



Research Article

A sensitive electrochemical aptasensor for cypermethrin detection using a screen-printed carbon electrode modified with polypyrrole/gold nanoparticles

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Abstract

The synthetic pyrethroid cypermethrin (CM) is employed as an insecticide. It has a long half-life, is simply excreted as residue, and pollutes food products derived from agriculture, leading to various health problems. To monitor pesticides in agricultural products, an electrochemical aptasensor based on polypyrrole/gold nanoparticles (Ppy/AuNPs) modified screen-printed carbon electrode (SPCE) for CM detection was developed. Modification of SPCE with Ppy/AuNPs as a sensing platform was accomplished via dropcast-polymerisation, whereas electrochemical testing was achieved with a direct format where CM in solution would bind with an anti-cypermethrin aptamer (Apt) immobilised onto the surface of modified SPCE. A linear regression of CM with $R^2 = 0.9668$ and a low limit of detection (LOD) of 0.256 ppm was reported. Analysis of vegetable samples showed that this electrochemical aptasensor could identify CM in actual samples, and the results were in close agreement with those obtained via liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Keywords: cypermethrin, polypyrrole, gold nanoparticles, aptasensor, electrochemical

Introduction

Cypermethrin has activity that mimics natural pyrethrins, which are extracted from chrysanthemum flowers in the form of pyrethrum and are the source of synthetic pyrethroid pesticides [1]. Chrysanthemic acid is a natural monocarboxylic acid found in certain plants, notably *Chrysanthemum indicum*, and is a key component of pyrethrins, which are natural insecticides. Chrysanthemic acid esters (ethyl 2,2-dimethyl-3-(1-isobutenyl) cyclopropane-1-carboxylate) are the chemical basis of pyrethroid pesticides. Since the 1980s, pyrethroid pesticides, a class of synthetic organic insecticide have been used all over

the world because they are more effective and less hazardous than the carbamate and organophosphate families of pesticides [2, 3].

Neurotoxins known as pyrethroid insecticides target the receptor site of voltage-gated sodium channels selectively [4]. They act by binding to sodium channels, leading to excitatory paralysis in insects. In addition, they cause hyperexcitable nerve cells to enter an unusually stable state through alterations in membrane potential. These alterations cause insects to experience sub-lethal, "knockdown" paralysing effects [5]. Cypermethrin acts similarly in humans, by

attacking the human nervous system, interfering with the formation of antibodies against disease, reducing the body's immunity and being carcinogenic [6].

Malaysia's reputation as an agricultural producer was tarnished in 2018 after Singapore authorities found high levels of pesticide residues in iceberg lettuce imported from Malaysia, as reported by the Ministry of Health (MOH) [7]. This has damaged the export opportunities of other Malaysian products, leading to financial and economic losses. Consumers are now truly prioritising food safety, and concerns about pesticide residues in vegetables are increasing among them.

The dynamic and quickly developing field of biosensor technology has arisen in response to the urgent demand for accurate, quick and affordable measures to solve issues with, for instance, food safety, environmental monitoring and healthcare [8]. A biosensor is an analytical device that integrates a biological molecule (the recognition element) with a transducer, which acts as an energy converter [9]. This combination allows the detection, measurement and reporting of the existence or concentration of specific chemical compounds or target molecules in a certain sample [10].

The recognition element is accountable for specifically binding to or interrelating with the target analyte (the substance being measured). Examples include enzymes [11], antibodies [12], nucleic acid [13], aptamer [14], or even whole cells [15]. Basically, a biosensor translates a biochemical response into a computable physical signal [16]. The underlying transduction mechanisms can employ a variety of technologies, including optical [17], electrochemical [18], magnetic [19], piezoelectric [20] and colorimetric [21] approaches.

Current standards for detecting and identifying cypermethrin primarily rely on laboratory-based techniques, specifically Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC). When coupled with Mass Spectrometry (MS/MS) or Electron Capture Detection (ECD) [22], these methods offer the high sensitivity and definitive molecular fingerprinting required to distinguish cypermethrin from other synthetic pyrethroids. However, these traditional approaches necessitate intensive sample preparation, often using the QuEChERS protocol [23] and are inherently costly, time-consuming, and laboratory-bound. The significant delay between sample collection and result reporting often precludes real-time intervention in

food safety crises. This creates a critical demand for portable electrochemical biosensors that can provide rapid, on-site quantification without the logistical burden of conventional chromatography.

This research introduces a novel electrochemical biosensor platform specifically engineered to address the critical need for rapid, high-sensitivity detection of cypermethrin residues in Malaysian produce. While traditional analytical techniques are often laboratory-bound and time-consuming, the development of this electrochemical approach offers a significant advancement by providing real-time, on-site monitoring capabilities with high detection limits. By integrating a specific recognition element with an electrochemical transducer, this study provides a localised solution to the 2018 agricultural trade crisis, allowing for the direct and precise quantification of synthetic pyrethroids against the maximum residue limit (MRL) set by the Malaysian Food Regulations of 1985.

The novelty of this work lies in its ability to bridge the gap between sophisticated electroanalytical chemistry and practical food safety enforcement, offering a scalable and cost-effective framework to restore consumer confidence and ensure regulatory compliance in the regional vegetable supply chain. Therefore, the purpose of this work was to evaluate the incidence of cypermethrin residues in vegetables from one of the five main chemical groups (synthetic pyrethroid). The detected concentrations were then compared with the maximum residue limit (MRL) specified under the Malaysian Food Regulations 1985.

Materials and Methods

Materials

Certified reference standards (> 98% purity) of pesticide (cypermethrin) PESTANAL[®], analytical standard was purchased from Sigma-Aldrich, United States. ASTM Type 1 ultrapure water, with a conductivity of 0.055 $\mu\text{S}/\text{cm}$ (18.2 $\text{M}\Omega\text{cm}$), was obtained from an Arium[®] Comfort I Combined Water System (Sartorius, Germany). Gold nanoparticles (AuNPs) were purchased from Sigma Aldrich, United States. An APTfold buffer and screen-printed carbon electrode (SPCE, DRP C110) was bought from Biogenes Technologies, Malaysia. Aptamer (Apt) was obtained by in silico with cypermethrin (CM), following the procedures described in the literature [24].

Herein, we first design and synthesise the aptamer via an in-silico approach. During this first step, the binding energy and length of the aptamer produced

will be studied (see **Schematic 1**). Next, we investigate the interaction between cypermethrin (CM) and its aptamer (Apt) electrochemically using a modified screen-printed carbon electrode (SPCE/Ppy/AuNPs) via the differential pulse voltammetry (DPV) technique. This non-labelling technique offers direct detection of CM at the molecular level with a strong binding. Theoretically, the binding between the aptamer and its target analyte will result in a higher binding force and stronger charge that will affect the surface resistance for the redox-active substance.

Buffers and solutions

Phosphate buffer solution (PBS) was made by dissolving 0.01 M disodium hydrogen phosphate (Na_2HPO_4) and 0.01 M sodium dihydrogen phosphate (NaH_2PO_4) in ultrapure water (18.2 M Ω ·cm) and adjusting the pH to the required value. The redox solution, containing 5.0 mM ferricyanide/ferrocyanide ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$) was similarly prepared using ultrapure water obtained from the

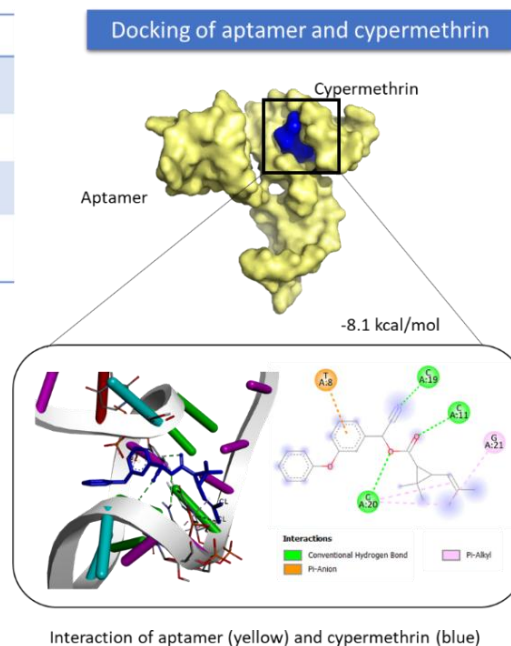
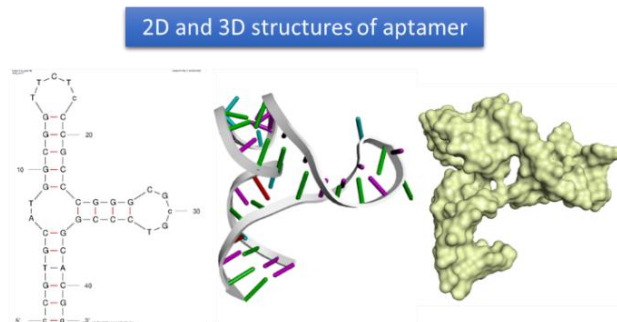
Arium® Comfort I Combined Water System (Sartorius, Germany).

Preparation of polypyrrole/gold nanoparticles modified screen-printed carbon electrode

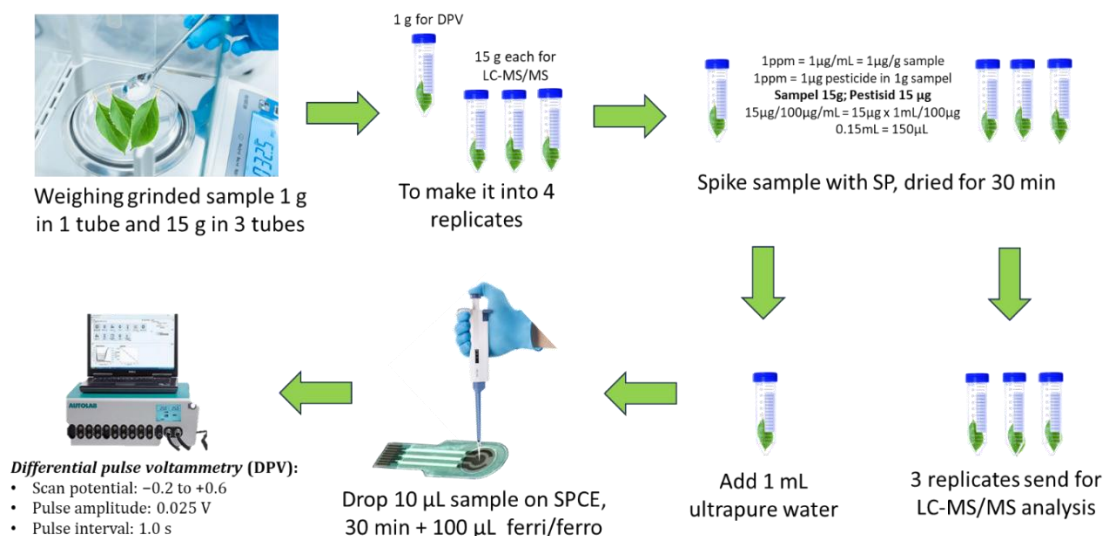
SPCE was modified by adding polypyrrole (Ppy) and gold nanoparticles (AuNPs) solution (1:1 ratio) via the drop-casting method and incubated for 30 minutes at room temperature. The electrode was then washed with PBS and ultrapure water to remove any residual polymers and nanoparticles. Next, 10 μL of the CM aptamer solution was pipetted onto the electrode and incubated for 30 minutes at 25 $^\circ\text{C}$. Before immobilisation, the 5'-thiolated aptamer was activated by heating at 95 $^\circ\text{C}$ for 5 minutes, followed by cooling to room temperature for another 5 minutes. Then the working electrode surface was rinsed with PBS, resulting in SPCE/Ppy/AuNPs/Apt. Some experimental conditions of the fabricated aptasensor were systematically optimised, including aptamer concentration and incubation time to attain the exceptional analytical performance.

Aptamer Design for Cypermethrin	
Sequence	CCGTGCATGGCGGTTCTCCCGCCGGGCGCGTCCCGGCACGG
Length	42-mers
Folding energy	-13.71 kcal/mol
Binding energy	-8.1 kcal/mol

Aptamer criteria:
 Length : 32-50 mers
 Folding energy: <-8.0 kcal/mol
 Binding energy: <-7.0 kcal/mol



Schematic 1. Aptamer design and structural analysis for cypermethrin detection. (Top left) Aptamer criteria for selection. (Top right) Docking model showing the aptamer (yellow surface) interacting with cypermethrin (blue). (Bottom left) Predicted 2D secondary structure and 3D models of the aptamer. (Bottom right) Detailed view of the molecular interactions between the aptamer and cypermethrin



Schematic 2. Illustration of the steps of spiked standard

Characterisation and standard curve development of an aptasensor

The direct DNA-based assay was used for recognition of cypermethrin (CM) by using 10 µL of standard CM with concentration range of 0.2 to 1.0 ppm. The unbound CM was removed by rinsing with PBS and ultrapure water. The cyclic voltammetry (CV) test was performed in a redox solution with a scan rate of 10 mV/s and a potential range from -0.2 to +0.6 V. This study focuses exclusively on the current anodic peak height (I_{pa}), one of the CV parameters. Then it was measured electrochemically by DPV at an amplitude of 0.025 V and at a scan potential range from -0.2 to +0.6 V, followed by electrochemical impedance spectroscopy (EIS) to monitor the charge transfer resistance (R_{ct}). EIS was performed at a formal potential of open circuit voltage in alternating current (AC) amplitude of 5 mV using a frequency range of 0.1 to 100 000 Hz. All measurements were executed at room temperature in 5.0 mM ferricyanide/ferrocyanide ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$) redox solution. The standard calibration curve was constructed and tailored using a linear regression calculation and the limit of detection (LOD) was calculated based on the formula $\text{LOD} = 3.3SE/b$, where SE is the standard error measurement with $n = 3$ and b is the calibration plot slope.

Recovery analysis by the electrochemical method

Schematic 2 illustrates the matrix extraction in sample preparation. The fresh vegetables were cut into small pieces before the extraction. Then, an amount of 1 g and 15 g of sample were weighed for both electrochemical and liquid chromatography-mass

spectrometry (LC-MS/MS) analysis, respectively, in a Falcon tube. The sample was spiked with the standard solutions of CM at different concentrations. The spiked samples were allowed to dry for 30 min, followed by resuspension in 1 mL of ultrapure water for electrochemical analysis. Prior to measurement, 10 µL of spiked sample was pipetted onto the modified screen-printed carbon electrode (SPCE).

Sample extraction for recovery study

For biosensor analysis, a simple extraction method using ultrapure water with minimal sample processing was used (**Schematic 2**). Meanwhile, for LC-MS/MS analysis, the extraction protocol by Anastassiades et al. [25] was followed by some modification. Weigh 15 g of the sample in a 50 mL Falcon tube. Add 15 mL of 0.1% (v/v) formic acid in acetonitrile (MeCN). Add 6.0 g magnesium sulphate (MgSO_4) + 1.5 g sodium chloride (NaCl) and shake vigorously for 1 min. Centrifuge at 4000 rpm for 5 min. Decant 10 mL extract into a 15 mL centrifuge tube, add 0.5 g primary secondary amine (PSA) and 1.5 g MgSO_4 , then shake for 30 s, centrifuge at 4000 rpm for 1 min. Filter the extract with a polytetrafluoroethylene (PTFE) membrane filter 0.2 µm.

Recovery analysis by liquid chromatography–mass spectrometry (LC-MS/MS)

The detection of cypermethrin was performed using ultra-performance liquid chromatography (Agilent 1200 Infinity HPLC, USA) combined with a tandem MS system (AB Sciex QTRAP 5500). Chromatographic separation was accomplished using a reversed-phase Synergi C18 LC column (Synergi 4

μm Fusion-RP 80Å, 4 μm pore size, 50 mm length, 2 mm outer diameter). Gradient elution was used, with Eluent A consisting of 0.1% (v/v) formic acid in acetonitrile and Eluent B consisting of 0.1% (v/v) formic acid in water. The analytes were separated at a flow rate of 0.30 mL/min, with a total separation time of 12 minutes. A 5 μL sample was injected into the column, and the column temperature was set at 45 °C. Detection was carried out in positive electrospray ionisation (ESI) mode. Data were acquired in multiple reaction monitoring (MRM) mode, with two transitions monitored for each pesticide. The ion spray voltage was set at 3,500 V and the MS capillary temperature was maintained at 450 °C. This LC-MS/MS method was applied as a reference for validating the performance of the developed electrochemical aptasensor.

Results and Discussion

Characterisation: Cyclic voltammetry (CV)

Figure 1 shows the cyclic voltammograms in 5.0 mM ferricyanide/ferrocyanide ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$) redox solution of each modified electrode. The electrochemical behaviour of cypermethrin at the modified electrode surface is characterised by a dominant oxidation process. Given that the anodic peak current (I_{pa}) provided a more pronounced and reproducible response than the corresponding reduction peak, the study focuses exclusively on the oxidation potential to ensure the lowest possible limit of detection (LOD). The SPCE/PPy/AuNPs electrode exhibited higher peak currents ($I_{pa} = 34.56 \mu\text{A}$, $I_{pc} = -33.94 \mu\text{A}$) compared to the bare SPCE ($I_{pa} = 21.96$

μA , $I_{pc} = -20.44 \mu\text{A}$), due to the enhanced conductivity provided by Ppy/AuNPs, which facilitated electron transfer at the electrode surface [26]. Modification of SPCE with Ppy/AuNPs increases the surface area of the electrode. The high affinity of AuNPs for thiol (S-H) groups allows many aptamers (Apt) to bind to AuNPs through Au-S bonds and occupy the SPCE surface, thus enabling bioactivity [27]. Apt is not inherently conductive, and therefore its presence results in a decrease in peak current ($I_{pa} = 25.57 \mu\text{A}$, $I_{pc} = -19.31 \mu\text{A}$) of SPCE/PPy/AuNPs/Apt. The immobilised Apt has a high affinity towards and forms a bond with the target compound, CM. Following the loading of Apt and CM on the SPCE surface, the SPCE/PPy/AuNPs/Apt-CM became less conductive but not as low as the bare SPCE, and the peak current of the electrode modified with the target also decreased ($I_{pa} = 25.48 \mu\text{A}$, $I_{pc} = -23.35 \mu\text{A}$).

Characterisation: Differential pulse voltammetry (DPV)

The results of electrochemical analysis by using different pulse voltammetry (DPV) techniques of the SPCE modified with polypyrrole (Ppy) and gold nanoparticles (AuNPs) (SPCE/PPy/AuNPs) and modified SPCE immobilised with 10 nM cypermethrin aptamer (Apt) (SPCE/PPy/AuNPs/Apt) and aptamer with target compound (Apt-CM) (SPCE/PPy/AuNPs/Apt-CM) are shown in the voltammograms (**Figure 2**).

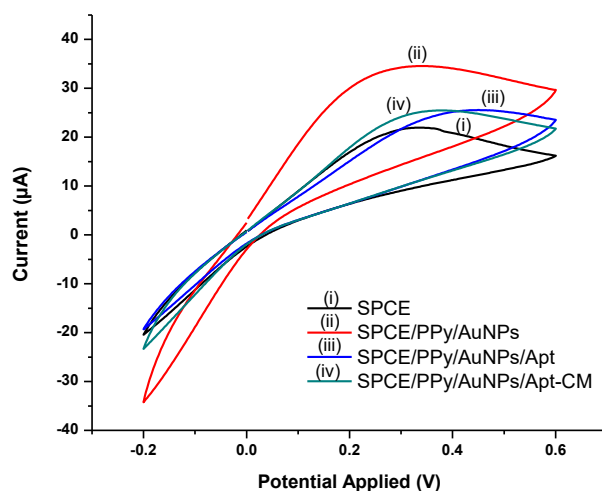


Figure 1. Cyclic voltammogram (CV) of (i) bare screen-printed carbon electrode (SPCE); (ii) modified SPCE with polypyrrole/gold nanoparticles (SPCE/PPy/AuNPs); (iii) modified SPCE immobilised with aptamer (SPCE/PPy/AuNPs/Apt); and (iv) with target compound (SPCE/PPy/AuNPs/Apt-CM) in 5.0 mM ferricyanide/ferrocyanide ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$) redox solution and 0.1 M KCl

The variations in peak potential (E_p) observed in **Figure 2** indicate changes in electron transfer kinetics and surface resistance at each modification stage. The negative shift in E_p for SPCE/PPy/AuNPs and Apt layers suggests that the synergistic effect of the PPy matrix and AuNPs reduces the overpotential required for oxidation by facilitating faster electron transfer. Conversely, a significant anodic (positive) shift is observed upon the addition of cypermethrin (CM). This shift is attributed to the formation of the Aptamer-CM complex, which creates a steric barrier and increases the charge transfer resistance (R_{ct}) on the electrode surface. Consequently, a higher potential is required to drive the electrochemical reaction,

confirming the successful binding of the non-conductive target analyte to the biosensor. It was shown that the peak of SPCE modified with PPy and AuNPs (SPCE/PPy/AuNPs) was the highest, 24.35 μA , compared to bare SPCE, which had the lowest peak, 4.28 μA . Thus, proving that AuNPs enhance the conductivity of SPCE while the polymer (PPy) provides a conductive lattice for AuNPs to be loaded on the SPCE surface. The peak of modified SPCE immobilised with CM aptamer (SPCE/PPy/AuNPs/Apt) then decreased, 17.03 μA and further decreased, 6.08 μA with the addition of target compound (SPCE/PPy/AuNPs/Apt-CM).

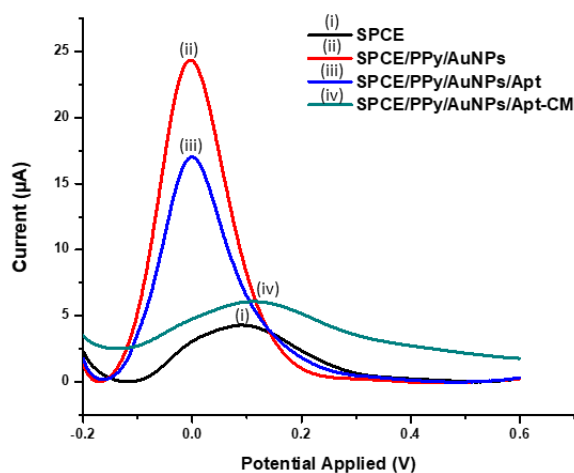


Figure 2. The voltammograms of (i) bare SPCE; (ii) modified with PPy and AuNPs (SPCE/PPy/AuNPs); (iii) immobilised with 10 nM cypermethrin aptamer (SPCE/PPy/AuNPs/Apt) and then with (iv) target, CM (SPCE/PPy/AuNPs/Apt-CM), which was measured electrochemically by using differential pulse voltammetry (DPV)

Characterisation: Electrochemical impedance spectroscopy (EIS)

To study the electrical behaviour of the modified electrode, the EIS technique was used. As shown in Figure 3, the impedance spectrum, also known as the Nyquist plot, consists of a semicircle region and a linear region. The semicircle region was located at high frequency and was associated with resistive charge transfer (R_{ct}). While the linear region is located in the low frequency region and represents a diffusion process. The interfacial properties of the modified electrodes were characterised using EIS, as shown in the Nyquist plots (**Figure 3**). The bare SPCE exhibited the lowest charge transfer resistance ($R_{ct} = 6540.37 \Omega$) due to its unmodified surface providing unimpeded access for the redox probe ($[\text{Fe}(\text{CN})_6]^{3-/4-}$). Upon the deposition of PPy/AuNPs, the R_{ct} increased to 8366.63

Ω . While these materials are intrinsically conductive, the formation of the multi-component film creates a physical mass-transport barrier that slightly hinders diffusion compared to the pristine carbon surface. The characterisation of the intermediate SPCE/PPy step was omitted to streamline the discussion toward this optimised composite, as the synergistic effect of AuNPs within the PPy matrix provides the necessary biocompatible surface for stable aptamer attachment. Subsequent immobilisation of the aptamer (Apt) further increased the resistance to 8687.89 Ω , attributed to the non-conductive nature of the oligonucleotide layer [28]. The final step, the binding of cypermethrin (CM), resulted in a significant jump in impedance to 14981.66 Ω . This substantial increase confirms the successful capture of the target analyte, which forms a dense, non-conductive Apt-CM

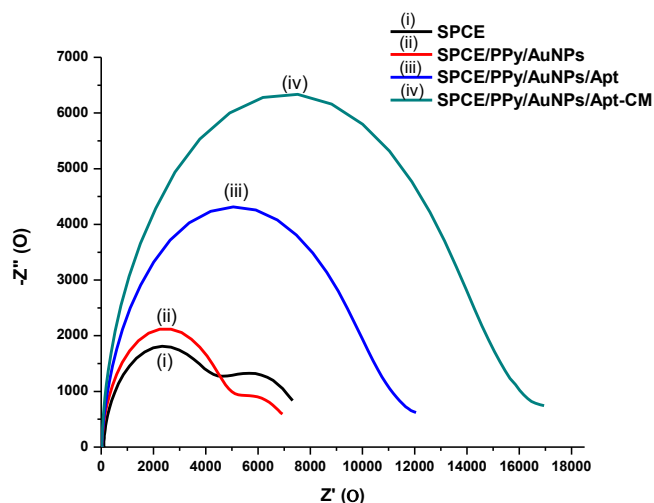


Figure 3. Nyquist plot of (i) SPCE; (ii) SPCE/PPy/AuNPs; (iii) SPCE/PPy/AuNPs/Apt; (iv) SPCE/PPy/AuNPs/Apt-CM in 5.0 mM ferricyanide/ferrocyanide ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$) redox solution and 0.1 M KCl

complex that effectively obscures electron transfer at the electrode interface.

Characterisation: Morphology

The morphological appearance of each modified electrode was viewed under the field emission scanning electron microscopy (FESEM) with 50,000 $\times$ magnification. The deposition of polymer, polypyrrole (Ppy) and nanoparticles, AuNPs on SPCE made the rough surface of bare SPCE (**Figure 4a**) transform into a cheese-like structure with large clusters of granules on top (**Figure 4b**). Immobilisation of Apt on the electrode transformed the modified SPCE/PPy/AuNPs into SPCE/PPy/AuNPs/Apt. The presence of smaller clusters of granules on the cheese-like surface (**Figure 4c**) indicates that the CM Apt was successfully loaded into the polymer lattice on the SPCE surface. Further changes in electrode morphology were observed following immobilisation with the target compound (SPCE/PPy/AuNPs/Apt-CM) with a more uniform distribution of granule-like structure on the electrode surface (**Figure 4d**). The transition from the irregular clusters in Figure 4c to a more uniform distribution of granule-like structures in Figure 4d is attributed to the conformational reorganisation of the aptamers upon interaction with cypermethrin. In the absence of the target, the immobilised aptamers may exist in a relatively disordered or loosely clustered state on the

AuNP-polymer lattice. However, the binding of cypermethrin triggers an induced-fit mechanism, causing the aptamers to fold into more compact and stable three-dimensional structures around the target molecule. This folding process facilitates the formation of discrete, well-dispersed Apt-CM complexes across the electrode surface, resulting in a more homogenous surface topography. The observed shift toward a uniform granular morphology provides visual confirmation of the successful and widespread capture of the target analyte across the functionalised sensing platform.

Optimisation of aptamer concentration and incubation time

Figure 5 shows the bar graph of the modified SPCE immobilised with cypermethrin aptamer (Apt) as a result of electrochemical analysis using the differential pulse voltammetry (DPV) technique. The bar graph shows that at the aptamer concentration and incubation time of 10 nM and 0.5 hours were the optimal conditions for electrochemical analysis. The optimal Apt concentration and incubation time were selected based on the highest current that the Apt exhibited when capturing a specific target compound (CM). From the bar graph, it is clear that at a concentration and incubation time of 10 nM and 0.5 hours, the current value (peak height) of Apt is higher compared to the others.

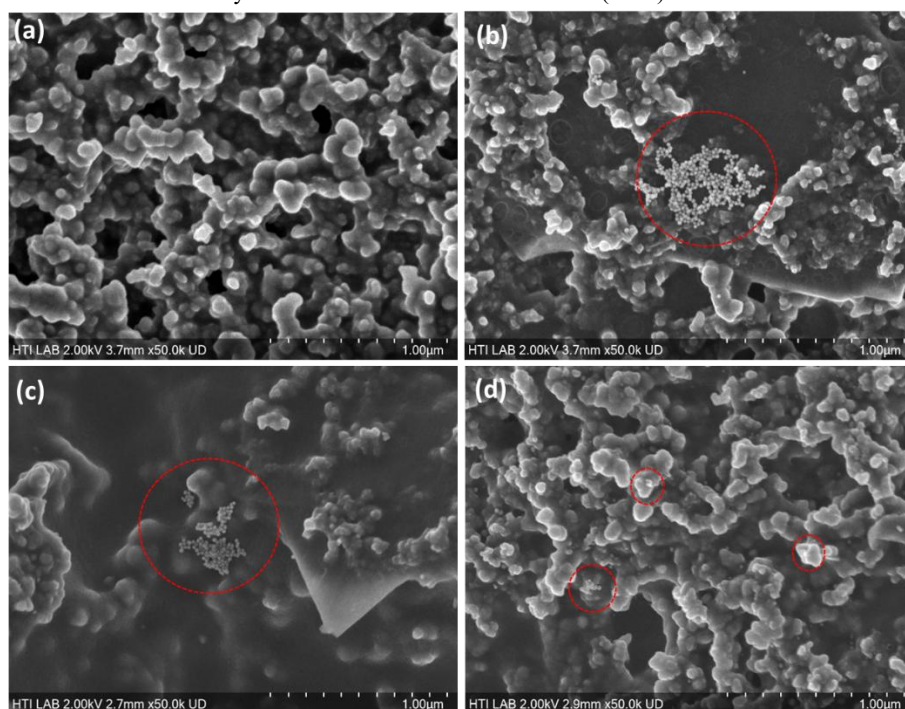


Figure 4. FESEM image at 50,000 $\times$ magnification, (a) rough surface of SPCE, (b) cheese-like structure with large clusters of granules of SPCE/PPy/AuNPs, (c) SPCE/PPy/AuNPs/Apt with smaller clusters of granules on the cheese-like surface and (d) SPCE/PPy/AuNPs/Apt-CM with a more uniform distribution of granule-like structure

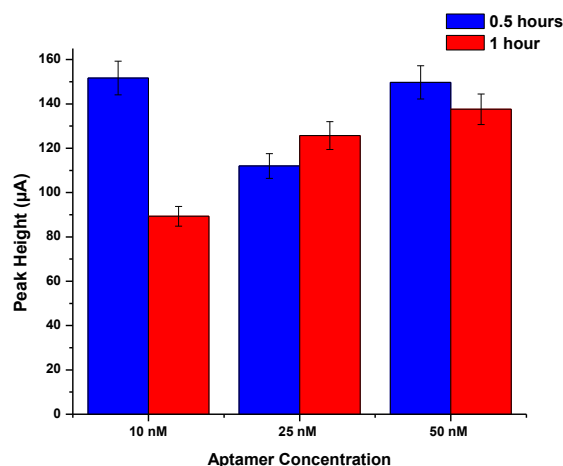


Figure 5. The bar graph of SPCE immobilised with cypermethrin aptamer, that were measured electrochemically by using the different pulse voltammetry (DPV) technique

Analytical performance of the aptasensor

The detection mechanism of the developed biosensor follows a signal-off electrochemical principle, corroborated by the synergistic trends in CV, DPV, and EIS data. Initially, the PPy/AuNPs composite enhances electron transfer by providing a high-

surface-area conductive lattice, significantly increasing peak currents and reducing overpotential. The subsequent immobilisation of aptamers introduces a non-conductive biological layer, which slightly elevates charge transfer resistance (R_{ct}). The definitive recognition event occurs when

cypermethrin (CM) binds to the aptamer, forming a stable aptamer-CM complex. This complex acts as a dense, insulating barrier that creates significant steric hindrance, effectively masking the electrode surface. This obstruction leads to a dramatic jump in R_{ct} (EIS) and a proportional decrease in anodic peak current (DPV). This measurable suppression of electron transfer serves as the analytical signal, where the magnitude of current reduction directly correlates to the concentration of cypermethrin captured.

Direct electrochemical measurements were conducted, and the CM standard was used to generate a linear regression calibration curve (**Figure 6**). A linear regression calibration curve is attained with R^2 of 0.9668, demonstrating that the concentration of 10

nM Apt is suitable for this study. The limit of detection (LOD) was estimated according to three point three times of standard error, $3.3SE/b$ ($n = 3$), and was 0.256 ppm, which complies with the requirement of maximum residue limit (MRL) regulations (0.7 ppm) [29]. The low LOD obtained in this study shows that the developed method has a high possibility of application in the detection of CM compound. Modification of the electrode with Ppy/AuNPs has improved the electrochemical characteristics of this aptasensor, thus enhancing the sensitivity and sensing response. Polypyrrole (PPy) is among the most widely used conductive macromolecules for electrode modification due to its convincing properties as a conductive polymer and its capacity to support the adsorption of metal ions, including AuNPs [30].

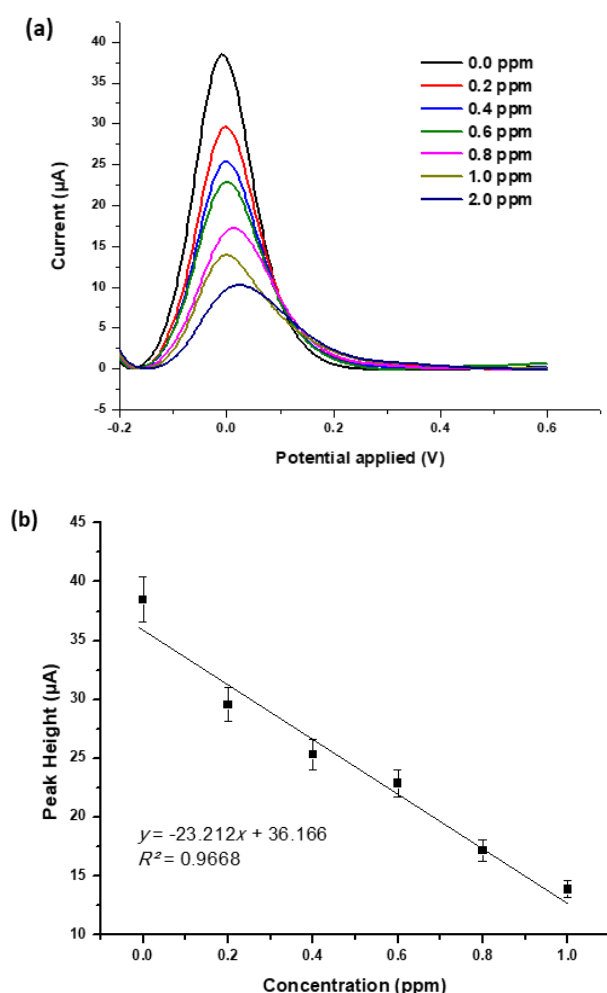


Figure 6. The (a) voltammograms and (b) linear regression of immobilised SPCE with 10 nM cypermethrin aptamer towards standard CM compound (SPCE/Ppy/AuNPs/Apt-CM) in a concentration range of 0.0 ppm to 1.0 ppm

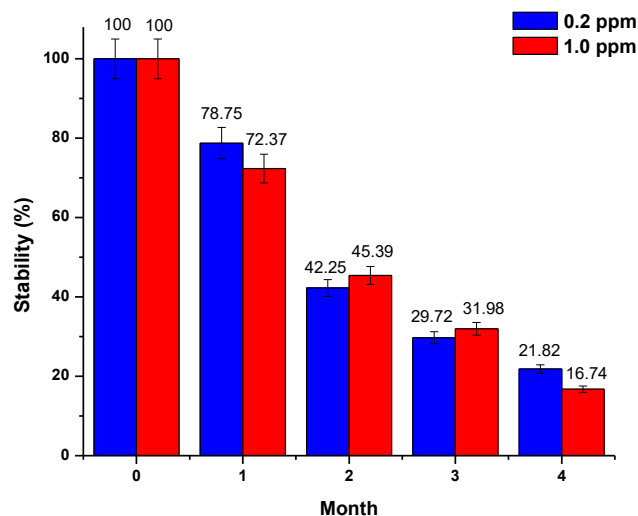


Figure 7. The storage stability performance of the aptasensor electrode (SPCE/Ppy/AuNPs/Apt) towards the standard CM compound at a concentration of 0.2 ppm and 1.0 ppm within 4 months

The storage stability performance of this aptasensor electrode (SPCE/Ppy/AuNPs/Apt) was also evaluated against 0.2 ppm and 1.0 ppm concentrations of CM compound (**Figure 7**). The fabricated aptasensor electrode retained 72 - 79 % of its initial current response after one month at 4 °C, confirming good storage stability and comparable to a previous study [31].

Selectivity of the aptasensor

Selectivity of the aptasensor was evaluated by testing other synthetic pyrethroids, including Type I and II compounds such as permethrin (PM) and deltamethrin (DM). The aptasensor exhibited minimal cross-reactivity toward PM (8.82 %) and DM (12.66 %),

demonstrating its exceptional selectivity for CM detection (100 %) (**Figure 8**). In measuring cross-reactivity, the ratio of concentrations of the primary analyte and cross-reactant that causes a 50 % decrease in the detected signal is considered acceptable [32]. Although the tested pyrethroids have a very similar basic structure (chiral compounds), where the difference is the additional cyano group in Type II [33], the analysis of these pesticides did not find significant or interfering signals throughout the measurements. The aptamer cast off as a biorecognition molecule in this work is specific towards CM, making the aptasensor highly selective for CM detection.

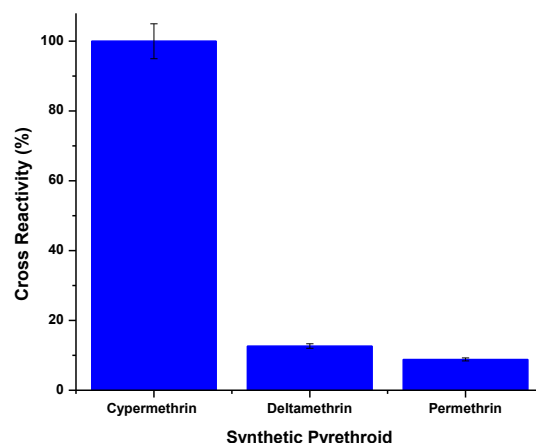


Figure 8. Cross reactivity study of 10 nM cypermethrin aptamer towards other synthetic pyrethroid, permethrin (PM) and deltamethrin (DM)

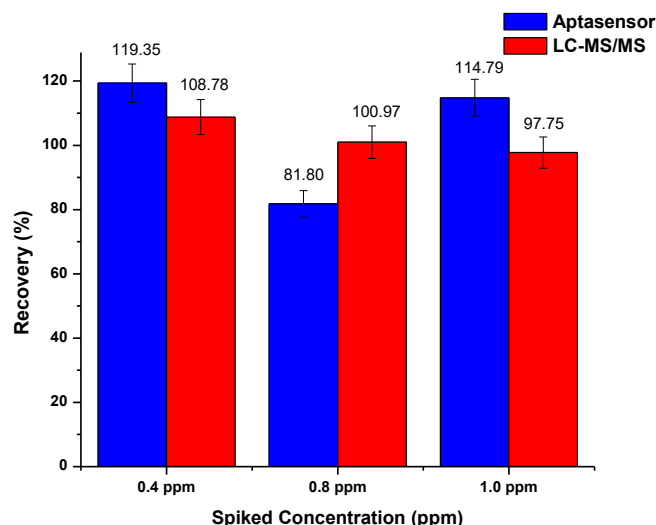


Figure 9. The bar graph of the recovery study for cypermethrin using the developed biosensor compared with liquid chromatography–mass spectrometry (LC-MS/MS)

Recovery analysis

Based on the examination of vegetable samples that had been tampered with, the developed aptasensor's validity for the detection of CM in actual samples (green leafy vegetables) was assessed. Linear regression plots were interpolated with measured current levels for analysis of CM spiked samples. As indicated in **Figure 9**, the analysis's findings were contrasted with those of the LC-MS/MS analysis. The significant reliability for CM detection in actual samples via this aptasensor is indicated by the recovery of spiked samples, which falls between 81.80 % and 119.35 %. In comparison to LC-MS/MS (97.75 % to 108.78 %), comparable results were obtained, indicating the reliability of this aptasensor for CM screening and monitoring in actual samples. All analytes fell within the recovery range of 70 % to 120 %, consistent with SANTE guidelines for initial validation [34].

Conclusions

An electrochemical aptasensor that is highly sensitive towards CM detection has been successfully developed. The use of SPCE modified with Ppy/AuNPs as a sensing platform and the establishment of a direct aptamer assay format has been effectively applied for CM detection. Morphological study discovered an even fabrication of Apt on top of Ppy/AuNPs, demonstrating the aptness of this sensor platform towards biosensor development. The low LOD (0.256 ppm) obtained using this aptasensor complies with the requirements

of the Food Act 1983 (Act 281) and Regulations. CM detection in spiked green leafy vegetable samples yielded 81.80 % and 119.35 % recoveries, proving the developed aptasensor is dependable for real sample analysis.

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References

1. Matsuo N. (2019). Discovery and development of pyrethroid insecticides. *Proceeding Japan Academy Series B Physical Biological Sciences*, 95(7):378-400.
2. Feng J, Shan G, Hammock BD, and Kennedy IM. (2003). Fluorescence quenching competitive immunoassay in micro droplets. *Biosensor Bioelectronic*, 18:1055-1063.
3. Ribeiro EB, Ribeiro DB, dos Santos Soares AM, et al. (2022). A novel glutathione-S-transferase-based biosensor for pyrethroid insecticides: From inhibition study to detection. *Sensors and Actuators Reports*.,4(November 2021).
4. Kaushik A, Solanki PR, Ansari AA, Malhotra BD, and Ahmad S. (2009). Iron oxide-chitosan hybrid nanobiocomposite based nucleic acid

- sensor for pyrethroid detection. *Biochemical Engineering Journal*, 46(2):132-140.
5. Sinha V, and Shrivastava S. (2024). Cypermethrin: An emerging pollutant and its adverse effect on fish health and some preventive approach—a review. *Indian Journal of Microbiology*, 64(1):48-58.
6. Nurdin M, Maulidiyah M, Salim LOA, Muzakkar MZ, and Umar AA. (2019). High performance cypermethrin pesticide detection using anatase TiO₂-carbon paste nanocomposites electrode. *Microchemical Journal*, 145:756-761.
7. People Food Connection. (2018). Malaysia's Health Ministry recalls lettuce over pesticide levels. Food Regulatory. Access from <https://dpointernational.com/food-industry-news/health-nutrition/malaysias-health-ministry-recalls-lettuce-over-pesticide-levels/>
8. Silva LMDC, Santos VPS Dos, Salgado AM, and Pereira KS. (2013). Biosensors for contaminants monitoring in food and environment for human and environmental health. In: Rinken T, ed. *State of the Art in Biosensors - Environmental and Medical Applications*. Edited Vol. InTech;:151-168.
9. Farzin MA, and Abdoos H. (2021). A critical review on quantum dots: From synthesis toward applications in electrochemical biosensors for determination of disease-related biomolecules. *Talanta*;224(November 2020):121828. doi:10.1016/j.talanta.2020.121828
10. Gaviria-Arroyave MI, Cano JB, and Peñuela GA. (2020). Nanomaterial-based fluorescent biosensors for monitoring environmental pollutants: A critical review. *Talanta Open*, 2:100006.
11. Amir Hafiz MF, Ahmad FB, and Akmal MHM. (2024). Novel *Rhizopus oryzae* esterase-immobilised chitosan/carbon nanomaterials as sensing support for enzyme inhibition-based biosensor in the detection of organophosphorus pesticide residues on crops. *Journal Natural Pesticides Research*, 10(July):100092.
12. Awaludin N, Abdullah J, Salam F, Ramachandran K, Yusof NA, and Wasoh H. (2020). Fluorescence-based immunoassay for the detection of *Xanthomonas oryzae* pv. *oryzae* in rice leaf. *Analytical and Biochemistry*, 610(7):113876.
13. Saad SM, Abdullah J, Rashid SA, Fen YW, Salam F, and Yih LH. (2019). A fluorescence quenching based gene assay for *Escherichia coli* O157:H7 using graphene quantum dots and gold nanoparticles. *Microchimica Acta*, 186(12):804-813.
14. Guo X, Wen F, Zheng N, Saive M, Fauconnier ML, and Wang J. (2020). Aptamer-based biosensor for detection of mycotoxins. *Frontier Chemistry*, 8:195.
15. Yu X. (2018). Rapid and sensitive detection of *Escherichia coli* O157:H7 using a QCM sensor based on aptamers selected by whole-bacterium SELEX and a multivalent aptamer system. Access from <http://scholarworks.uark.edu/etdhttp://scholarworks.uark.edu/etd/2776>
16. Endo H, and Wu H. (2019). Biosensors for the assessment of fish health: A review. *Fisheries Sciences*, 85(4):641-654.
17. Mohd Saad S, Abdullah J, Abd Rashid S, Wing Fen Y, Salam F, and Han Yih L. (2020). A carbon dots based fluorescence sensing for the determination of *Escherichia coli* O157:H7. *Measurement*, 160: 107845.
18. Ismail S, Yusof NA, Abdullah J, and Abd Rahman SF. (2020). Electrochemical detection of arsenite using a silica nanoparticles-modified screen-printed carbon electrode. *Materials (Basel)*, 13(14).
19. Xu M, Wang R, and Li Y. (2017). Electrochemical biosensors for rapid detection of *Escherichia coli* O157:H7. *Talanta*, 162:511-522.
20. Tsai SC, Fang HC, Lai YL, Lu CH, and Liu CP. (2016). Efficiency enhancement of green light emitting diodes by improving the uniformity of embedded quantum dots in multiple quantum wells through working pressure control. *Journal of Alloys Compounds*, 669:156-160.
21. Chaisri N, Jaengphop C, Hirono I, and Unajak S. (2024). Rapid apta-chromogenic detection method for nitrofurantol metabolite determination. *Molecules*, 29(8):1-13.
22. Munawar N, Farina Y, Yaqoob M, Nabi A, and Shah SM. (2021). Distribution of pesticides in different commonly grown vegetables of Cameron Highlands, Pahang, Malaysia. *Sains Malaysiana*, 50(10): 2937-2944.
23. Ishak A, Pak-Dek MS, Rukayadi Y, Ramli NS, and Wasoh H. (2023). Evaluation of pesticide residues in selected vegetables from Kuala Lumpur, Malaysia using modified QuEChERS and assessment of washing methods. *International Food Resesearch Journal*, 30(5):1159-1170.
24. Buglak AA, Samokhvalov A V., Zherdev A V., and Dzantiev BB. (2020). Methods and applications of in silico aptamer design and modeling. *International Journal Molecular Sciences*, 21(22):1-25.
25. Anastassiades M, Lehotay SJ, and DS, Schenck

- FJ. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *Journal of AOAC International*, 86(2):412-431.
26. Xu G, Hou J, Zhao Y, et al. (2019). Dual-signal aptamer sensor based on polydopamine-gold nanoparticles and exonuclease I for ultrasensitive malathion detection. *Sensors Actuators, B Chemistry*, 287(1):428-436.
27. Villalonga A, María Pérez-Calabuig A, and Villalonga R. (2020). Electrochemical biosensors based on nucleic acid aptamers. *Analytical and Bioanalytical Chemistry*, 412:55-72.
28. Azri FA, Selamat J, Sukor R, et al. (2021). Determination of minimal sequence for zearalenone aptamer by computational docking and application on an indirect competitive electrochemical aptasensor. *Analytical and Bioanalytical Chemistry*, 413(15):3861-3872.
29. Board LR. (2023). *Laws of Malaysia: Food Act 1983 (Act 281) & Regulations*. 5 Feb 2023. International Law Book Services.
30. Xu T, Dai H, and Jin Y. (2020). Electrochemical sensing of lead(II) by differential pulse voltammetry using conductive polypyrrole nanoparticles. *Microchimica Acta*, 187(1): 4027.
31. Fu J, An X, Yao Y, Guo Y, and Sun X. (2019). Electrochemical aptasensor based on one step co-electrodeposition of aptamer and GO-CuNPs nanocomposite for organophosphorus pesticide detection. *Sensors Actuators, B Chemistry*, 287(2):503-509.
32. Sotnikov D V., Zherdev A V., Zvereva EA, Eremin SA, and Dzantiev BB. (2021). Changing cross-reactivity for different immunoassays using the same antibodies: Theoretical description and experimental confirmation. *Applied Sciences*, 11(14): 6581.
33. Zhang Q, Zhang W, Wang X, and Li P. (2010). Immunoassay development for the class-specific assay for types I and II pyrethroid insecticides in water samples. *Molecules*, 15(1):164-177.
34. Nimako C, Ikenaka Y, Akoto O, et al. (2021). Simultaneous quantification of imidacloprid and its metabolites in tissues of mice upon chronic low-dose administration of imidacloprid. *Journal of Chromatography A*, 1652:462350.