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Original Research Article

Aptamer-Based Sensor Using Direct Electrochemical for Sensitive Deltamethrin Detection

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Abstract: A sensitive aptamer sensor based on electrochemical using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques were established for detection of deltamethrin. Compared with conventional sensing practicalities, aptamer has many compensations, such as relaxed modification, higher specificity, stronger affinity and well-made stability. The passive deposition of aptamer on gold electrode (SPGE) offered decent biocompatibility and electrical conductivity on the sensor. Following the addition of deltamethrin to the electrode surface, the aptamer is forced to fold, prompting the formation of a 3D structure to specifically interact with deltamethrin. Redox solution was added to encourage the target binding which significantly enhanced the current change of electrochemical signal. Therefore, the auspicious approach provides a signal current and resistance readout using the DPV and EIS technique of aptasensor. In this work, the developed biosensor showed high sensitivity towards deltamethrin via the signal design and autofold target binding. Under the optimized conditions, the anticipated sensor exhibited a good linear regression with R^2 value of 0.9932 and 0.9543 via DPV and EIS, respectively for calibration curves of 0.0 to 0.6 ppm. A reproducible and sensitive DPV and EIS on SPGE is developed, reaching a limit of detection of 0.07 ppm (n = 3) and 0.17 ppm (n = 3), respectively compared to the maximum residue limit (MRL) for deltamethrin of 2.0 ppm.

Keywords: aptamers; in silico; computational docking; electrochemical; deltamethrin

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1. Introduction

The conventional analytical methods that are used for the detection of pesticides are chromatographic techniques attached with numerous detectors (e.g. LC-MS, GC-MS, GC-ECD, HPLC-UV) (Albanis & Hela, 1995; Thurman *et al*., 2001). These instrumentation-based methods have the advantages of being accurate and automated, with high specificity and can be used for concurrent detection. However, the drawbacks of these method are that they are costly in both instrumentation and maintenance part, laborious and tedious sample pre-treatment that require hazardous organic solvents (Fu *et al*., 2019). Enzyme-linked immunosorbent assay or ELISA (Bronshtein *et al*., 2012), another technique widely used for pesticides detection, also depends on a laboratory platform and requires a relative long assay time and several steps operation. Immunochromatography assay (ICA) and enzymatic/affinity paper-based assay (Shrivas *et al*., 2020), though is very useful as a screening method for pesticides, are often laden by insignificant color changes thus making the quantification difficult (Hossain *et al*., 2009; Kim *et al*., 2018).

With this regard, biosensor technique lends itself well as a potential tool for simple, rapid, sensitive and cost-effective monitoring that allow sample testing to be performed on-site with minimal sample preparation (Reynoso *et al*., 2019; Zamora-Sequeira *et al*., 2019). Until today, biosensor approach in detecting pesticides merely rely on immunosensor and enzyme approach. For instance, immunoassay and amperometric biosensor were employed in the detection of deltamethrin in seawater (Fruhmann *et al*., 2018). Screen-printed acetylcholinesterase-based biosensors for inhibitive determination of permethrin was also reported in 2021 (Domínguez-Renedo *et al*., 2012).

Over the span of 15 years, our research group in MARDI have successfully developed enzyme-based biosensor for the detection of over 20 compounds of pesticides comprising organophosphates, carbamates, dithiocarbamates (Puat *et al*., 2022) and neonicotinoids. However, some of the pesticides are becoming obsolete and became persistent in environment thus were replaced with new class of compounds. On another note, the specificity of an enzyme-based biosensor also disputable and may react with other substance in the sample. At that time, antibody production for synthetic pyrethroids was deemed challenging due to the non-antigenic site available in synthetic pyrethroids structure.

Synthetic pyrethroids are used widely in agriculture, forestry, domestic, veterinary and public health applications (Farina *et al*., 2018; Pitzer *et al*., 2021). The high insecticidal activity and low toxicity to mammals exhibited by synthetic pyrethroids has led to their

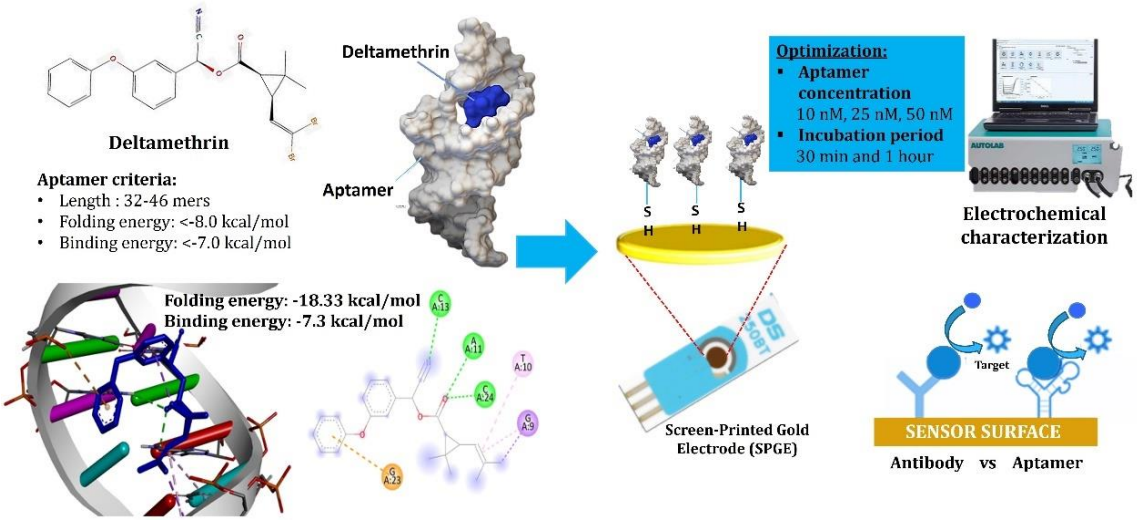
increased use over other classes of insecticides. There have been reports of reversible poisoning symptoms and suppressive effects on the immune system after exposure to pyrethroids even though they were initially considered safe for humans (Chrustek *et al*., 2018). Some pyrethroids can cause splenic and lymph node damage, even worse they are carcinogenic (Mak *et al*., 2005; Oudou *et al*., 2001). Pyrethroids bring tremendous compensations to agricultural ecosystem if used reasonably, however still pose a risk for environmental destruction (Maulidiyah *et al*., 2017). A sensitive, selective and rapid method for monitoring residue levels of pyrethroids in agricultural ecosystems is therefore required.

Aptamer, a new tool in biosensor recognition molecule, has been regarded as next generation for smart biosensing (Ruscito & DeRosa, 2016; Yoo *et al*., 2020). Aptamer has the ability to imitate antibody structure (Scheller *et al*., 2013) without the need to perform tedious in vivo immunization in small animals that may require animal ethic consideration (Bauer *et al*., 2019). Until today, no aptamer has been designed for synthetic pyrethroids detection. As reviewed, the only direct electrochemical and photoelectrochemical aptasensor that has been reported were for the detection of acetamiprid, chlorpyrifos and malathion (Li *et al*., 2019).

Herein, we first design and synthesis the aptamer via in silico approach. During this first step, the binding energy and length of aptamer produced will be studied. Next, we investigate the direct detection between the synthesized aptamer and for synthetic pyrethroids via electrochemical measurements namely differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) on screen printed gold electrode (SPGE) (Schematic 1). Both of these techniques are able to probe detection at the molecular level, do not require labels and thus offer direct detection for target analytes. Nowadays, EIS is a powerful tool in electroanalysis because it offers information about its structure, the electrode interface and the response, i.e. the modulation of the blocking potential on the sensor surface. Theoretically, the binding between aptamer and its target analyte will result in higher binding force and stronger charge that will affect surface resistance for the redox-active substance.

Based on expertise developed from past experience in electrochemical biosensor (using antibody and enzyme as bioreceptors), this aptamer-based biosensor will be a new study area and expertise development for our group to venture and embark on. This study will bring up a new interest area for bio-sensing at molecular level in biosensor technology. In addition, this finding may become a platform and new knowledge in establishing the

detection of synthetic pyrethroid in agricultural products including for food safety and environment monitoring.



Schematic 1. Illustration of the steps of aptamer design and synthesis via an in silico approach and aptamer immobilization on a screen-printed gold electrode (SPGE). Electrochemical study for aptamer and its target pesticide (synthetic pyrethroids) on a modified gold electrode (modified SPGE). The electroanalytical techniques chosen in this study are EIS and DPV.

2. Materials and Methods

2.1 Materials and Reagents

An APT fold buffer and Dropsens ceramic based screen printed gold electrode (SPGE) (250BT) were provided by Biogenes Technologies, Malaysia. Standard synthetic pyrethroid, deltamethrin (MW 505.20), permethrin, cypermethrin, and fenvalerate (Sigma-Aldrich) were purchased from Synergy Scientific, Malaysia. Ammonium persulfate (APS), potassium phosphate monobasic (KH_2PO4), disodium hydrogen phosphate (Na_2HPO_4), ethanol, potassium chloride (KCl) and sodium chloride (NaCl) were from Sigma-Aldrich, Japan. All of the chemicals were analytical standard grade and were used without further purification. All solutions were prepared with ultrapure water (Arium Comfort I system, Sartorius) with a resistivity of 18.2 MΩcm. Synthetic aptamer was supplied by Apical Scientific (Malaysia). Aptamer stock solution was prepared in the nuclease free water meanwhile the dilution was prepared in APT fold buffer that has been purchased from Biogenes Sdn. Bhd. Then the aptamer was heated at 90℃ for 5 minutes using the Thermo Scientific thermo shaker and heat block instrument. After that, the aptamer needs to cool down to the room temperature. The purpose of heating is to unfold the aptamer before used

as a biological element in biosensor development. The sequence of the aptamer used is as follows 5'-GCGCATCCGTAGCGC GCGTCGCGCTATCCCGGATGCGCG-3'.

2.2 Synthetic Pyrethroid (Deltamethrin) Structure and Ligand Preparation

The active insecticide of the proinsecticide tralomethrin is deltamethrin. It has a function as an agrochemical, a pyrethroid ester insecticide, an antifeedant, a calcium channel agonist and an EC 3.1.3.16 (phosphoprotein phosphatase) inhibitor. The 3D compound structures were retrieved from PubChem database (Figure 1). These structures were undergone an energy minimization process using OpenBabel software prior to docking.

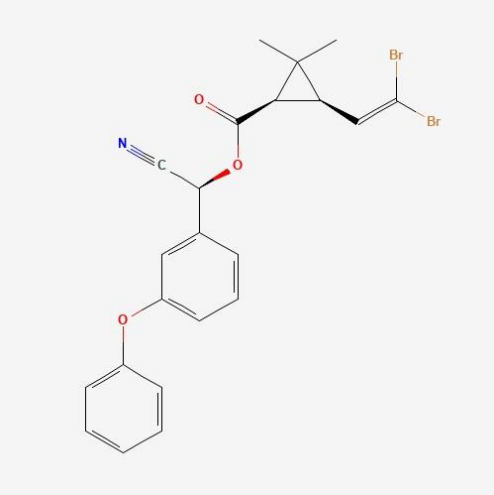


Figure 1. 2D structure of deltamethrin (CID: 40585) (Source: PubChem).

2.3 In Silico Aptamer Design and Receptor Preparation

The aptamer was designed based on the tRNA sequences retrieved from Genomic tRNA database (GtRNAdb, gtrnadb.ucsc.edu). The tRNAs with hairpins or stem loops which possessing folding capability were selected. Folding of the tRNAs was carried out through UNAfold (http://www.unafold.org/) (Zuker, 2010) and folding energy was measured. The RNAs were then converted to DNAs and the 3D DNA structures were formed. The aptamer was set to non-rotatable bonds using Autodock Tool.

2.4 Molecular Docking Analysis and Mechanism of Action of the Synthetic Pyrethroids With the Aptamers

Docking simulation was performed using Autodock Vina version 2.5.4. Grid box was set to cover the entire surface of the protein. To improve accuracy, the grid box size was then reduced to target the binding site with an x-, y- and z- sizes of 22 × 16 × 22 and centered at coordinates of x = 2.466, y = -26.519 and z = -3.198. Exhaustiveness was set at 8. The

interaction of docked complexes was visualized using PyMOL v2.5.4 (Schrodinger, LLC) and Biovia Discovery Studio Visualizer v21.1.0.20298 (BIOVIA, San Diego, CA, USA). Selection is based on the docked complex with the lowest binding energy (kcal/mol). The selected aptamer was then synthesized for use in electrochemical analysis.

2.5 Electrochemical Set-up and Measurement

Sensor characterization and analysis of the modified electrodes were conducted using an Autolab PGSTAT 20 potentiostat (Eco Chemie, Netherlands). The cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) method were used to investigate the electron transfer capability of the sensor. The CV tests was performed in a redox solution of 1 mM ferrocene carboxylic acid (FCA) in PBS 0.01 M, pH 7.4 with scan rate of 10 mV/s and potential range from -0.2 to +0.6 V. The DPV was tested under pulse amplitude of 0.025 V with the pulse interval of 1.0 s and scan potential from -0.2 to +0.6 V. The same solution was used for EIS at a formal potential of open circuit voltage in alternating current (AC) amplitude of 5 mV using a frequency range of 0.1 to 100 kHz. All measurements were executed at room temperature in 5.0 mM ferricyanide/ferrocyanide redox solution containing 5.0 mM $K_3[Fe(CN)_6]$ and 5.0 mM $K_4[Fe(CN)_6]$.

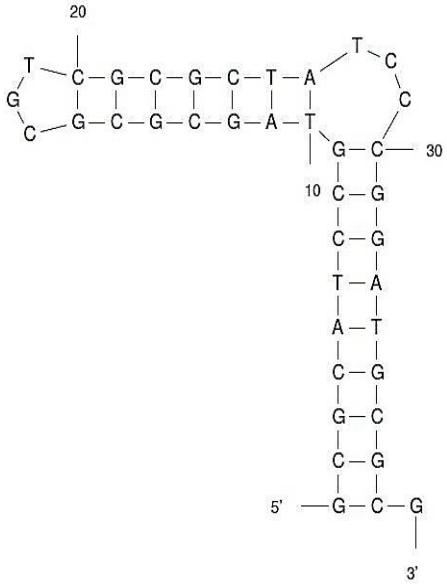
3. Results and Discussions

3.1 In Silico Design and Customized Synthesis of Desalted Oligo-Based Aptamer on Target Molecule

The in silico method is used to design aptamers because it is less tedious and less rigorous than the conventional method, SELEX (Navien *et al*., 2021). The in silico method offers details of aptamer-ligand interactions that are difficult to explain experimentally. Thus, aptamers can be designed more flexibly and quickly in a virtual environment (Buglak *et al*., 2020). The data of target molecule structure were obtained from PubChem. Among the features that was optimized in the aptamer design are secondary structure, hairpins/stem-loops, folding capability and 3D structure stability (Table 1).

Table 1. Data of in silico design for aptamer.

	Deltamethrin
Aptamer Sequence	5’-GCGCA TCCGT AGCGC GCGTC GCGCT ATCCC GGATG CGCG-3’
Aptamer Length	39 mers
Folding Energy	-18.33 kcal/mol

Binding Energy	-7.3 kcal/mol
2D Structure	

3.2 Molecular Docking Analysis and Mechanism of Action of the Aptamer and Deltamethrin

Computational techniques, molecular docking are used to predict ligand to receptor protein binding affinity (Agu *et al*., 2023). Molecular docking analysis revealed that the aptamer was able to interact with deltamethrin with a binding energy of -7.3 kcal/mol. Three hydrogen bonds were formed between deltamethrin and nucleotide C13, C24 and A11 of the aptamer with a distance of 2.61 Å, 3.07 Å and 2.37 Å, respectively (Figure 2). This interaction was strengthened with the formation of two hydrophobic interactions with G9 and T10 of the aptamer.

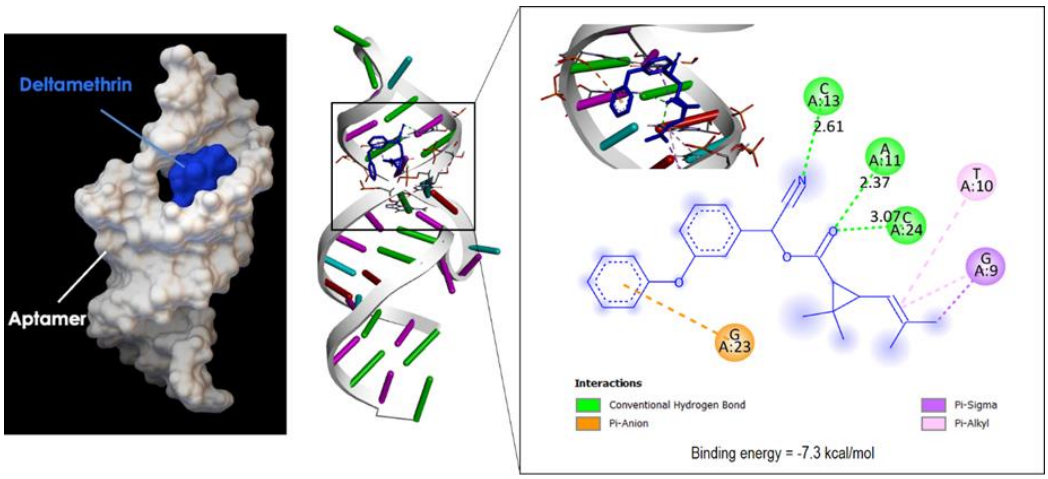


Figure 2. The 3D and 2D interactions of deltamethrin with aptamer. The interactions were enlarged in box where the hydrogen bonds and hydrophobic interactions were shown in green and pink dashed lines, respectively.

3.3 Electrochemical Characterisation and Optimization of SPGE/DM Bioelectrode

The preparation of the synthesized aptamer and its characterisation has been observed by cyclic voltammetry (CV), different pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) respectively. A Dropsens ceramic based screen printed gold electrode (SPGE) (250BT) (Biogenes Technologies) was used as a transducer where the 10 nM, 25 nM and 50 nM of aptamer solution were immobilized on the surface of working electrode via physical adsorption technique for 0.5 hour and 1 hour of incubation time, respectively. These immobilized deltamethrin aptamer (DM) was characterized and compared with the bare SPGE. All electrochemical studies were performed in redox solution containing 5.0 mM $K_3[Fe(CN)_6]$ and 5.0 mM $K_4[Fe(CN)_6]$. Figure 3 exhibits the cyclic voltammograms of the bare electrode, immobilized SPGE with 10 nM, 25 nM and 50 nM deltamethrin aptamer.

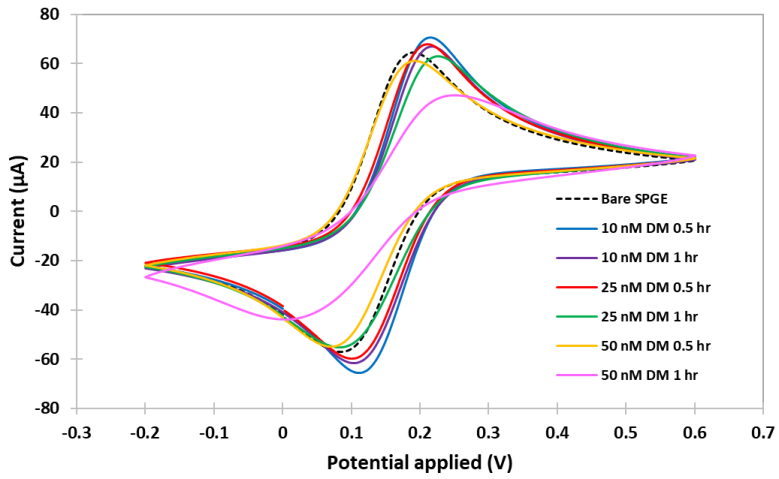


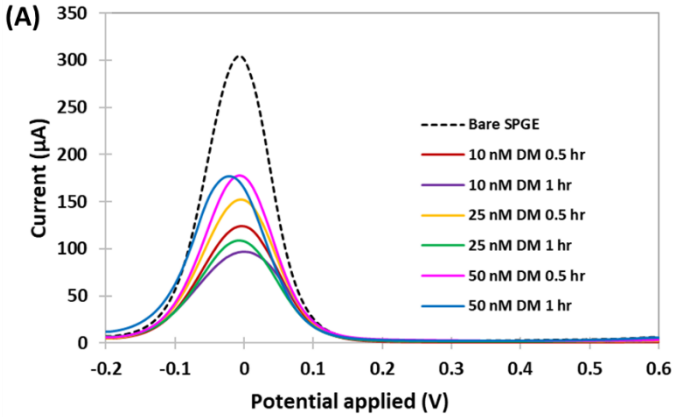
Figure 3. Cyclic voltammograms of bare SPGE, immobilized 10 nM, 25 nM and 50 nM deltamethrin aptamer at 0.5 hour and 1 hour in a range of potential of -0.2 V to +0.6 V.

A pair of redox peaks were observed for each condition with different peak position, *E* (V) values as shown in Table 2. Table 2 showed the oxidation peak (E_{pa}) and the reduction peak (E_{pc}) of immobilized aptamer for deltamethrin (DM) was slightly shifted into the right compared to the oxidation and reduction peak of bare SPGE. The $E_{1/2}$ (V) was calculated as the sum of E_{pa} (anodic peak potential, V) and E_{pc} (cathodic peak potential, V) then divided by two. The calculated $E_{1/2}$ is then used as a set potential for other electrochemical analysis (electrochemical impedance spectroscopy, EIS).

Table 2. The comparison of oxidation and reduction peak of bare and modified SPGEs.

	Oxidation E_{pa}**, V**	***Reduction*** E_{pc}**, V**	$E_{½}$**, V =(**E_{pa}**+**E_{pc}**)/2**
Bare SPGE	0.1928	0.0903	0.14155
10 nM DM, 0.5 hr	0.2148	0.1123	0.16355
10 nM DM, 1 hr	0.2172	0.2148	0.216
25 nM DM, 0.5 hr	0.2124	0.1025	0.15745
25 nM DM, 1 hr	0.2148	0.0976	0.1562
50 nM DM, 0.5 hr	0.1928	0.0708	0.1318
50 nM DM, 1 hr	0.0048	0.0415	0.02315

Figure 4(A) shows the voltammograms of the bare SPGE, immobilized SPGE with 10 nM, 25 nM and 50 nM deltamethrin (DM) aptamer as results of electrochemical analysis by using different pulse voltammetry (DPV) techniques. From the graph, it shows the peak of gold electrode that has been immobilized with deltamethrin (DM) aptamer were slightly shifted to the right compared to the bare gold electrode. Meanwhile, the different value of peak height (current, *I*) was observed for each condition as shown in Table 3. From the voltammogram, it shows that at the aptamer concentration and incubation time of 50 nM; 0.5 hour was optimal conditions for deltamethrin aptamer. The bar graph in Figure 4(B) also proves that the most optimal conditions of electrochemical analysis using the DPV technique for deltamethrin aptamer on gold electrodes are at a concentration and incubation time of 50 nM and 0.5 hour.



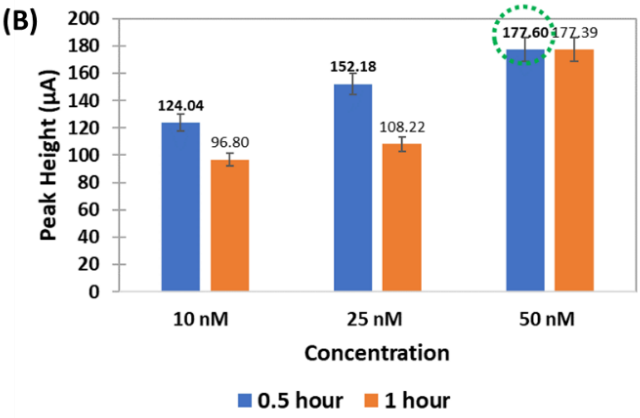


Figure 4. The voltammograms (A) and bar graph (B) of bare SPGE, 10 nM, 25 nM and 50 nM immobilized deltamethrin aptamer that were measured electrochemically by using different pulse voltammetry (DPV) technique.

Table 3. The comparison of peak height and peak position for bare and modified SPGEs.

	Peak height, *I* (µA)	**Peak Position, *E* (V)**
Bare SPGE	304.73	-0.01526
10 nM DM, 0.5 hr	124.04	-0.00565
10 nM DM, 1 hr	96.80	0.000305
25 nM DM, 0.5 hr	152.18	-0.00366
25 nM DM, 1 hr	108.22	-0.00366
50 nM DM, 0.5 hr	177.60	-0.01526
50 nM DM, 1 hr	177.39	-0.02151

The semicircular and linear parts make up the EIS spectrum. The electron transfer resistance at higher frequencies was represented by the semicircular diameter and the diffusion process at lower frequencies was represented by the linear section. Figure 5(A) shows the Nyquist Plots of bare SPGE and immobilized SPGE with 10 nM, 25 nM and 50 nM deltamethrin (DM) aptamer as results of electrochemical analysis by using electrochemical impedance spectroscopy (EIS) technique. From the graph, it shows the different value of R_s values (solution resistance) and R_{CT} values (charge transfer resistance) as shown in Table 4. Figure 5A shows the semicircle of the bare gold electrode was smaller because the unmodified surface has no resistance therefore allowing the redox solution to reach the surface freely. However, when immobilized with 10 nM, 25 nM and 50 nM deltamethrin (DM) aptamer, the semicircles are increasing indicating the increment of

resistance barrier on the gold surface due to the existence of the adsorbed aptamers. The semicircle of 50 nM concentration and 0.5 hour incubation time of deltamethrin aptamer on gold electrode was the largest compared to other conditions. This indicates that it is the most optimal condition where the adsorbed aptamer provides the best resistance barrier on the gold surface compared to other conditions. The bar graph in Figure 5(B) also proves that the most optimal conditions of electrochemical analysis using the EIS technique for deltamethrin aptamer on gold electrodes are at a concentration and incubation time of 50 nM and 0.5 hour, respectively.

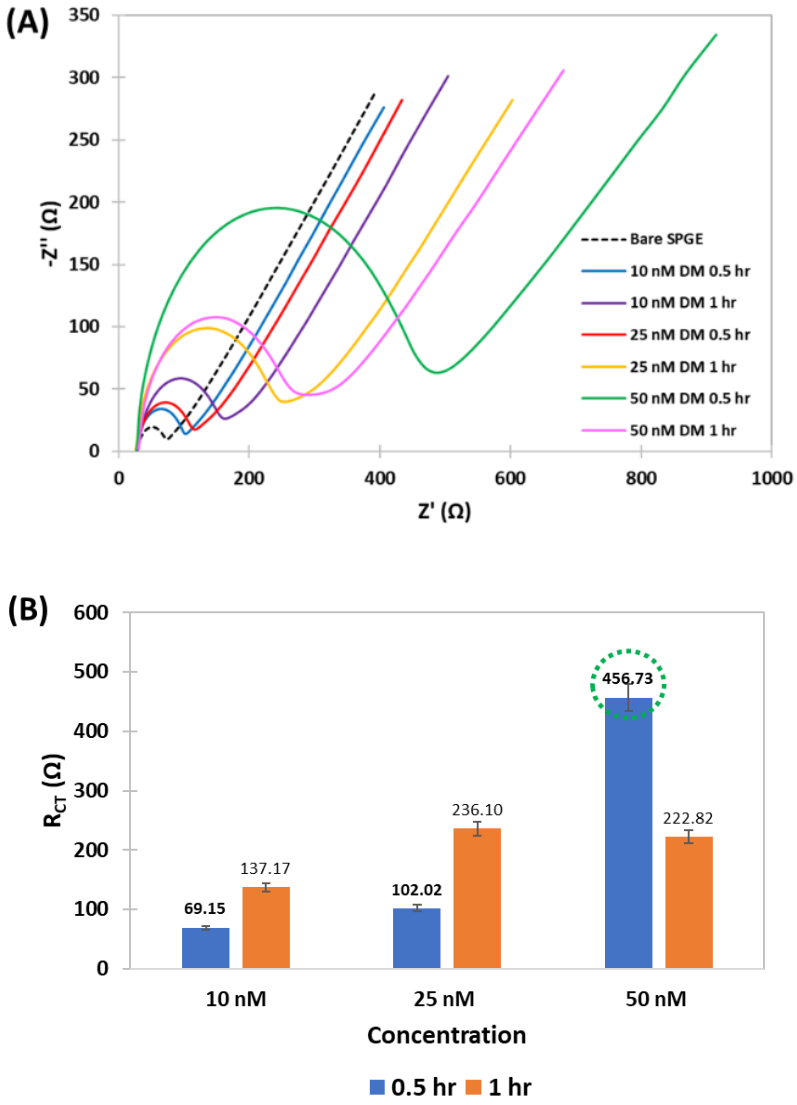


Figure 5. The Nyquist plots (A) and bar graph (B) of immobilized SPGEs with 10 nM, 25 nM and 50 nM deltamethrin aptamer in 5.0 mM $K_3[Fe(CN)_6]$ and 5.0 mM $K_4[Fe(CN)_6]$.

Table 4. The comparison of solution resistance and charge transfer resistance for bare and modified SPGEs.

	R_S (Ω)	R_{CT} (Ω)
Bare SPGE	23.1093	46.2186
10 nM DM, 0.5 hr	34.5759	69.1519
10 nM DM, 1 hr	68.5861	137.1722
25 nM DM, 0.5 hr	51.0108	102.0215
25 nM DM, 1 hr	118.0477	236.0953
50 nM DM, 0.5 hr	228.3651	456.7303
50 nM DM, 1 hr	111.4115	222.8231

3.4 Electrochemical Performance of SPGE/DM Bioelectrode

To evaluate the performance of this electrochemical sensor, a series of DPV analyses were carried out at different standard deltamethrin concentrations under optimal experimental environments. Figure 6 shows the voltammograms of the bare SPGE and immobilized SPGE with 50 nM deltamethrin (DM) aptamer by increasing the standard deltamethrin concentration in the range of 0.0 to 0.6 ppm using different pulse voltammetry (DPV) technique. Reduction of the current signal due to saturation occurred between the immobilized aptamer and serial dilution of standard deltamethrin towards a fixed amount of aptamer binding sites (50 nM). Electrochemical detection was performed using the DPV technique and the signal was analysed based on the peak height signal. As shown in Figure 6, the current decreased with the increase concentration of standard deltamethrin in the analyte solution. This is due to the aptamer immobilized on the gold electrode surface being completely occupied by the additional target standard deltamethrin, which then increases the blocking of electron transfer to the electrode surface. Therefore, with increasing of analyte concentration, a lower change in the electron transfer response of the peak height was obtained.

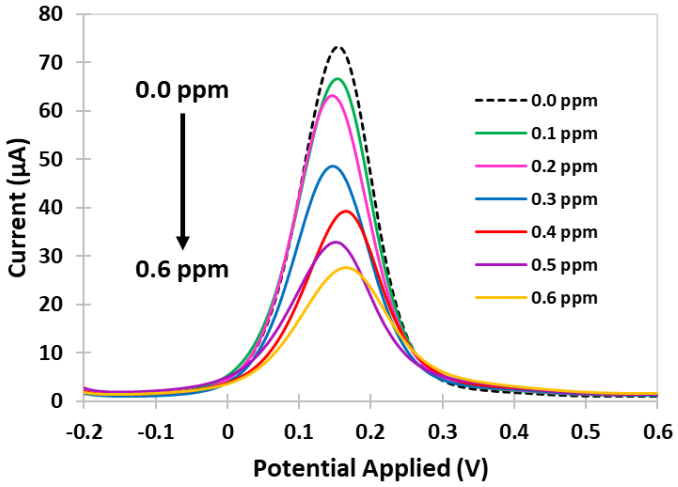


Figure 6. The voltammograms of bare SPGE and 50 nM immobilized deltamethrin (DM) aptamer that were measured electrochemically by using different pulse voltammetry (DPV) technique.

The linear relationship of the electrochemical response between the immobilized aptamer and standard deltamethrin was shown in Figure 7. The regression equation is as follow: y = -79.609x + 73.244, R^2 = 0.9932 which magnitude of the current response decreases linearly with increasing of standard deltamethrin concentration. According to three point three times of standard error, 3.3SE/b (n = 3), the SPGE/DM bioelectrode shows low detection limit, 0.07 ppm (~0.07 mg/L) with linear range of 0.1 ppm to 0.6 ppm for standard deltamethrin concentration. The result indicate that the developed SPGE/DM sensor was comparable to the sensor reported by Zhu *et al*. (2022) with an acceptable electrochemical performance.

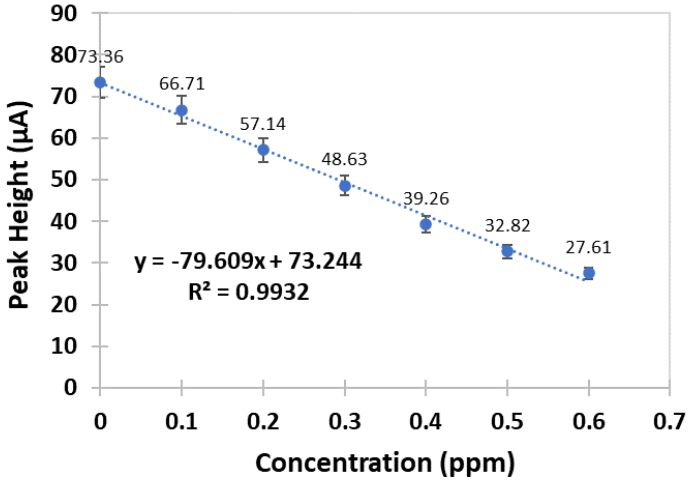


Figure 7. The linear regression of deltamethrin aptamer with linear range of 0.0 ppm to 0.6 ppm for standard deltamethrin concentration by differential pulse voltammetry (DPV) technique.

Figure 8 shows the Nyquist Plots of immobilized SPGE with 50 nM deltamethrin aptamer as results of electrochemical analysis by using electrochemical impedance spectroscopy (EIS) technique. The semicircle of 0.1 ppm was smaller because as the unmodified surface has lower resistance that allows the redox solution to reach the surface. However, when the added target concentration of standard deltamethrin was increased, the semicircles was increased indicating the increment of resistance barrier on the gold surface due to the increase of the binding between target and immobilized aptamer which implies an increasing in electron-transfer resistance of the redox probe and provides a guarantee for subsequent electrochemical detection (Xu *et al*., 2020).

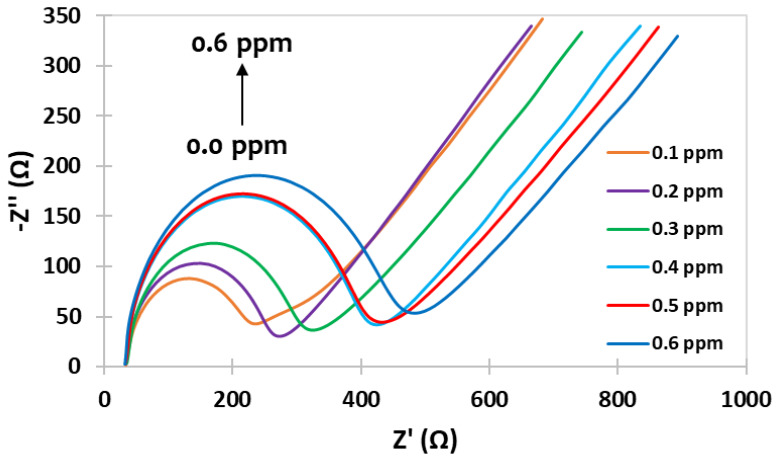


Figure 8. The Nyquist plots of immobilized SPGEs with 50 nM deltamethrin (DM) aptamer in standard concentration range of 0.0 ppm to 0.6 ppm.

Good linear regressions of deltamethrin aptasensor were achieved with $y = 579.5x + 91.586$, R^2 value of 0.9543 via electrochemical impedance spectroscopy (EIS) technique for standard curves of 0.0–0.6 ppm (Figure 9). A reproducible and sensitive EIS on modified gold electrode is developed, reaching a limit of detection (LOD) of 0.17 ppm (n = 3) compared to the maximum residue limit (MRL) for deltamethrin of 2.0 ppm. The LOD of the method were considerably lower than the maximum residue limits (MRL) established by the Codex Alimentarius International Food Standards.

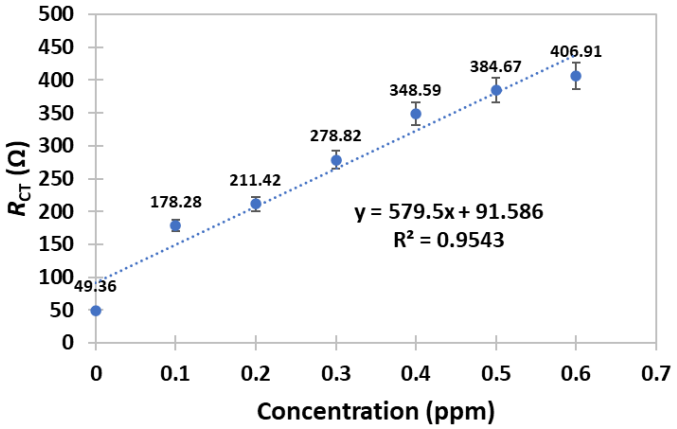


Figure 9. The linear regression of deltamethrin aptamer with linear range of 0.0 ppm to 0.6 ppm for standard deltamethrin concentration by electrochemical impedance spectroscopy (EIS) technique.

Therefore, the proposed sensor showed a lower detection limit compared to other studies that also used electrochemical sensors to detect pesticide. These parameters were also compared with those obtained for other types of sensors, such as fluorescence quenching and chemiluminescence, as shown in Table 5. The results showed that the electrochemical aptasensor in this study has a low LOD and a wide detection linear range, demonstrating that this study has a certain meaning and was possible. This study differs from the study by Ribeiro *et al*. (2022), in terms of detection mode. This study used a direct approach to detect deltamethrin while they used an inhibitory approach. The advantage of this approach is that it is directly targeted towards the deltamethrin compound while in the inhibitory approach it involves competitive binding for the same active site.

Table 5. Comparison of proposed sensor with other types of biosensors for pesticide detection.

Method	Pesticide	Linear Range (µM)	LOD (µM)	Reference
Electrochemistry	Propamocarb	1.0–5.0	0.6	(Taşaltın *et al*., 2022)
Fluorescence	Deltamethrin	0.01–5.0	0.006	(Bhamore *et al*., 2019)
Chemiluminescence	Deltamethrin	0.6–19	0.2	(Yahyai *et al*., 2021)
Electrochemistry	Cypermethrin	0.2–2.4	0.2	(Nurdin *et al*., 2019)
Electrochemistry	Deltamethrin	0.01–0.1	0.02	(Ribeiro *et al*., 2022)
Electrochemistry	Endosulfan	32.3–77.6	6.8	(Masibi *et al*., 2021)
Electrochemistry	Deltamethrin	0.0–0.6	0.17	This study

3.5 Cross Reactivity Study

Structurally related compounds such as permethrin (PM), cypermethrin (CM) and fenvalerate (FV) were tested to evaluate the specificity of the deltamethrin (DM) aptamer.

Cross reactivity study of 50 nM DM aptamer shows that the highest magnitudes of response of SPGE/DM was towards 0.2 ppm of standard deltamethrin with 100% cross reactivity percentage (Figure 10). The magnitude of response is found to decrease on addition of 0.2 ppm of others synthetic pyrethroid with cross reactivity percentage of 17.45%, 20.87% and 9.83% towards permethrin (PM), cypermethrin (CM) and fenvalerate (FV), respectively.

In this study IC_{50} value was calculated to indirectly determine the ligand affinity. A competition binding assay in which the concentration of ligand is needed to shift 50% of a fixed concentration of the reference ligand was used to determine the IC_{50}. Therefore, the combination of aptamer and target with the lowest IC_{50} was chosen as more selective (Mak *et al*., 2005). Table 6 summarizes the IC_{50} and cross-reactivity results for each compound, showing that the designed aptamer is more selective to deltamethrin than to type I (PM) and type II (CM and FV) pyrethroids analog. However, a slight recognition (%CR < 21%) of pyrethroids was also observed due to they are structurally related compounds where the difference is only in the addition of a cyano group to type II pyrethroids (DM, CM and FV). The IC_{50} value found in this study was comparable to the report by Fruhmann *et al*. (2018). Additionally, this result showed that the designed aptamer was specific and selective towards synthetic pyrethroid, deltamethrin.

Table 6. the IC_{50} and cross-reactivity of related compounds in the SPGE/DM.

Compound	IC_{50} (ppm)	Cross Reactivity (%)
Deltamethrin	0.1109	100
Permethrin	1.5836	17.45
Cypermethrin	6.4661	20.87
Fenvalerate	5.3803	9.83

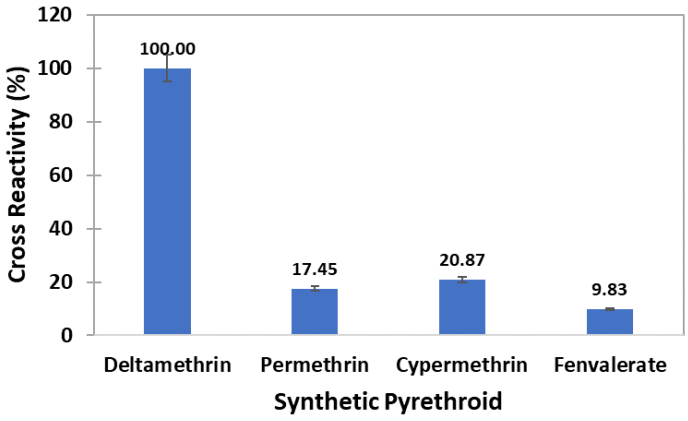


Figure 10. Cross reactivity study of 50 nM deltamethrin (DM) aptamer towards standard deltamethrin (concentration at 0.2 ppm) and others synthetic pyrethroid, standard permethrin (PM), cypermethrin (CM) and fenvalerate (FV), respectively.

4. Conclusions

Generally, any interaction between biological components will elicit signals in terms of current or mass changes. This signal could be amplified and transmitted into biosensor system using either electrochemical, acoustic wave or optical approach. Electrochemical biosensor is favoured over other fore mentioned approaches as it is more sensitive, specific, stable and feasible. By utilizing the designed aptamer, we are keen to study the aptamer interaction with its targeted synthetic pyrethroids in terms of electrochemical sensor. In this study, a specific and selective signal aptasensor based on electrochemical for deltamethrin detection was successfully fabricated. The aptasensor has been effectively functional in the determination of standard deltamethrin with acceptable results. Promisingly, by changing the aptamer sequence, this strategy can be extended for the detection of other synthetic pyrethroids, showing great potential in aptasensor design.

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