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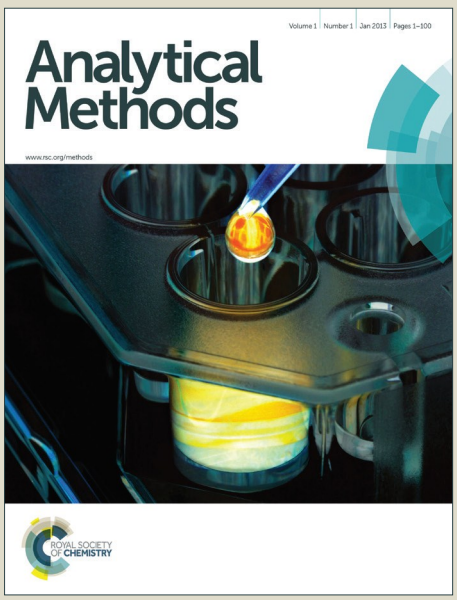

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Development of silver functionalised Polyaniline electrochemical Immunosensor for Polychlorinated biphenyls

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An electrochemical immunosensor based on the silver nanoparticles (Ag NPs)-doped polyaniline (PANI) modified glassy carbon electrode (GCE) transducer where polyclonal anti- polychlorinated biphenyls (PCB) antibody (*Ab*) was immobilized by covalent linkage with glutaraldehyde (GA) was developed. Optimum conditions for the fabrication of the immunosensor were; immersion, 1.0% GA and 30 min GA incubation period. While electrochemical measurement of PCB 28 were best done under square wave voltammetry (SWV) technique. The optimum methodology conditions were a scan rate of 20 mV/s, sweep potential range of -1 to 1 V and PCB incubation period of 2 hrs. The electrochemical response obtained under these optimum conditions was linear within 0.2 and 1.2 ng/mL with limit of detection(LOD) and limit of quantitation (LOQ) of 0.063 ng/mL and 0.209 ng/mL respectively. The specificity of the developed sensor towards PCB 28 against benzyl chloride (BnCl) and PCB 180 was poor due to structural similarities. However , the PCB 180 results corresponds to total PCB thus indicating the sensors applicability to a regular total PCB determination. Cations and anions tested had minimum effect on the sensor. Recoveries studies in water and guava juice ranged from 90 to 102%. Thus, proving the immunosensor´s selectivity to PCBs and its possible application in detection and monitoring of PCBs in food, water bodies as well as general environmental samples.

Introduction

Among the many environmental threats, polychlorinated biphenyls (PCBs) still remain a challenge and a major public health concern. This is because of their persistence[1] and toxic health effects.[2] PCBs have had a widespread usage, as a result, there are still detectable amounts in the environment[3] to date. Conventional analytical methods such as chromatographic techniques coupled with either electron capture detector (ECD) or mass spectrometry (MS) have shown several disadvantages on the analysis of PCBs. They are costly to run, often complex to use, time-consuming and utilize expensive and toxic solvents[1]. They typically require elaborate sample preparation before analysis.[4] In addition their instrumentation is not applicable for on-site analysis.[5] Again, these methods require high knowledgeable personnel. Therefore, in addition to lowering and/or removing the levels of PCBs and their toxic discharges into the environment, there still remains a need to develop techniques and methods that can detect and monitor these environmental pollutants in a cheap, sensitive and selective manner for ensuring safety and quality of food, water, and environment especially in on-point analysis.

Biosensors are well known as complementary tools for cost effective on-point analysis tools for pollutants including PCBs. They have shown a capability to overcome chromatography analytical constraints [6,7] hence, there is a growing research interest in their development. These analytical techniques are known for their high performance and generation of high sample throughputs with significant decrease in analysis time.[1] They are specifically suitable for continuous, rapid and in-situ on-site screening of environmental pollutants. Immunosensor technologies are selective biosensors utilising antibodies or antigens (*Ag*) as specific probes. These technologies are easily adaptable by incorporation of nanotechnology, polymer science and electronics. Thus, widening their application in detection of a broad spectrum of analytes even in complex sample matrices with minimal sample preparation or pre-treatment.[8-10] The incorporations offer improved immobilization and detection steps. Thus, also extending the possibilities of assay procedure simplification by steps reduction. More importantly, their use guarantees miniaturization and portability of immunosensor systems which therefore, assure inexpensive and simple production of these analytical devices.[11,12]

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Electronic supplementary information ; EDX plots

This research focuses on the development of label-free reagentless electrochemical immunosensor based on integration of an *Ab* on Ag NPs-doped polyaniline (PANI) modified GCE (GC/PANI/Ag NPs) coupled with electrochemical detection. The adoption of electrochemical detection techniques is based on several advantages over their optical, piezoelectric and thermal counterparts. Immunosensors coupled with electrochemistry can offer and guarantee sensitivity, flexible label-free and reagentless detection methods with less interferences and low detection limits.[13,14] Nanomaterials act as electron transfer "mediators" or "electrical wires", e.g. Ag and Au nanoparticles, which have good conductivity and have been used to enhance electron transfer between proteins and electrodes.[15]

Silver nanoparticles have been extensively studied due to their unique physiochemical and electronic properties which have lead to enormous applications in sensing, biosensors, electronics, catalysis, pharmaceutical and therapeutics.[16,17] Additionally, PANI is a leader in the study of conducting polymers as a result of its ease of modification, processing, adaptability and stability.[18,19] It is these benefits of both materials that influenced their choice in this study

The proposed sensor was designed and tested utilizing PCB 28. This congener is reportedly one of the seven persistent and prominent PCBs which are used as indicator congeners because they form the basis of reference for evaluation and analysis of the entire PCB family due to their believed high potency of toxicity and adverse health effects.[20,21] Notably it has been implicated among the endocrine disrupting PCBs of global concern.[22] It has been studied and determined mostly as total PCBs with other congeners using different methods.[20,21,23-25] These imply that the knowledge and information about PCB 28 is significant. There are two key steps that are important for the accomplishment of this piece of work. First is the initial development of the immunosensor and second is the ultimate application of this tool in the analysis of PCBs. These steps involve the immobilization of *Ab* on the transducer and the electrochemical detection of PCB respectively. Factors that affect the *Ab* immobilization and the detection were identified and optimized. Optimum conditions were validated and the immunosensor performance tested in spiked real samples.

Experimental

Materials and reagents

Aniline (99%, Sigma Aldrich) was pre-distilled and stored refrigerated at -4^{oc} before use, hydrochloric acid, HCl (32%), alumina (Al_2O_3) powder (0.05, 0.3, 1.0 µm) (Buhler), ethanol (99%, analytical grade), N, N - dimethyl formamide (DMF) (99.8 %, analytical grade), Benzyl chloride (BnCl) ($C_6H_5CH_2Cl$) (analytical grade), micro - cloth pad (Buhler) and analytical grade nitrogen gas were used as they were. PCBs 28 and 180 were obtained from Sigma Aldrich prepared by Dr Ehrenstorfer GmbH, Germany as 100 µg lyophilized in phosphate buffered saline (PBS). Glutaraldehyde (25% v/v) as a cross linker was also from Sigma Aldrich. NaCl, KCl, $Na_2HPO_4{\cdot}12H_2O$, KH_2PO_4 and NaOH (analytical grades) were used for PBS preparation.. Tap water and guava fruit juice were used without prior treatment as PCB-free matrices. Distilled water was used for preparation of all aqueous solutions. Citrated capped Ag NPs which were synthesized by the reduction of $AgNO_3$ with trisodium citrate according to described procedures [17] were used. CV and TEM were used to confirm presence of Ag NPs in the suspension.

Preparation of (PBS) (10x = 0.1 M) and PCB 28 standard solutions

PBS solution, used as a supporting electrolyte as well as for preparation of solutions, was prepared using Sambrook Fritsch & Maniatis preparation method.[26] PCB 28 standard solutions were prepared in acetonitrile (ACN).

Apparatus

Electrochemical measurements were done using Autolab PGSTAT 101 (*Metrohm*, SA). computer interfaced with Nova software. A three compartment electrochemical cell with GCE (A=0.071 cm^2) working electrode (WE), Pt wire(3.0 mm diameter) auxiliary (counter) electrode (AE) and silver/silver chloride (Ag/AgCl, 3 M KCl) reference electrode (RE) system was utilized. Prior to modification the GCE was polished repeatedly with 1, 0.3 and 0.05 µm alumina slurries. After each polishing, electrode cleaning adopted was as follows; successive 5 minutes rinsing with doubly distilled water, ultrasonication in ethanol and finally with another doubly distilled water to remove any adsorbed substances on the electrode surface.

A high resolution transmission electron microscope (HRTEM) from Tecnai G2F20 X-Twin MAT (US) was used for transmission electron microscopy (TEM) analysis. TEM samples were prepared by placing a drop of methanol suspensions of the polymers on a carbon-coated standard copper grid (300 mesh) operating at 80 kV. An EDX analyzer attached to the TEM was used to analyze the chemical compositions of the synthesized nanoparticles.

Construction of the immunosensor

The GC/PANI/Ag NPs transducer was prepared by modification on GCE surface through 10-cycle CV electropolymerization of 0.2 M aniline 1MHCl medium in the presence of Ag NPs. The polymer was air-dried and activated by immersion in GA solution, dried and rinsed with distilled water (GC/PANI/Ag NPs/GA) followed by the immobilization of *Ab* on the GA activated platform to yield GC/PANI/Ag NPs/GA/*Ab*. This was also dried and rinsed with distilled water and used or stored at -4 ^{0}C. The steps were monitored by CV in PCB free electrolyte, PBS (pH = 7.4)/ ACN (99.6:0.4 v/v %) at a sweep range of -1 to +1 V and 20 mV/s scan rate.

Optimization of the immunosensor construction parameters

Fabrication steps were optimised for immobilization method, time required for effective interation of the GA, Ab and PCB as well as the optimum concentration of GA and Ab. These parameters were optimized by comparing the response of the immunosensor when varying the parameter under study while keeping others constant.

The type of immobilization was determined by comparison of immersion (dipping the modified electrode in solutions of 1 % GA and 1 µg/mL Ab for 1 hour) and drop-coating (5 µL of the GA and *Ab* solutions on modified electrode and left for an hour). The effect of concentration of GA was studied by treatment of GC/PANI/Ag NPs with 1, 2 and 4 % prepared from 25 % GA stock solution in distilled water. The optimum period for the activation of GC/PANI/Ag NPs with GA was determined by using immersion times of 0.5, 1, and 2 hours. Time effect on interaction of *Ab* and *PCB* was investigated using 0.5, 1, and 2 hrs.

Optimization of factors which have an affect on the signal intensities such as; sweep potential, scan rate and detection method were evaluated. The suitable potential range was determined by ramping the modified electrode using CV within -4 and +1 V. The sweep rate was optimized in the range 5 - 100 mV/s while the methods of detection was by comparison of CV, SWV and DPV signals. These optimization were carried out using PCB and *Ab* concentrations of 1 µg/mL each.

PCBs detection

Under the optimum conditions, the sensor was incubated with 10 mL of different concentrations of PCB (0.2 – 1.2 ng/mL) prepared with PBS (pH = 7.4)/ ACN (99.6:0.4 v/v %) in an electrochemical cell. The solution was deoxygenated using nitrogen gas for 10 min and left static for a specific time. A potential sweep of -1.0 to +1.0 V, scan rate of 20 mV/s, amplitude of 20 mV and frequency of 25 Hz were used to run the solutions SWV voltamogramms.

Reproducibility, specificity, interference and validation of the immunosensor

For the fabricated immunosensor, reproducibility was evaluated by the intra- and inter-assay measurements and relative standard deviation (RSD) at 1 µg/mL PCB concentration using SWV. The intra-assay RSD was determined by running 5 replicate measurements using one immunosensor while inter-assay for the same PCB concentration was done using 5 individual immunosensors fabricated in the same manner.

Cross-reactivity was tested by spiking pure sample of the potential cross-reactants, BnCl and PCB 180 (in the ratios 1:5, 1:10 and 1:20) to the PCB 28 solution before incubation followed by measuring the current response and determining the cross-reactivity. The test was done for the same concentration of PCB solution (1 ng/mL). Each measurement was completed through three replicates. The matrix ionic interference studies were done by spiking the PCB standard sample with the ionic compounds of Na and K at the concentration ratios as in the cross-reactivity above and determining the voltammetric current response. The extent (%) of matrix effect was then determined. Method validation involved the use of the new method on analysis of samples in real life and testing the recoveries. It was done by spiking real water and fruit juice samples with 1.0 ng/mL PCB 28 standard. The analysis was made with three replications for each sample.

Results and discussions

The fabrication of an immunosensor was as depicted in Fig. 1. Which shows the steps for transducer fabrication by electropolymerization of aniline in the presence of Ag NPs followed by step by step activation of the transducer with the GA cross-linker and the immobilization of *Ab* on the GA activated platform. During these steps PANI formed enamines when it interacts with GA, while addition of *Ab* resulted in the formation of imines.

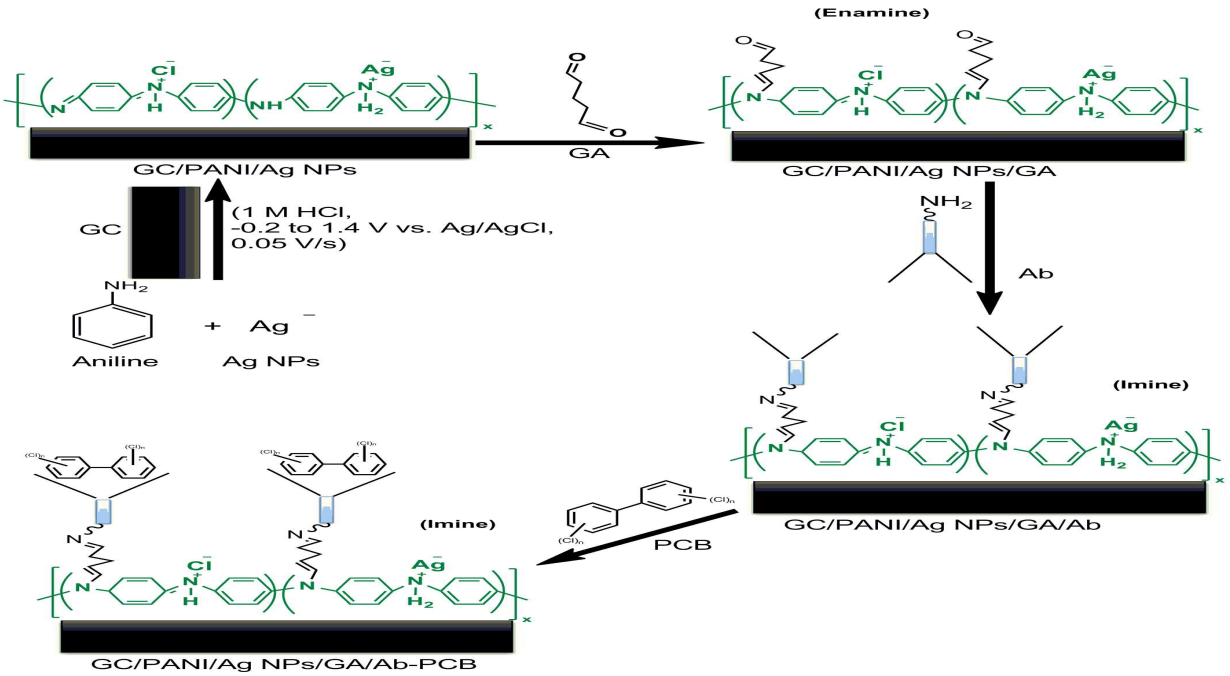

Fig. 1 Immunosensor fabrication mechanism scheme

Formation of GC/PANI/Ag NPs

Fig. 2 (A) depicts the first (inset) and tenth CV cycles voltammograms during the electropolymarisation of PANI in the presence (GC/PANI/Ag NPs) and absence of AgNPs (GC/PANI). Both polymer CV voltammograms had characteristic PANI peaks. The current peaks increased with successive cycles(not hown). Electropolymirasation and free monomer CV voltammograms show that Ag NPs-doped PANI currents were larger than those of PANI indicating improved conductivity (Fig. 2 (B)). However, presence of Ag NPs was not evident from the Voltammograms peaks. This absence of Ag NPs peaks may be due to the PANI peaks intensity and proximity relevant to the Ag redox couple at 0.1V and -0.3 V (Fig. 2(C)) or a result of the potential range applied. It is also possible that there is no free Ag NPs due to its interaction with the polymers functional groups or it being embedded within the polymer.

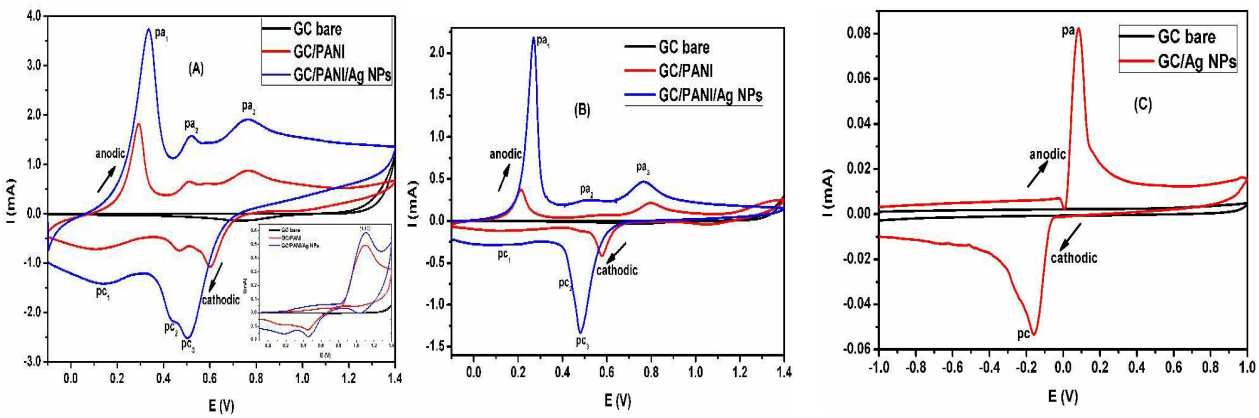

Fig. 2 Cyclic voltammograms obtained with the modified GC electrode; during electropolymerization (A), Polymer in monomer free 1 M HCl (B) and Ag NPs in 1 M HCl (C)

The characteristic morphologies of the materials (PANI, PANI/Ag NPs and Ag NPs) are depicted in Fig. 3 (A) to (C). The micrographs in Fig 3 (B) clearly show the Ag NPs embedded within the polymer as dispersed particles of the metallic silver in the polymer matrix as stated in the literature.[27] The presence of the Ag is confirmed by the EDX analysis S1 (B). Both EDX plots (S_1 (A) and (B)) indicate presence of Cl^- dopant from the HCl during polymerization process. The presence of both Ag and Cl (S_1 (A) and (B)) substantiates the concept of Ag NPs and Cl^- simultaneously participating in PANI doping. In addition, the EDX show the presence of carbon, nitrogen, oxygen and chlorine atoms in both plots (PANI and PANI/Ag NPs) which constitute the backbone of PANI, citrate-capped Ag NPs and protonic acid doping effect on the polymer. While the presence of copper is contributed by thecopper coated micro-plates used for sample preparation. Fig 3(C) is the image of the Ag NPs used in PANI fuctionalisation, from the image various morphologies are observed with an average size of 60.0 nm.

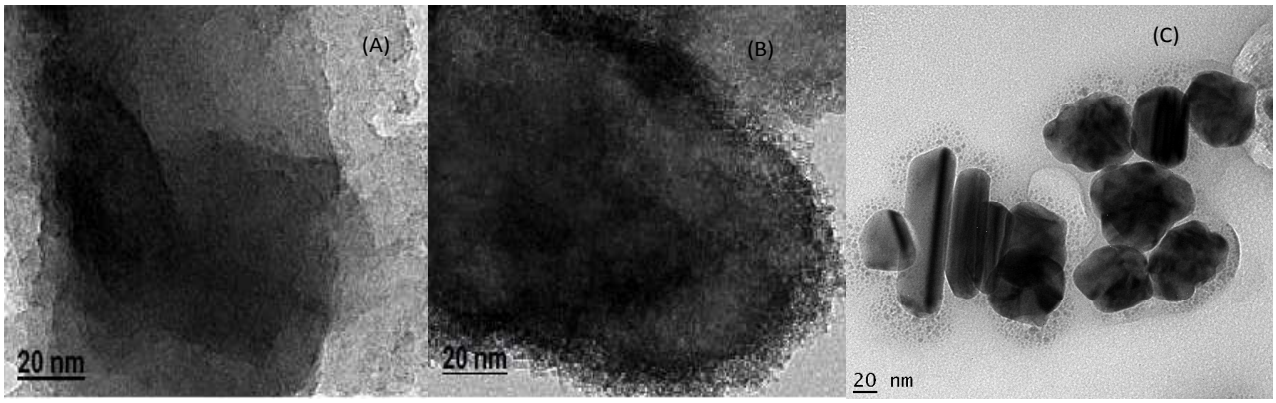

Fig. 3 TEM images of PANI (A), PANI/Ag NPs (B) and Ag NPs (C)

Optimization of immobilization and immunological parameters

In development of an immunosensor, immobilization of the *Ab*/*Ag* on the transducer is a crucial step that determines the analytical performance. Therefore, sufficient time and concentration needed to attain maximum interaction, stability and reproducible response must be optimized.

Effects of different coating, incubation time and concentration of *Ab* and GA

Fig. 4 (A) depicts CVs of the electrode GC/PANI/Ag NPs/GA/*Ab* fabricated by drop coating or immersion of linker, antibody and PCB in each step. Immersion fabricated electrode does not exhibit peaks while reduced intensity PANI cathodic peak is observed in drop-coated electrode. The prevalence of peaks under drop coating suggests inadequate and inefficient modifications with GA and *Ab* while immersion offered better activation of the supporting transducer by the GA and ultimate success in the immobilization of the *Ab*. Hence, immersion was a method of choice in the immunosensor fabrication.

The effect of GA was assessed by insolubilizing the various concentrations of *Ab.* The square wave voltammetry responses of the PCB 28 measurements decreased with an increase in GA concentration. (Fig. 4 (B)). This change could be explained as follows: Increasing GA concentration at constant *Ab* results in increased rigidity which possibly destructed the *Ab* attachement. Destruction of *Ab* led to ultimate underperformance of the immunosensor as evidenced by the low signal. The 1% GA was the optimum concentration for the device. The *Ab* to transducer integration is achieved through covalent linkage using GA. This offers strong linkage that eludes loss of *Ab* and promotes its stability.[28] Previous studies have reported the successful application of GA as a cross-linker in fixation of *Ab*[29-32] and it has been shown to enhance the stability of the biomolecule.[29,33] However, there are contradicting reports on the effect of variation of GA concentration; some indicate no significance effect on the performance of the sensor[34] while others[35,36] proclaimed that the concentration of the cross-linking material has an influence on the cross-linkage in that the strength and efficiency of the cross-linking are directly proportional to the concentration of the cross-linker thus supporting our results.

Variation of time needed to activate the transducer (GC/PANI/Ag NPs) with GA from 0.5, 1.0 and 2 hrs resulted in 1.49, 1.52 and 1.50 μA peak current responses respectively. This indicates insignificant influence of the time needed to immerse the electrode in GA solution. Therefore, 30 min was used for the rest of the analysies. The following optimum immobilization of *Ab* on the transducer were adopted; the immersion as a way of introducing GA and *Ab* on the transducer and 0.5 hrs incubation in 1 % GA solution.

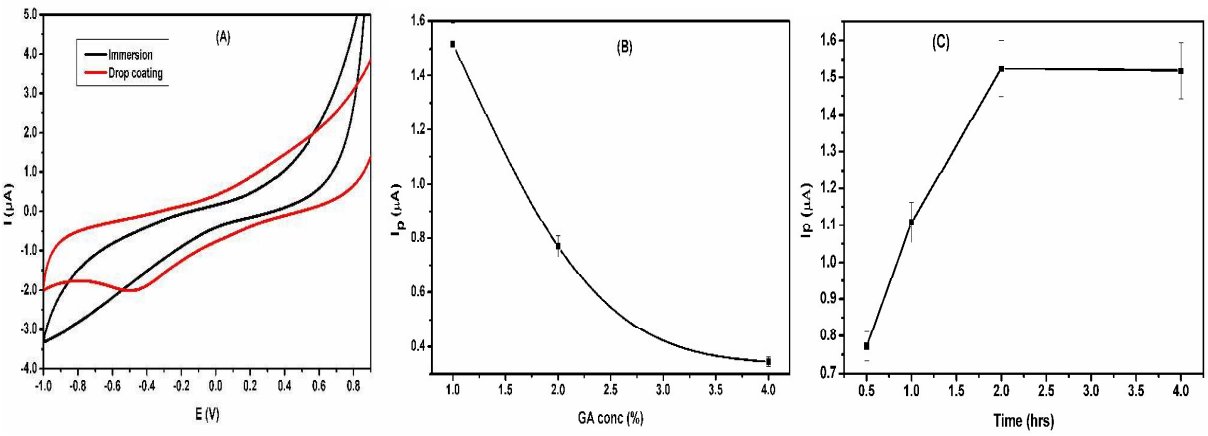

Fig. 4 Effects of various parameters during immunosensor fabrication: CV of GC/PANI/Ag NPs/GA/*Ab* in PBS/ACN (96.6: 0.4 v/v %) at 20 mV/s using different coating methods (A), GA concentration (B) and incubation period for PCB28 (C)

Effect of PCBs incubation time on the *Ab* modified electrode is depicted in Fig. 4 (C). From the figure, the current response increased with time from 0.5 to 2 hrs and then remained constant. This phenomenon could be due to saturation of the *Ab* binding sites with the PCB leaving no more room for more PCB molecules to bind to the *Ab* and be accompanied by increase in peak current. Thus, 2 hrs incubation period was considered optimum for PCB detection with the developed analytical tool.

Immunosensor fabrication

Voltammograms for monitoring the immunosensor fabrication process using optimum conditions are depicted in Fig. 5. These Voltammograms depicted changes with each step in the characteristic emeraldine form of PANI peaks in GC/PANI/Ag NPs which were observed at anodic peak 0.5 V and the cathodic peak at -0.4 V. These peaks intensities were reduced with the introduction of GA (CV for GC/PANI/Ag NPs/GA) and *Ab* (CV for GC/PANI/Ag NPs/GA/*Ab*) as shown in Fig. 5. This decrease in the original PANI peaks intensities is due to effective interactions between GA and PANI resulting in GA-PANI complex that has different electrochemical properties. The disappearance of PANI peaks does not necessarily imply loss of the PANI conductivity because there is still a flow of charge due permeable flow of ions. This behaviour was explained by de Melo et.al.[37] Wher they indicated that in near neutral medium, GA reduces PANI, but that has little effect on the loss of permeability of PANI for ions. GA rather, activates the transducer thereby rendering it heterofunctional for anion exchange. The disappearance of peaks with further electrode modification with *Ab* denotes the successful immobilization of *Ab* and good compatibility of the biomolecule with the transducer. These have reportedly be made possible by high rapid reaction of GA through its carbonyl functional groups with an amine groups of PANI in the transducer and those of *Ab*[37,38] respectively and the high stability of the resultant bonds between GA and amine groups.[36] The CV voltammograms of each electrode modification step during the immunosensor construction confirm the proposed mechanisms scheme in Fig. 1.

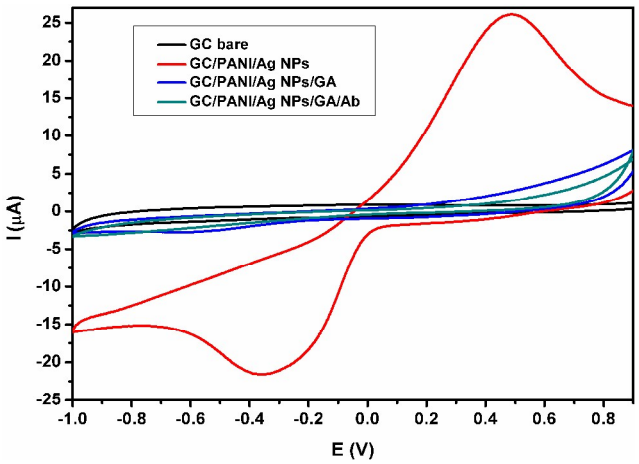

Fig. 5 Stepwise immobilization CV responses in PBS/ACN (99.6:0.4 v/v %)

Optimisation of sweep potential, scan rate and detection method

The electrodes potential was screened from -4 to 1.4 V in PBS/ACN solution. Within -4 to -1.5 V there was noise, while at the range of -1 to 1.4 V, distorted and shifted PANI peaks prevailed (first anodic and cathodic peaks) while between -1.5 and -1 V, PANI gets detached from the electrode surface as disgruntled layers falling into the solution. The suitable range therefore was taken as -1 to 1 V. This was then used for the rest of the analyses. The scan rate of 20 mV/s was also found suitable for the assay. The optimum voltammogram was assessed using CV, DPV and SWV (not shown) techniques. CV voltammograms showed very low-intense broad peaks at around -0.20 V with negligible current response changes when the concentration is increased. While DPV voltammograms had

intense current responses which increased with and increase in concentration. The peak positions were not representatively reliable and could lead to false interpretations. Contrarily, SWV showed a well resolved stable peak at around 0.0 V that increased with an increase in PCB concentration. Due to these promising results, SWV detection technique was used throughout the study.

Immunological detection and calibration studies of PCB 28

The voltammograms (Fig. 6 (A)) show the peaks due to various PCB concentrations at around 0.020 V for the lower concentration, which shifts to a lower potential with increasing concentration. Peak current increases with increasing PCB concentration, suggesting that the response in current was as a result of PCB detection by the immunosensor. A calibration graph (Fig. 6 (B)) shows linear relationship between the peak current and the concentration in the range 0.2 - 10 ng/m. The linear regression equation Y (nA) = 0.0291X + 0.160, with a linear correlation coefficient (R^2) of 0.968, and the detection limit (LOD of 0.063 ng/mL was obtained. The limit of quantitation (LOQ) was determined to be 0.209 ng/mL. These values are dependent on signal to noise ratio which is due to the electrolyte purity. Recently, PCBs were determined at levels of 0.150 ng/mL in South Africa.[24] These levels are normally higher in countries where PCBs were manufactured or found wide application such as 0.250 ng/mL in France.[21] Based on the analytical parameters exhibited by the proposed electrochemical immunosensor, it could be a good analytical tool for detection of PCB 28 .

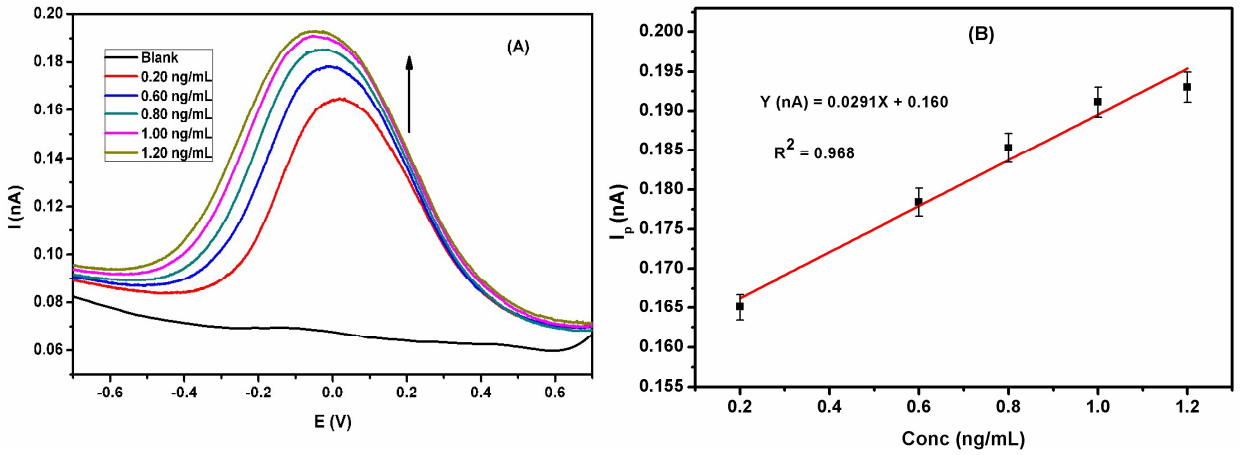

Fig. 6 Immunosensor square wave voltammograms with PCB increasing concentration (A), calibration graph (B)

Reproducibility, specificity/cross-reactivity

Reproducibility was evaluated by calculation of RSD for the intra- and inter- assay measurements for 1 µg/mL PCB 28. The values were determined as 0.933 and 3.12 % respectively for intra and inter assays. These represent acceptable repeatability and reliability exhibited by the developed analytical immunosensor.

BnCl and PCB 180 were used to test the antibody specificity toward PCB 28 and the possible cross-reactivity. Both chemicals showed a signal increase that increased with their increasing amounts (Table 1). BnCl had less effect compared to PCB 180. This results strongly suggests cross reaction of these compounds with Some OF The Ab binding sites. Thus indicating lack of specificity of *Ab* toward PCB 28. This is possibility encouraged by the structural similarity between the PCB and BCl as they both bear the phenyl and chloride groups. In essence, the acceptable cross-reactivity level is 5%,[39] and the high level of cross-reactivity of the *Ab* tested against PCB 180 implies that the designed immunosensor measures the total PCB. This suggests that the immunosensor can generally be applicable for measaurements of total PCB family which a common required determination. The RSD ranges between 2.4 to 6%, which is within aceptable error limits. The effect of matrix interference was tested using the Na and K salts of chloride and phosphate respectively. The % interference (Table 1) ranged between 0.6 and 3.5 and was below the highest acceptable value (5 %). Therefore, it is reasonable to believe that NaCl and KH_2PO_4 have no (or have insignificant) effect on the analysis of PCB with RSD ranges between 2 to 5 %.

Table 1 Cross-reactivity and specificty studies at different ratios of PCB 28 and interent (n = 3)

PCB 28: Int	Interferents							
	BnCl		PCB 180		NaCl		KH_2PO_4	
	I (nA)	% CR	I (nA)	% CR	I (nA)	% Int.	I (nA)	% Int.
alone	0.19± 0.01	n/a	0.18 ± 0.01	n/a	0.17 ± 0.003	n/a	0.172 ± 0.003	n/a
1:5	0.20 ± 0.01	4.19 ± 0.12	1.04 ± 0.03	474.59 ± 12.78	-	-	-	-
1:10	0.21 ± 0.01	8.38 ± 0.20	1.57 ± 0.04	764.64 ± 25.19	0.18 ± 0.004	2.33 ± 0.08	0.173 ± 0.003	0.58 ± 0.03
1:20	0.23 ± 0.01	22.0 ± 1.01	2.50 ± 0.15	1281.22 ± 46.46	0.17 ± 0.004	1.16 ± 0.03	0.178 ± 0.009	3.49 ± 0.08

CR - cross reactivity, Int – interferent, I - current response

Validation and comparison with literature

The developed method was successfully validated through spiking and recoveries of the PCB 28 into tap water and guava juice. Table 2 shows the % recoveries which range from 90 to 102 %. This values are within the acceptable spike and recovery range of 80-120 %.[39] This validates the applicability and accuracy of the developed immunosensor for measurement and monitoring of PCBs. RSD ranges between 3 to 5.4%. From Table 3, the developed immunosensor analytical parameters are comparable to literature. This technique is valuable in analysis of total PCBs and it is less affected by high concentration of salts which are commonly found in the enviroment.

Table 2 Recovery studies

Analyte	I (nA)	% recovery
PCB 28 alone	0.17± 0.01	n/a
PCB 28 in tap water	0.16 ± 0.01	90.10 ± 3.10
PCB 28 in juice	0.18 ± 0.01	102.3 ± 3.16

Table 3 Present work comparison with previously reported electrochemical PCB immunosensors

Immunosensor	Amplification	Method	Analyte PCB/Aroclor	Matrix	LOD	Linear range	Reference
GC/Ppy	-	Pulsed amperometry	1016, 1242, 1248, 1254	Water	1.66, 3.3, 1.56 0.39 ng/ mL	0.3-100 ng/mL	1
SPCE	HRP	Chrono amperometry	15, 1242, 1248	-	0.2 µg/mL	0.0-10 µg/mL	5
SPCE	AP	DPV	28, 101, 118	Food	-	0.01-50 ppm	23
SPCE/MBs	AP	DPV	1242, 1248, 1016	Marine sediments	0.3, 0.4 and 0.8 µg/L	-	40
GC/PANI-Ag NPs	-	SWV	28	Water, juice	0.269 ng/mL	0.2-1.2 ng/mL	Present work

SPCE-screen printed carbon electrode , HRP-horseradish peroxidase , AP-alkaline phosphatase , MBs- magnetic beads

Conclusions

An electrochemical immunosensor as a unique method for PCBs analysis was developed. This was successfully achieved through immobilization of *Ab* on the GC/PANI/ Ag NPs using GA cross-linker. The development protocol demonstrated a good biocompatibility of the transducer with *Ab*. From the construction optimization it is clear that the efficiency of this immunosensor depends on activation method, incubation time at each step and the concentrations of the *Ab* and cross-linker. However, construction time can be taken care of by use of printed electrodes. The method was tested against PCB 28 and exhibited good analytical parameters with limit of detection (LOD) and limit of quantitation (LOQ) being 0.063 ng/mL and 0.206 ng/mL respectively. The linear range was within 0.2 - 1.2 ng/mL. A good repeatability from the intra and inter assay measurements with RSDs of 0.933 and 3.12% were achieved. Good specificity was demonstrated with interferences less than the highest accepted level of 5% for compounds other

than BnCl and PCB 180. Whose data indicated that the immunosensor has a potential for detection and monitoring of the entire PCB family as it measures total PCB. In most cases total PCB measurements are necessary, therefore a wide applicability of this immunosensor in environmental analysis and food is possible.

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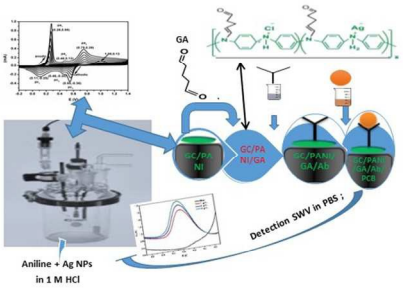
References

1 Bender and O.A. Sadik, *Environ Sci Technol.*, 1998, **32**, 788
2 D.W. Bench, *WM'99 CONFERENCE*, Tucson, Arizona, USA. 1999.
3 J. Kucklick, L. Schwacke, R. Wells, A. Hohn, A. Guichard, J. Yordy, L. Hansen, E. Zolman, R. Wilson, J. Litz, D. Nowacek, T. Rowles, R. Pugh, B. Balmer, C. Sinclair and P. Rosel, *Environ Sci Technol.*, 2011 **45**, 4270.
4 J. Ferrario, C. Byrne and A.E.J. Dupuy, *Chemosphere*, 1997, **34**, 2451.
5 S. Laschi, M. Franek and M. Mascini, *Electroanalysis*, 2000, **12**, 1293.
6 S. Laschi and M. Mascini, *Ann Chim.*, 2002, **92**,425.
7 M.P. Kreuzer, M. Pravda, C.K. O'Sullivan and G.G. Guilbault, *Toxicon*, 2002, **40**, 1267.
8 R. Draisci, G. Volpe, L. Lucentini, A. Cecilia, R. Federico and G. Palleschi, *Food Chem.*, 1998, **62**, 225.
9 D. Moscone, R.A. Bernardo, E. Marconi, A. Amine and G. Palleschi, *Analyst*, 1999, **124**, 325.
10 G. Panfili, P. Manzi, D. Compagnone, L. Scarciglia and G. Palleschi, *J Agric Food Chem.*, 2000, **48**, 3403.
11 B. Hock, *Anal Chim Acta.*, 1997, **347**, 177.
12 N. Bojorge Ramírez, A.M. Salgado and B. Valdman, *Brazilian J Chem Eng.*, 2009, **26**, 227.
13 M. Del Carlo, I. Lionti, M. Taccini, A. Cagnini and M. Mascini, *Anal Chim Acta.*, 1997, **342**, 189.
14 B. Piro, S. Reisberg, G. Anquetin, H.T. Duc and M.C. Pham, *Biosensors*, 2013, **3**, 58.
15 X. Luo, A. Morrin, A.J. Killard and M.R. Smyth, *Electroanalysis*, **18**, 2006, 319.
16 J. Ezzati Nazhad Dolatabadi and M. de la Guardia, *Anal Methods*, 2014, **6**, 3891.
17 K.M.M. Abou El-Nour, A. Eftaiha, A. Al-Warthan and R.A.A. Ammar, *Arab J Chem.*, 2010, **3**, 135.
18 S.Y. Park, M.S. Cho and H.J. Choi, *Curr Appl Phys.*, 2004, **4**, 581.
19 S.J. Kwon, M. Seo, H. Yang, S.Y. Kim and J. Kwak, *Bull Korean Chem Soc.*, 2010, **31**, 3103.
20 Y. Date, A. Aota, K. Sasaki, Y. Namiki, N. Matsumoto, Y. Watanabe, O. Naoya and M. Tomokazu, *Anal Chem.*, 2014, **86**, 2989.
21 S. Net, D. Dumoulin, R. El-Osmani, S. Rabodonirina and B, Ouddane, *Int J Environ Res.*, 2014, **8**, 1159.
22 B. Yurdakok, K. Tekin, A. Daskin and A. Filazi, *Reprod Domest Anim.*, 2014, **50**, 41.
23 S. Laschi, M. Mascini, G. Scortichini, M. Franek and M, Mascini, *J Agric Food Chem.*, 2003, **52**, 1816.
24 R. Amdany, L. Chimuka, E. Cukrowska, P. Kukučka, J. Kohoutek and B. Vrana, *Water SA*, 2014, **40**, 425.
25 J. Lv, Y. Zhang, X. Zhao, C. Zhou, C. Guo, Y. Luo, W. Meng, G. Zou and J. Xu, *Environ Sci Pollut Res Int.*, 2015, **22**, 4256.
26 R. Dulbecco and M. Vogt, *J Exp Med.*, 1954, **99**, 167.
27 G. Neelgund, E. Hrehorova, M. Joyce and V. Bliznyuk, *Polym Int.*, 2008, **57,** 1083.
28 Q. Yu, Q. Wang, B. Li, Q. Lin and Y. Duan, *Crit Rev Anal Chem.*, 2014, **45**, 62.
29 F. Lopez-Gallego, L. Betancor, C. Mateo, A. Hidalgo, N. Alonso-morales, G. Dellamora-ortiz, J.M. Guisan and R. Fern, *J Biotechnol.*, 2005, **119**, 70.
30 K. Arora, G. Sumana, V. Saxena, R.K. Gupta, S.K. Gupta, J.V. Yakhmi, M.K. Pandey, S. Chand and B.D. Malhotra, *Anal Chim Acta.*, 2007, **594**, 17.
31 N.S.K. Gunda, M. Singh, L. Norman, K. Kaur and S.K. Mitra, *Appl Surf Sci.*, 2014, **305**, 522.
32 H.J. Kang, E.J. Cha and H.D. Park, *Appl Surf Sci.*, 2015, **324**, 198.
33 L. Betancor, F. Lopez-Gallego, A. Hidalgo, N. Alonso-morales, G.D.C. Mateo, R. Fernandez-Lafuente and J.M. Guisan, *Enzyme Microb Technol.*, 2006, **39**, 877.
34 V.V.R. Sai, S. Mahajan, A.Q. Contractor and S. Mukherji, *Anal Chem.*, 2006, **78**, 8368.
35 G.B. Broun and K. Mosbach, *Methods in Enzymology*, Ed. Academic Press, New York 1976.
36 W.K. Chui and L.S.C. Wan, *J Microencapsul.*, 1997, **14**, 51.
37 J.V. de Melo, M.E. Bello, W.M. de Azevedo, J.M. de Souza and F.B. Diniz, *Electrochim Acta.*, 1999, **44**, 2405.
38 K. Okuda, I. Urabe, Y. Yamada and H. Okada, *J Ferment Bioeng.*, 1991, **71**, 100.
39 B.H. Garcia II, A. Hargrave, A. Morgan, G. Kilmer, E. Hommema, J. Nahrahari, B. Webb and R. Wiese, *J Biomol Tech.*, 2007, **18**, 245.
40 S. Centi, B. Rozum, S. Laschi, I. Palchetti and M. Mascini, *Chem Anal (Warsaw)*, 2006, **51**, 963.



Graphic table of contents

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