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Analysis of Bisphenol A in drinking water bottles and baby feeding bottles using Polyaniline/Bentonite Modified Glassy Carbon Electrode Biosensor.

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Abstract: *This work sought to investigate the presence of BPA in water placed in some plastic drinking water bottles and baby feeding bottles obtained randomly in the Kenyan market using polyaniline/ bentonite modified glassy carbon electrode biosensor. The aim was to analyse the levels of BPA that leached from the bottles when water maintained at known temperature and time was analyzed. The control consisted of the same water placed in similar bottles but made of glass at the same time and temperature. The hypothesis of the work was that BPA would leach into the water from the plastic bottles. The BPA content was investigated using electrochemical methods. A calibration curve was used to determine the amount of BPA that leached in to the water.*
The results obtained indicated that all the bottles investigated contained immediate detectable amounts of BPA amounting to 0.030mM, 0.021 mM and 0.035mM for first, second and third drinking water bottles respectively and 0.019mM and 0.030 mM first and second feeding baby bottles respectively on average for water in the bottles kept at 70 degrees for five minutes.

Keywords: *Bisphenol A, biosensor, polyaniline, glassy carbon electrode, surfactant, bentonite, cyclic voltammetry, square wave voltammetry, differential pulse voltammetry and tyrosinase.*

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I. Introduction

Bisphenol A is a Phenolic compound with two phenol groups (Willhite, 2008) and was among the top 50 chemicals produced in manufacturing industries worldwide a few years ago (Chang *et al.*, 2002).

It is a typical product of the industrial society produced in large quantities worldwide (Qiu et al. 2010). More than 80% of BPA is used as a monomer for the production of polycarbonate plastics, epoxy resins, and unsaturated polyester-styrene resins (Rodriguez- Mozaz, 2005). As a monomer, BPA is used as coatings on cans, as powder paints, as additives in thermal paper, in dental fillings, and as antioxidants in plastics (Qiu et al. 2010).

During production of polycarbonate, epoxy, unsaturated polyester, and polysulfone resinsbisphenol A is used as the main ingredient (Rufus, 1994). BPA is used in the synthesis of a number of products for example Polyethylene terephthalate (PET), High-density polyethylene, Vinyl/polyvinyl chloride (PVC), Polypropylene and Polystyrene. Polycarbonate plastics (PC) are characterized by great strength, stability, elasticity, and low density. For these reason they have been widely used for production of food packaging, bottles of water, kitchen utensils, medical equipment (Hao-Chang et al., 2010; Rivas et al., 2009; Schecter et al., 2010).

BPA residues have been detected in water stored in packages made of PC since it can be released from PC and then migrate to the water inside the bottle. This migration is promoted by acidity of the water stored, elevated temperature, mechanical cleaning, and use of detergents for cleaning. (Coulier et al., 2010; Carvalho et al., 2015; Lane et al., 2015).

BPA is known to cause problems to humans by interfering with endogenous hormones in the human body (Castillo, 1997). Some of the effects of BPA include neurological blastoma especially in children,(Braun *et al.*, 2009) breast and prostate cancer, sexual dysfunction (Li. *et al*., 2011 (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). Studies linked maternal BPA exposures to an increase in premature births (Chou et al., 2011***).***

II. Experimental

Tyrosinase was drop-coated on a bentonite and polyaniline - modified electrode laced with surfactant and phosphate buffer. This was used in a 3-electrode potentiostat to qualitatively and quantitatively analyse bisphenol A in water placed in various bottles randomly purchased from the Kenyan market. Specifically, three drinking water bottles, pink, blue and green and two feeding baby bottles, pink and green were used. Different colours of bottles were used to differentiate them. The production and working of this biosensor is described in the journal, Fabrication of a biosensor for analysis of Bisphenol A from modified glassy carbon electrode modified using Kenyan bentonite, polyaniline and tyrosinase (Kiio *et al* 2019).

To reduce the organic and inorganic contaminants, all beakers, calibrated flasks, and other glassware used in the experiments were cleaned sequentially with tap water, neutral detergent, and tap water, then soaked in nitric acid for 48 hrs after which they were cleaned with distilled water.

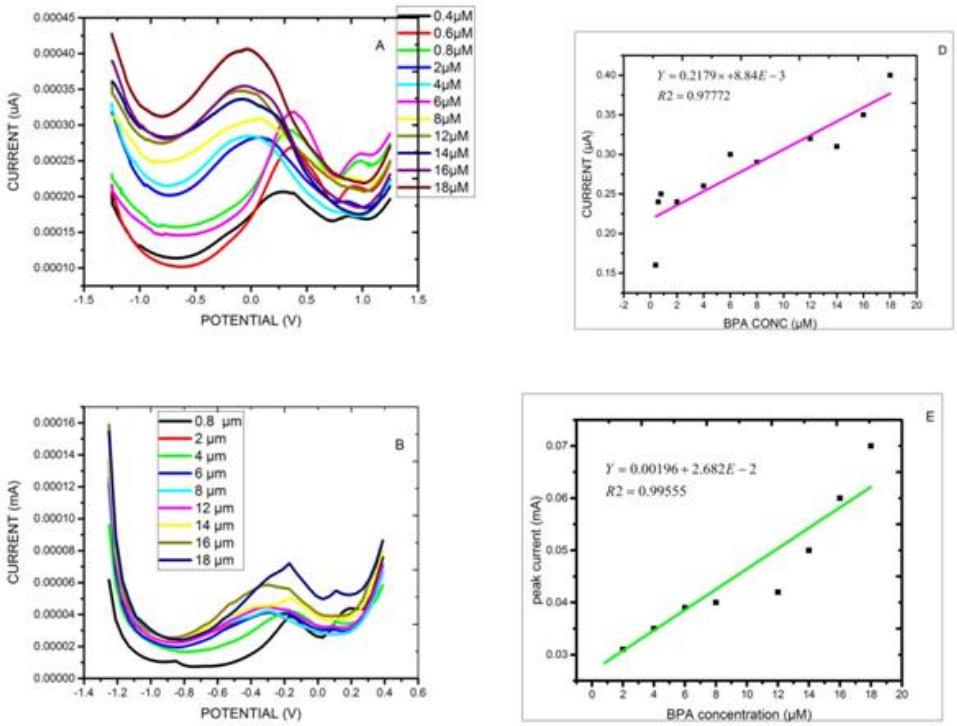
Forty (40) ml of water at 50° C, 70°,C80° C and 95° C was placed in the bottles for investigation, and allowed to equilibrate at that temperature for five minutes. The water was then cooled to room temperature, since enzymes are de-natured by inappropriate temperatures. 40 ml of 0.1M phosphate buffer pH 7.2 was then added to the water.

The biosensor was used to investigate the presence of BPA in the water using cyclic voltammetry (-0.1 V and +1.0 V) square wave voltammetry (– 1.5V and + 1.5V) and differential pulse voltammetry (– 1.5 V and + 1.5V) modes, at scan rates of 10, 20, 30, 40, and 60 mV s^{-1}.

III. Results and discussion

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 1 (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 at a scan rate of 40 mV s^{-1}.The DPV results showed one reduction peak at -0.1V at a scan rate of 40 mV s^{-1}. In cyclic voltammetry one reduction peak was observed at +0.5V and one oxidation peak at +0.20 V by cycling the potential repeatedly between -0. 1V and +1.0 V at a scan rate of 40 mV s^{-1} for 4 voltammetric cycles.



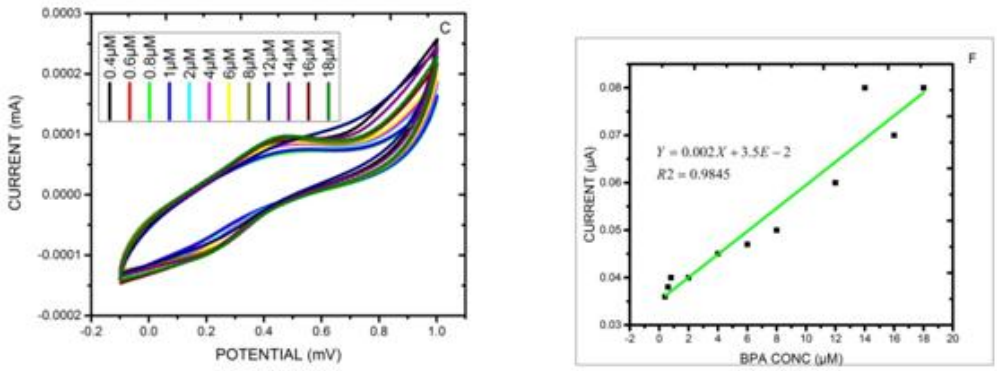


Figure 1 (A) Square wave voltammetry (SWV) (B) DPV (C) CV (D) SWV Linear calibration curve reduction peak (E) DPV Linear calibration curve of reduction peak (F) CV Linear calibration curve of reduction peak results for the biosensor in response to various concentrations of BPA.

The peak observed at +0.5V from the results obtained is the expected peak for BPA in the presence of tyrosinase, which electrochemically reduces the diphenol to *o*-quinone at relatively low potentials.

Linearity of calibration curves and correlation coefficients

The linear fit calibration curve for reduction peak potentials versus BPA concentrations was obtained for the biosensor and the correlation coefficient (R^2) obtained was 0.985 (figure 1 (f)). The correlation coefficient (R^2) for SWV and DPV using the biosensor was 0.978 and 0.996 respectively (figure 1 d and e).

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 using a retention time of five minutes, and within a concentration range of 0.4 -18.0 µM BPA. The detection limit value obtained in this study was found to be similar to the value reported by Matyholo, (2011) which was 1.9 x 10^{-8} M at a range of 1.0-16.0 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. The detection limit value obtained in this study was higher but very close to those reported in literature for other techniques such as Elisa, which was calculated to be 4.4 x 10^{-10} M for Real water samples with retention time of 6 minutes (Zhao *et al.*2002).

HPLC-MS was calculated to be 2.5 x 10^{-9} M for water samples with retention time of 15 minutes (Jiang *et al.* 2011). GC-MS, detection limit was calculated to be 2.6 x 10^{-9} M for water samples with retention time being 8 minutes (Del Olmo *et al.* 1997) and LC-MS detection limit was calculated to be 8.74 x 10^{-10} M with retention time of 10.5 minutes (Jiménez-Díaz *etal.* 2010).

Detection of BPA in first (blue) drinking water bottle

When cyclic voltammetry was done at a potential between -0. 1V and +1.0 V and a scan rate of 40 mV s^{-1} ,, only one reduction peak was observed at potential of +0.50 V and one oxidation peak at +0.20 V . The peak current obtained for the first drinking water bottle was approximately 0.00025 **mA**.The results obtained were shown in figure 2 (a) - (c) below.

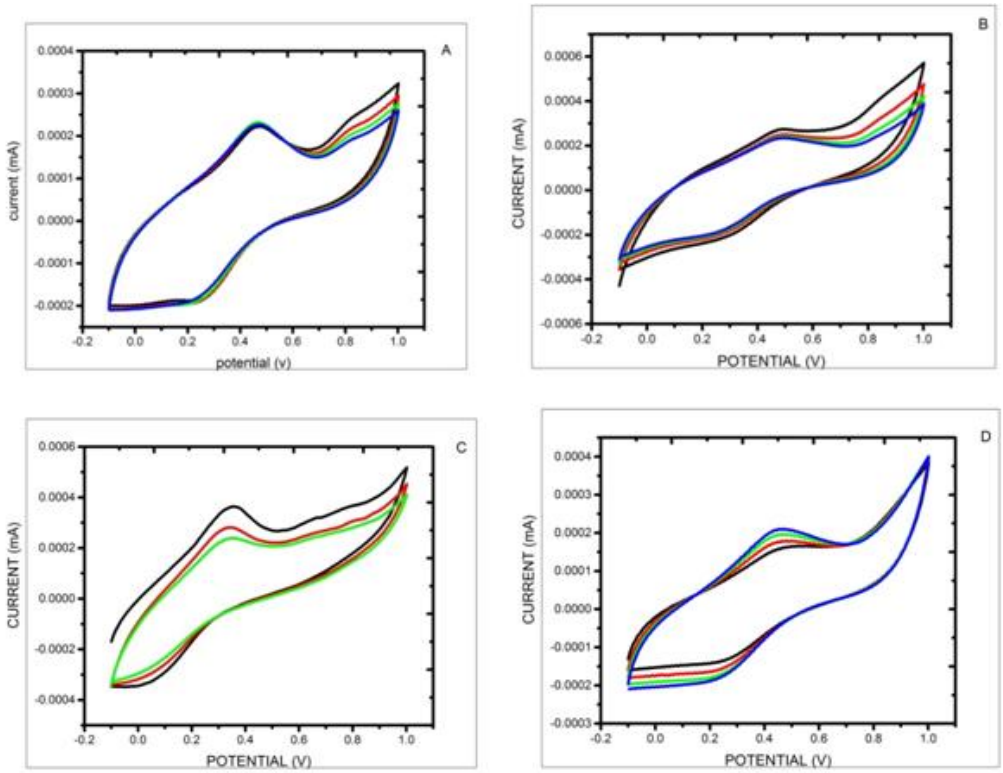


Figure 2: cyclic voltammetry of 40ml water in drinking water bottle in 40ml 0.1M PBS and pH 7.2 at temperatures of (a) 95 ^{0}C and (b) 70 ^{0}C (c) 80 ^{o}C (different scan rates) (d) Water in drinking water bottle in 0.1 M PBS left for 5 days at Room temperature.

The peak current was observed to increase as the number of cycles increased. From the calibration curve, the concentration of BPA in the water bottles at the three temperatures did not vary considerably and was estimated at 0.035mM BPA .

Detection of BPA in second (pink) drinking water bottle.

The results obtained were as shown in figure 3. From the results obtained using cyclic voltammetry, only one reduction peak was observed at +0.5 V and one reduction peak at + 0.2V. The reduction and oxidation peaks obtained are very similar to those obtained when 0.03 mM BPA in 0.1 M Phosphate buffer P H 7.2 were determined using biosensor discussed in the journal,Fabrication of a biosensor for analysis of Bisphenol A from modified glassy carbon electrode modified using Kenyan bentonite, polyaniline and tyrosinase (Kiio,*et al* 2019), and the detection limit for BPA was 2.1 x 10 $^{-9.}$ The SWV linear graph obtained when peak current was plotted against scan rate indicated that reaction is diffusion –controlled. The peak current obtained was about 0.00022, which may corresponded to approximately 0.021mM BPA. When linear graph of peak current verses scan rate was drawn the correlation coefficient obtained was $R^2 = 0.9924$.

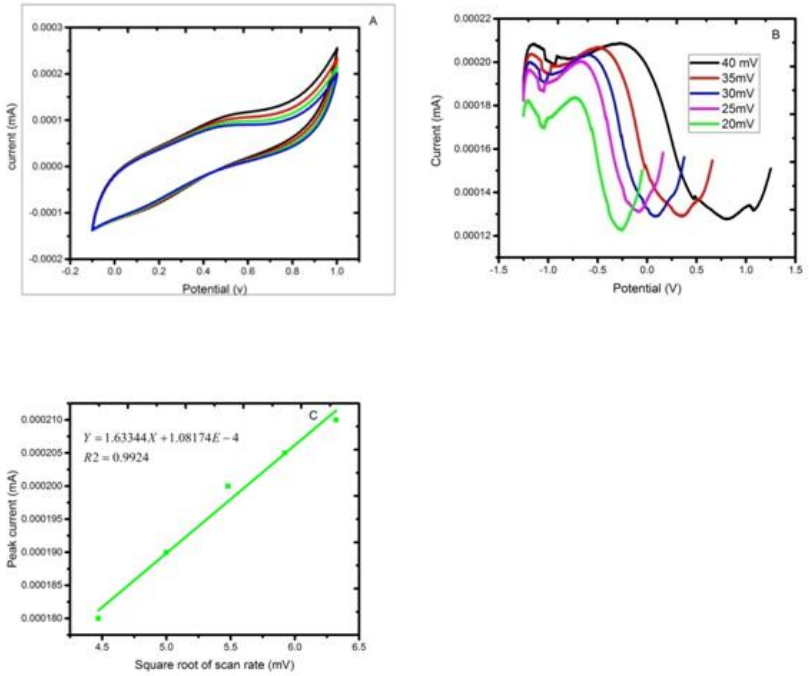


Figure 3: Water in second drinking water bottle at 70 ^{0}c at different scan rates (A) CV (B) SWV (C) SWV linear graph peak current verses scan rate.

Detection of BPA in the third (green) drinking water bottle

The results obtained when the third drinking water bottle was used were shown in figure 4. From the results obtained using CV at 70 ^{0}C and DPV at 70 ^{0}C, one reduction and one oxidation peaks were obtained at +0.49V and +0.23V respectively which was similar to the results obtained when the first and second bottles were used. Water was left in the bottle for seven days and BPA was tested in the water at different times. It was noted that the BPA concentration in the water increased with increase in contact time of the water and the plastic bottles. The results obtained showed one reduction peak at approximately +0.51V and one oxidation peak at +0.22V figure 4 (a) similar results were obtained when the first bottle was used (fig 2d)).After the fifth day there was no further increase in the peak current. This could have been attributed to occupation of active sites of the tyrosinase enzyme immobilized on the biosensor by BPA due to its high concentration.Figure 4 (d) results showed one reduction peak at approximately +0.51V and one oxidation peak at +0.22V, these results are similar to those obtained earlier in (fig 2d).

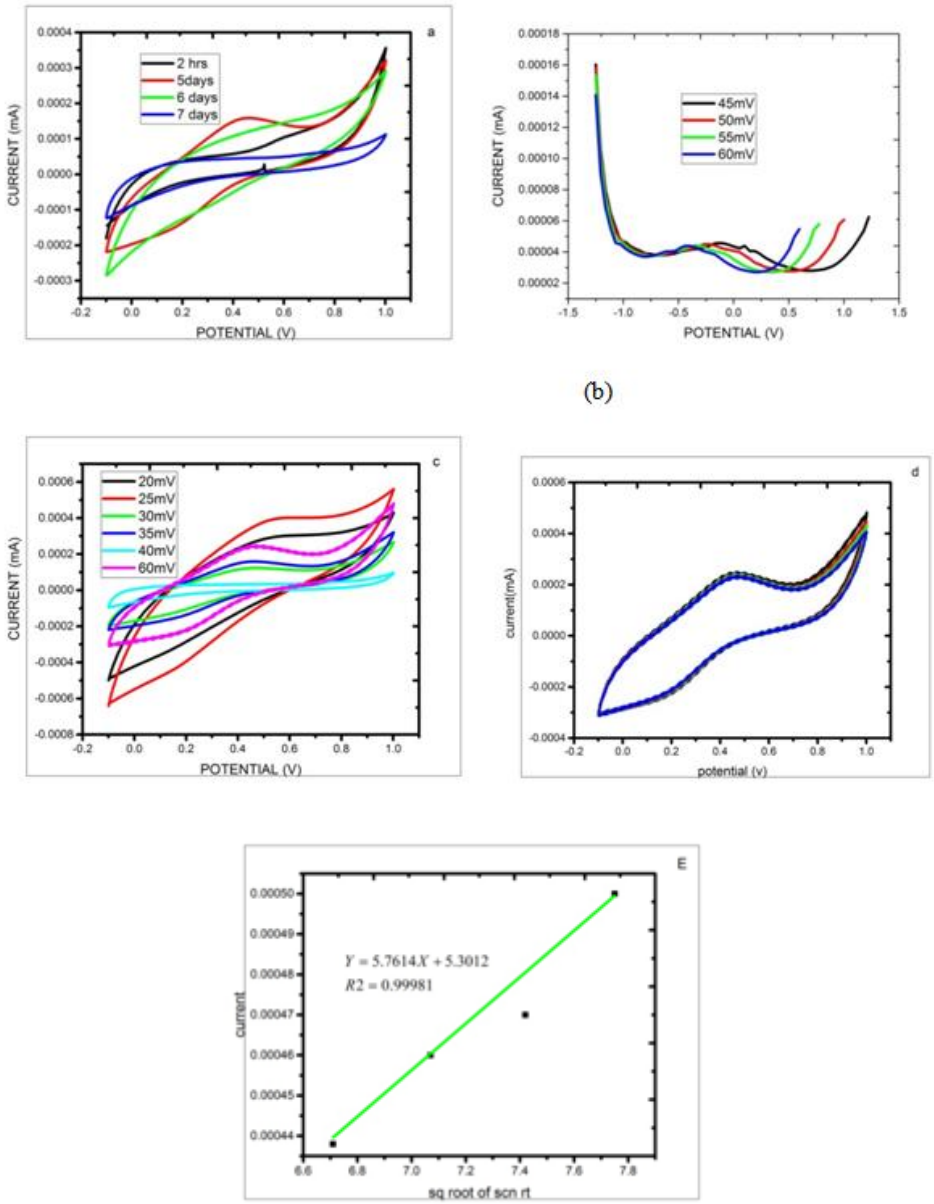


Figure 4 (a) water at room temp using biosensor between 2 hours- 7 days (b) DPV of water at a temp of 70 ^{0}c bottle at different scan rates (C) CV of water at temp of 70 ^{0}c using biosensor at different scan rates (d) water at room temp using biosensor after 5 days at a scan rate of 40 mV/s (e) peak current verses square root of scan rate DPV of water at70 ^{0}c

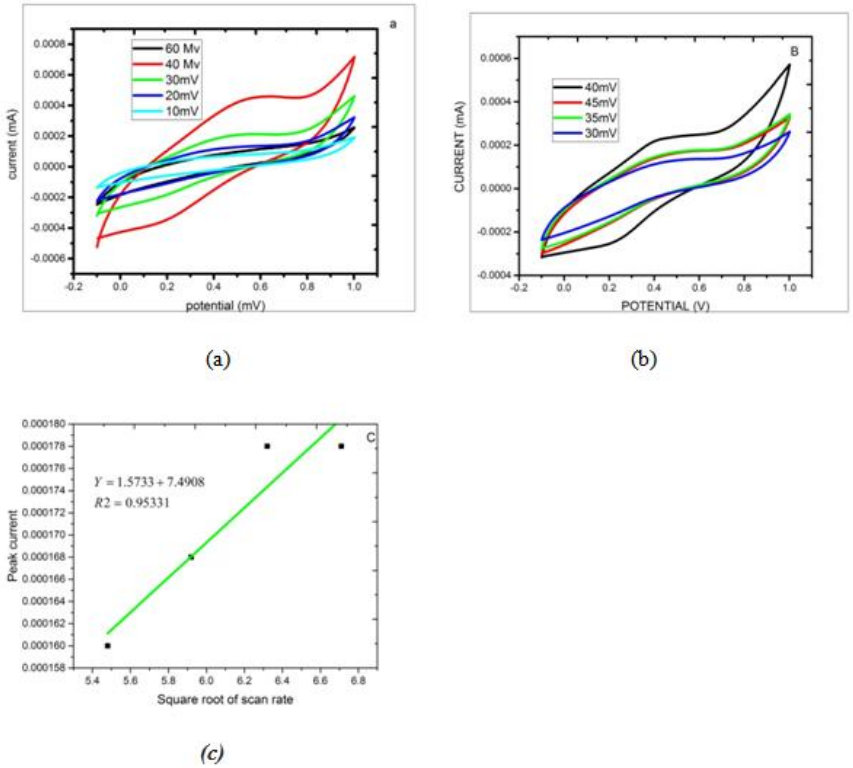
When linear graph of peak current verses scan rate was drawn the correlation coefficient obtained was R2 = 0.9998 which was very close to the one obtained for the second water bottle which was 0.9924, fig 3c. This value is similar to the value obtained when the biosensor was used to determine the presence of known concentration of BPA (Kiio,*et al* 2019) whose R2 value was 0.995. The detection limit for BPA in the green water bottle by the developed biosensor was calculated and found to be 0.03 mM.

Detection of BPA in baby feeding bottles.

The analysis of baby feeding bottles showed one reduction peak which was observed at potential of +0.50 V at a current of 0.0004 mA and one oxidation peak at + 0.21V at a scan rate of 40 mV s-1 in the first(green) baby feeding bottle. These results are similar to those obtained with the previous bottles. A graph of square of scan rare against current 5 (c) shows a linear relationship with a correlation coefficient of 0.953, which means that the reaction is diffusion-controlled.

The formula 3σ/slope was employed to calculate detection limit, where σ is the standard deviation of the blank. Under the optimized conditions, the detection limit of water in first feeding baby bottle was 3.112 x

10^{-10} M. The detection limit was found to be in good agreement with those reported in literature by Del Olmol *et al.*, 1997 which was 2.6 x 10^{-9} M for BPA determination in water samples using GC- MS technique and by Jiang *et al.*2011 which was 2.5 x 10^{-9} M for determination of BPA in water samples using HPLC-MS. From the calibration curve and the peak current, the amount of BPA in the water from the first (green) baby bottle was estimated to be 0.019.



(a) (b)

(c)

Figure: 5 (a) CV of water at 70 ^{o}C infirst baby bottle in 0.1m PBS pH 7.2 (b) water at 80 ^{o}C in 0.1m PBS pH 7.2 (c) Peak current verses square root of scan rate

When BPA in second (pink) baby bottle was determined using the biosensor at different scan rates, the same pair of peaks signaling BPA at +0.5V (reduction) and +0.22V (oxidation) were observed.

Clearer and more defined peaks were observed when the water was subjected to higher temperatures as shown in Figure: 6 (a) and (b) below. The correlation coefficient obtained when linear curve of second baby bottle at 70 ^{0}C was compared with the calibration curve of BPA was 0.993 and this value was in agreement with the previous studies done on determination of known concentration of BPA using biosensor fig 1 (c). The calculated detection limit of BPA in the second baby bottle using the calibration curve figure 6 (c) was 1.977 x 10^{-8}M.

Generally the peak current increased with increase in scan rate and decreased at high scan rate as observed in fig 5a and b.

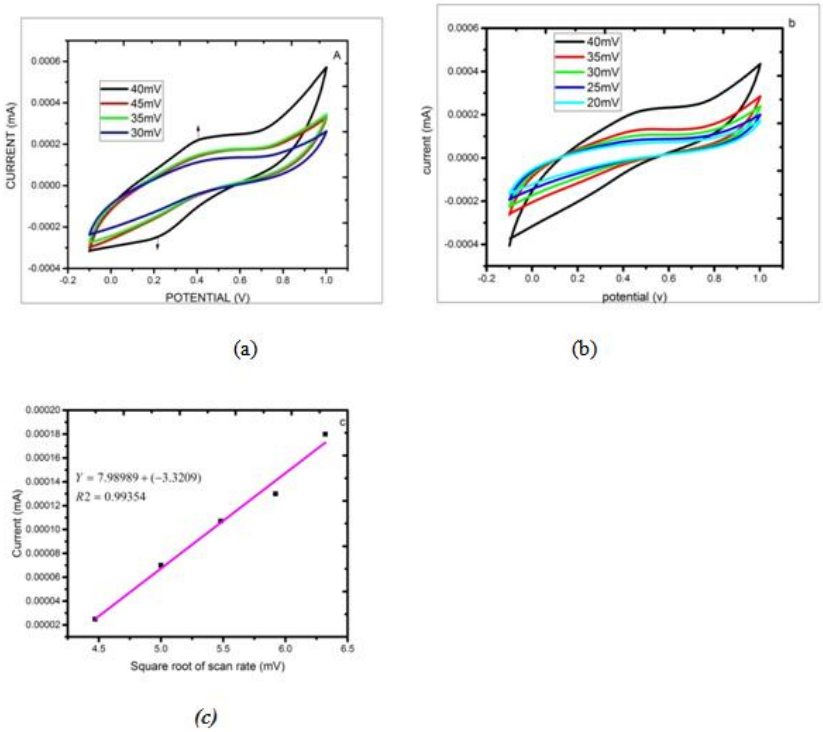


Figure 6: Water in the second baby bottle at a temperature of (a) 80 ^{0}C and (b) 70 ^{0}C (c) linear plot of peak current verses square root scan rate of second baby bottle at 70 ^{0}C

Comparison for the water bottles and the baby bottles.

A comparison was done for all the water bottles and the baby bottles using cyclic voltammetry, square wave voltammetry and differential pulse voltammetry. For cyclic voltammetry, this was achieved by cycling the potential repeatedly between -0. 1V and +1.0 V at a scan rate of 40 mV s^{-1}. The results obtained were as shown in figure 7 below**.** From the results obtained, one reduction peak was observed at + 0.48V with a shift to the positive potential. One oxidation peak was observed at +0.23V. The CV results obtained were found to be similar to those obtained earlier on voltammetric characterization of biosensor using a concentration of 0.002M BPA in 0.1M PBS at a pH 7.2. (Kiio, *at al*, 2019)

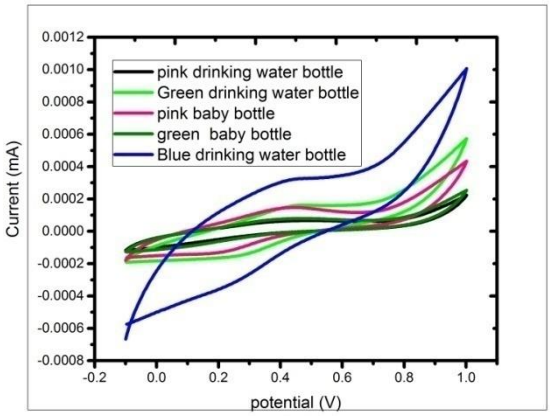


Figure: 7: Comparison of water in water bottles and baby bottle at a scan rate of 40 mV s^{-1} at 70 ^{0} c.

Figure 8 (a) and (b) illustrates the SWV results for the electrochemical behavior of BPA using the biosensor as a result of addition of water at a temperature of 70^{0}C and 50 ^{0}Cinto different bottles then letting it to cool in the bottles. 40 ml of the water was then added into 40 ml 0.1 M phosphate buffer. The study was performed at a potential range of -1.2V to +1.2 V. The SWV results for water at a temperature of 70^0 C showed one reduction peak centered at +0.50V while at a temperature of 50 ^{0}C the reduction peak centered at +0.49 V.There was no peak observed when water was put in glass flask and 0.1M PBS buffer added was investigated. This showed specificity of the biosensor in detecting BPA since BPA is not used to make glass. From the results

obtained it was clear that water in the glass bottle did not have BPA, this meant that, the BPA in the water came only from the plastic bottles. This results obtained are similar to those observed when CV was used.

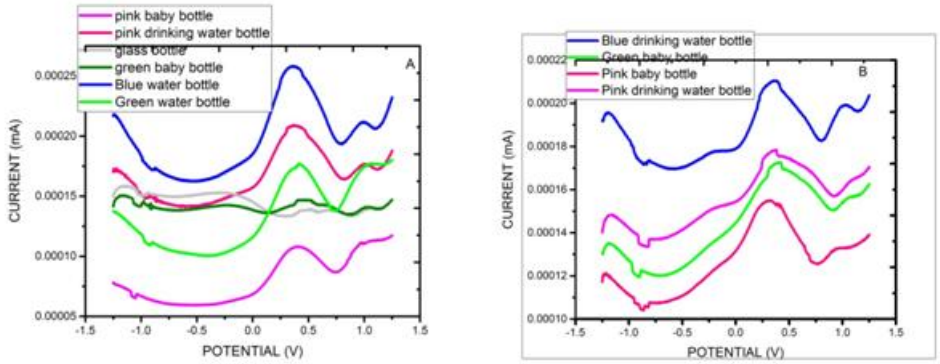


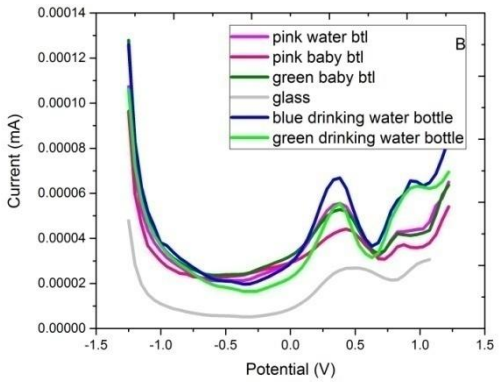
Figure: 8: SWV of the different bottles at temperatures of (a) 70˚ C (b) 50 ˚C using biosensor (GCE/ PANI-BTN/SLS/TYR).

The results for peak current for square wave voltammetry (figure 8 (a))using the five bottles used in this study at a temperature of 70˚ C using biosensor were plotted as shown in table 2 below. From the results obtained, the blue water bottle had the highest peak current of 0.00025(m A). These results indicated that the blue water bottle had the highest amount of BPA concentration that leached in the water.

Table 2: peak current for square wave voltammetry (figure 8 (a)) for different bottles at a temperature of 70˚C and contact time of 5 minutes.

Type of bottle	**Peak current (mA)**	**Detectable amount of BPA (mM)**	**Standard deviation (σ)**
second (Pink) baby bottle	0.00012	0.030	0.0013
Second (Pink) drinking water bottle	0.00022	0.021	0.0028
First (Green) baby bottle	0.00015	0.019	0.0017
First (Blue) water bottle	0.00025	0.035	0.0022
Third (Green) water bottle	0.00018	0.030	0.0042

Figure 9(b) illustrates the DPV results for the electrochemical behavior of BPA in drinking water bottles and feeding baby bottles using biosensor (GCE/ PANI-BTN/SLS/TYR) at a temperature of 70˚ C. The study was performed at a potential range of -1.2V to 1.2 V.



(a)

Figure: 9: DPV of different drinking water bottles using (a) GCE/ PANI-BTN/SLS/TYR (biosensor

The DPV results obtained for GCE/ PANI-BTN/SLS/TYRfig 9 showed one reduction peak centered at +0.49 V with slightly shift in peak potentials to more positive potentials.
It was noted that the BPA concentration in the water increased with increase in contact time of the water and the plastic bottles.

The presence of BPA in the plastic bottles echoed the findings of Tokunaga.*et al.,* 2008 who reported the presence of plasticizer residues in water stored in bottles. This could be attributed to the migration of BPA from the bottle material to the water. The variation in the amount of BPA in the various bottles varied since bottle quality usually depends on the raw material and the technology used in bottle production (Amiridou . *et al.,* 2011).

From this study, it can be concluded that, Bisphenol A was transferred from the plastic bottles into the water that was put into the bottle. The ester bonds in BPA-based polymers found in drinking water bottles, feeding baby bottles, etc. are subject to hydrolysis. Heat and repeated washing of polycarbonate products have been shown to result in an increase in the rate of leaching of BPA (Vom-Saal *et al.,* 2008) and therefore it leaches into food and drinks from their storage containers. This is dangerous because it implies that the people who ingest food and drink contained in the containers also ingest BPA in the levels present in the bottle.

IV. Conclusion and Recommendations

In this work, various bottles were investigated for presence of BPA. The results obtained indicated that all the bottles investigated contained immediate detectable amounts of BPA amounting to 0.030mM, 0.021 mM and 0.035mM for first, second and third drinking water bottles respectively and 0.019mM and 0.030 mM first and second feeding baby bottles respectively on average for bottles kept at 70 degrees.

It is recommended that, the biosensor parameters should be investigated further and optimized in order to achieve lower detection limits. This study only investigated the presence of BPA in water that has been put into the bottles. The amount of BPA in other drinks (carbonated water, milk, juices, etc) that are usually housed in plastic bottles should be investigated. Finally, we recommend that BPA content in body fluids of consumers could be investigated so as to determine whether the body absorbs the BPA consumed.

Acknowledgement

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