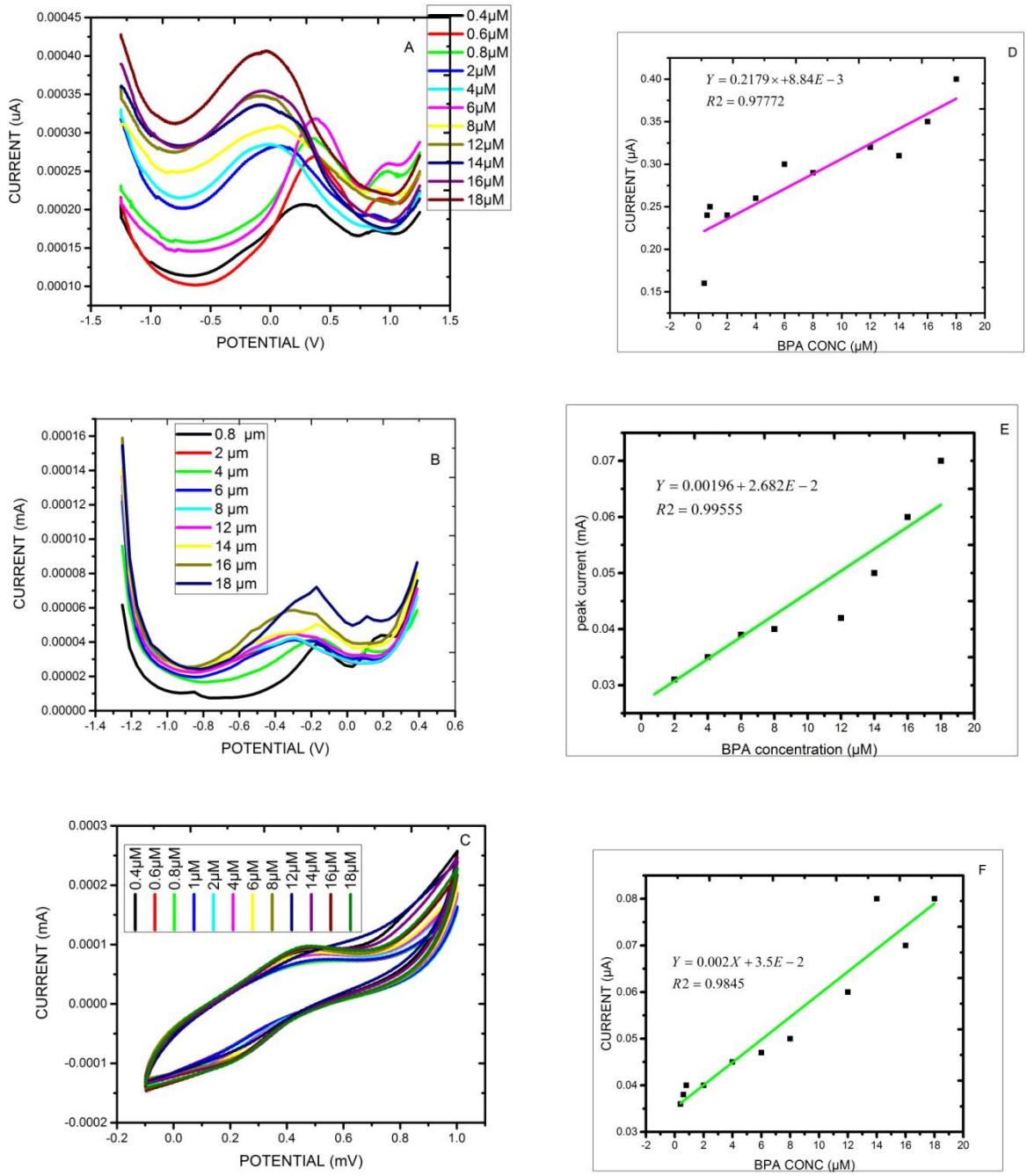


Figure 8: (a) Square wave voltammetry (SWV) (b) DPV (c) CV (d) SWV Linear calibration curve reduction peak (e) DPV Linear calibration curve of reduction peak (f) CV Linear calibration curve of reduction peak results for the biosensor in response to various concentrations of BPA.

The behavior observed for the peak at +0.5V from the results obtained is the expected behavior in the presence of tyrosinase enzyme, which electrochemically reduces the diphenol to *o*-quinone at relatively low potentials. The bentonite modified biosensor showed good response to detection of BPA (figure 8a, b and c) when compared to a surface without modification,(bare GCE)(figure 6(a), 7(a and b)). The good response to BPA when biosensor modified with bentonite is used is shown by the well developed peaks observed in fig 8 (a, b and c). Linear fit calibration graph of reduction peak potentials versus BPA concentration was obtained with correlation coefficient (R^2) of 0.926 for bare GCE, figure 6(b) while that of bentonite modified electrode (biosensor) was 0.985 figure 8(f). The correlation coefficient (R^2) for SWV and DPV using (Tyr/PANI-BTN/SLS/GCE) biosensor was 0.978 and 0.996 respectively (figure 8 d and e). This improved response of BPA to bentonite modified biosensor can be attributed to the pre-concentration of the BPA molecules as a result of

being trapped in the octahedral layers in the bentonite (Mbui *et al.*, 2014). The alignments of aniline in the clay montmorrilonite matrix not only enhanced the electrochemical signal, but also lead to reduction in Gibbs free energy resulting from entropic effects as a result of the realignment of the redox functional groups in the bentonite host matrix (Orata and Segor 1999). The surfactant used also influenced the rate of polymer formation, particle size, distribution, morphology and homogeneity (Armes *et al.*, 1990) leading to formation of enhanced peaks. The results obtained collaborate with those observed by Mbui*et al.*, (2014).

Based on these results, the detection limit for BPA which is given by; 3 x standard deviation of the blank)/Sensitivity (Matyholo, 2011)was calculated to be 2.1 x 10^{-9}M within a concentration range of 0.4 -18.0 μM BPA. The detection limit value obtained in this study was compared to literature and it was found to be similar to the value reported by Matyholo, (2011) which was 1.9 x 10^{-8} M at wide range of 1.0 x 10^{-16} μM estimated for Tyr/PDMA (Poly (2, 5-dimethoxyaniline)-PSS (Poly (4-styrenesulfonic acid)biosensor used to investigate the effect of BPA on activity of tyrosinase enzyme. The detection limit value obtained in this study was higher but very close to those reported in literature for other techniques such as Elisa, HPLC-MS, GC-MS and LC-MS.

IV. Conclusion and Recommendations

A biosensor for determination of Bisphenol Awas constructed using polished glassy carbon electrode (GCE), bentonite (BTN), Sodium lauryl sulphate (SLS) and Tyrosinase (TYR). The bentonite and the enzyme were drop-coated on the GCE using an electrochemically inert adhesive. Biosensor characterization and application was performed using cyclic, differential pulse voltammetry and square wave voltammetry. The optimum activation pH for tyrosinase enzyme was determined to be 7.2 proving that tyrosinase worked best at neutral pH. Straight calibration curves were obtained for determination of BPA using bare GCE and bentonite modified electrode. However, the bentonite modified electrode had a higher correlation (R^2) value when cyclic voltammetry was used of 0.986 compared to bare GCE which had 0.926. this means that the bentonite adsorbed and pre-concentrated the BPA.

It is recommended that, the biosensor parameters should be investigated further and optimized in order to achieve lower detection limits. The applicability of the developed biosensor should be determined by analyzing real samples in the market.

Researchers could adopt the methodology and optimization parameters investigated in this work to obtain a portable BPA detector for analysis of samples in the market.

Acknowledgement

The authors would like to acknowledge the National Commission for Science, Technology and Innovation (NACOSTI) for generously funding this study.

References

[1]. Armes, S. and Aldissi M. (1990). Preparation and characterization of colloidal dispersions of polypyrrole using poly (2-vinyl pyridine)-basedsteric stabilizers. Polymer, 33(31), 569-574.
[2]. Amiridou D. Voutsa D. (2011). Alkylphenols and phthalates in bottled waters. Hazardous materials. 185(1), 281-86.
[3]. Braun, J. Yolton, K., Dietrich, K.N., Hornung, R., Ye, X., Calafat, A.M. and Lanphear, B. (2009). Prenatal bisphenol A exposure and early childhood behavior. Environmental Health Perspect, 16 (9), 1945- 52.
[4]. Bard A.J. and Villemure G. A. (1990). Part 9: Electrochemical studies of the electroactive fraction of adsorbed species in reduced and preadsorbed clay films. Electroanal. Chem., 282(5), 107-121.
[5]. Bard A.J. and Rudzinski W.E.,(1986). Clay modified electrodes, Part VI: aluminium and silicon pillared clay modified electrodes, J. Electroanaltical Chemistry, 199(9), 323-340.
[6]. Fiege, H.and Heinz-Werner Voges, T. (2002). Ullmann's Encyclopedia of Industrial Chemistry. Phenol Derivatives., 35 (6), 110-112.
[7]. Fatoki. J. Olujimi.O.Odendaal .J.,(2009). Endocrine disrupting chemicals (phenol and phthalates). Water, 36 (1:), 117-126.
[8]. Galloway, T. Cipelli, R., Guralnik, J, A.M., Money, C., McCormack, P. and Melzer, D (2010). Daily bisphenol A excretion and associations with sex hormone concentrations: results from adult population study. Environmental Health Perspect, ,64 (4:), 1603-8.
[9]. Guo.k.,Chen.X.,Fregui.S.,Donose.B.,Dan.C. (2013). Spontaneous modification of carbon surfaces with neutral red from diazonium salts for bioelectrochemical systems. Biosensors and Bioelectronics, 47(2), 184-189.
[10]. Heinze.J. Muller. R. (1998). Direct electrochemical ditection of C60 in solution by steady state voltametry at micrielectrodes. J.Electrochemical soci., 145(21), 1227-32.
[11]. He'lie's- Toussaint, J. Rochester and Ashley L. Bolden (2014). Bisphenol S and F: A Systematic Review andComparison of the Hormonal Activity of Bisphenol A Substitutes. Comparison of the hormonal activities of BPA substitutes, 32(6), 3-4.
[12]. Jiménez-Díaz I,Zafra-Gómez A, Ballesteros O, Navea N, Navalón A, Fernández MF, Olea N, Vílchez JL. (2010). Determination of Bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography-tandem mass spectrometry. Chromatogr Analytical Technology Biomed Life Science., 878:(32), 3363-9.
[13]. Katayama. M Matsuda Y, S. K. (2001). Simultaneous determination of six adenyl purines in human plasma by high-performance liquid chromatography with fluorescence derivatization. chromatogram biomedical applied science, 760(1), 159-63.
[14]. Kuralay.F.,Erkut.Y.,Lokman.U.,Adil.D. (2013). Modified disposable pencil graphite electrodes for the investigation of affinity binding to bovine, albumin. Colloids surfaces, 110(1), 270-274.

[15]. Liao, C. and Kannan (2011(a). High levels of bisphenol A in paper currencies from several countries, and implications for dermal exposure. Environmental Science Technology, 45(7), Environ SciTechnol 6761-8.
[16]. Li, X. (2005). "Bioelectrochemical Response of the Polyaniline TyrosinaseElectrode to Phenol. Journal of Analytical Chemistry. 60(11), 1073-1077.
[17]. Meeker, J. (2010). Exposure to environmental endocrine disrupting compounds and men's health. Maturitas, 66(12), 236-41.
[18]. Melzer, D. Harries, L., Cipelli, R., Henley, W., Money, C., McCormack, P., Young, A., Guralnik, J., Ferrucci, L., Bandinelli, S., Corsi, A.M. and Galloway, T. (2011). Bisphenol A exposure is associated with in vivo estrogenic gene expression in adults. environmental health pespect, 119(7), 1783-93.
[19]. Melzer, D. Osborne, N.J., Henley, W.E., Cipelli, R., Young, A., Money, C., McCormack, P., Luben, R., Khaw, K.T., Wareham, N.J. and Galloway, T.S. (2012). Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. circulation, 125(10), 1482-90.
[20]. Mei.S Da.W, Ming.J.,Yong L. (2011). Determination of trace bisphenol A in complex samples using selective molecularly imprinted solid-phase extraction coupled with capillary electrophoresis. Microchemical Journal, 98(1:), 150-155.
[21]. Mita, D. (2007). Enzymatic determination of BPA by means of tyrosinase immobilized on different carbon carriers. Biosensors and Bioelectronics, 23(1), 60-65.
[22]. Matyholo. V. (2011,). polymeric tyrosinase nanobiosensor system for the determination of endocrine disrupting bisphenol A. Environmental Chemistry. 2 (1):33-41.
[23]. Mbui D., Orata.D., Jackson.G.,Kariuki.D (2014). Investigation of kenyan bentonite in adsorption of some heavy metals in aqueous systems using cyclic volumetric techniques. International journal of physical sciences, 9(5), 102-108.
[24]. Nada.F.,Soher.A.,Sayed.E.,Gala.A. (2007). Effects of surfactants on volumetric responses and determination of antihypesentive drug. J.Talanta, 72(4), 1438-1445.
[25]. Orata. D and Segor. (1999). Bentonite (clay montmorillonite) as a template for electrosynthesis of thyroxine. Catalysis Letters, 58(8), 157–162.
[26]. Oukil.D. Makhloufi.L.Saidani.B. (2007). Preparation of polypyrolle films containing ferrocyanide ions deposited into thermally pretreated and untreated iron substrate. Application in the electroanalytical detrmination of ascobic acid., 123(2), 1083-1089.
[27]. Pulgar. M. Fatima. O.Arancha .N. Ana .R., Patricia .P., Vicente P.,Jose-Manuel N., and Nicolas O. (2000). Determination of Bisphenol A and Related Aromatic Compounds Released from Bis-GMA-Based Composites and Sealants by High Performance Liquid Chromatography. Environmental Health Perspectives, 108(1), 21-27.
[28]. Rodriguez-Mozaz, S. L. (2005). Analysis of bisphenol A in natural waters by means of an optical immunosensor. Water Research, 5, 5071-5079.
[29]. Sui, Y. Ai, N., Park, S.H., Rios-Pilier, J., Perkins, J.T., Welsh, W.J. and Zhou, C (2012). (2012) Bisphenol A and its analogues activate human pregnane X receptor. Environ Health Perspect, 120(10), 399-405.
[30]. Tokunaga OH, Liu X, Takay, Matsushima A, Shimohigashi Y (2008). Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. Environ Health Perspect, 116(1), 32-38
[31]. Vom Saal FS, Myers JP (2008). Bisphenol A and Risk Metabolic Disorders , Electrochemica Science. 300(299): 1353-5.
[32]. Yin H. Cui L, Chen Q, Shi W, Ai S, Zhu L, Lu L (2011). Amperometric determination of bisphenol A in milk using PAMAM-Fe3O4 modified glassy carbon electrode. Food Chem 125:1097–1103., 125(6), 1097–1103.
[33]. Yina. H, Zhou, J. Xu, S. Ai, L. Cui and L. Zhu. (2010). Anaytical Chemistry. Acta, 659(11), 144-5.

IOSR Journal of Applied Chemistry (IOSR-JAC) is UGC approved Journal with Sl. No. 4031, Journal no. 44190.

Kiio, L. " Fabrication of a Biosensor for analysis of Bisphenol A from Modified Glassy Carbon Electrode modified using Kenyan Bentonite, Polyaniline and Tyrosinase." IOSR Journal of Applied Chemistry (IOSR-JAC) 12.2 (2019): 01-10.