Aptamer-conjugated gold nanoparticles/ reduced graphene oxide for electrochemical detection of malathion

Safura Taufik[1,a,*], Muhammad Amir Irfan Azizan[2,b] and Jahwarhar Izuan Abdul Rashid[1,c]

[1]Centre for Defence Foundation Studies, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 5700 Kuala Lumpur, Malaysia

[2]Faculty of Defence Science and Technology, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 5700 Kuala Lumpur, Malaysia

[a]safura@upnm.edu.my, [b]3211512@alfateh.upnm.edu.my, [c]jahwarhar@upnm.edu.my

Keywords: chemical sensor, electrochemical, organophosphorous compound

Abstract.
The widespread use of organophosphorous (OP) compounds in environment has raised serious human health and environmental concerns. The OPs are commonly used as pesticides and insecticides, but also display potential to be employed as chemical warfare agents (CWAs) by terrorist. Thus, the development of chemical sensor with high sensitivity and selectivity towards OPs is of vital importance. Electrochemical sensor is one of the most useful techniques that has been reported for the detection of OPs due to its stability, potential to be miniaturized for on-site detection, and simple measurement procedure. Herein, we have developed an electrochemical sensor for detection of malathion that employs gold nanoparticles (AuNPs) decorated reduced graphene oxide (rGO) modified on screen printed carbon electrode (SPCE) as the sensing platform. Graphene oxide was electrochemically reduced on SPCE and further modified with AuNPs to produce AuNPs/rGO-modified SPCE which was then utilized for the immobilization of thiolated DNA aptamer by self-assembly method. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron micrograph (SEM) were utilized to show the successful surface modification of SPCE. The detection was carried out by using EIS and the result shows that the value of charge transfer resistance increased upon binding of malathion to the aptamer-modified electrode indicating the successful formation of malathion-aptamer complex that blocks the electron transfer. The sensor also demonstrated good selectivity for malathion against other OP compounds such as DMMP, paraoxon and phorate. This proposed electrochemical sensor provides a sensitive and selective detection of malathion which can be potentially used for application in real samples.

Introduction

Malathion is an organophosphate (OP) insecticide commonly used in agricultural fields and domestic settings for eradication and control of pests. The toxicity level of malathion in birds and mammals are considered moderate, however, due to widespread usage, their persistence in environment has raised serious human health and environmental concerns (Liu et al., 2019; Moore et al., 2010). Malathion and other OPs nerve agents (sarin, soman, tabun and VX) inhibit the activity of acetylcholinesterase (AChE), the enzyme involved in the hydrolysis of neurotransmitter acetylcholIne (Ach) and impact to the function of the central nervous system, eventually leading to respiratory paralysis and death (Mulchandani & Rajesh, 2011). Therefore, a method for the easy, sensitive, and rapid detection of malathion is necessary to be developed for both human health and environmental safety. There are traditional analytical methods have been developed for the detection of OP compounds including gas or liquid chromatoghraphy (Cai et al., 2021; Khalifa et al., 2017;

Sapahin et al., 2019), mass spectrometry (Banoub et al., 1995; Picó et al., 2004; Sun et al., 2018), and enzyme linked immune system analysis (Hongsibsong et al., 2020; Yue et al., 2022). Most of these methods are selective and accurate, but they require expensive instrumentation and time-consuming, as well as limitation due to poor stability of the enzyme or antibodies. cc (McConnell et al., 2020). Recently, there are great numbers of research on aptamer-based biosensors (aptasensor) have been developed for the determination of OPs (Liu et al., 2019). In addition to the advantage of aptasensor, coupling with the fast development of nanotechnology and analyst methods, such as optical and electrochemical aptasensors, have tremendously enhance the performance of biosensor specifically developed for the detection of OPs (Rapini & Marrazza, 2017). Previous works done on colorimetric assays based on gold nanoparticles (AuNPs) for detection of omethoate and malathion have been reported by Wang et al. (P. Wang et al., 2016) and Bala et al. (Bala et al., 2017), respectively. The detection of omethoate was carried out by observing and analyzing the color changes in AuNPs resulted from the disconnection of aptamer molecules from AuNPs in the presence on analyte, and also showed in their aggregation. By using the aptamer, this biosensor showed high selectivity towards omethoate, and good linearity between 0.1 uM and 10 uM, with limit of detection (LOD) of 0.1 uM (P. Wang et al., 2016). A similar approach was developed for colorimetric detection of malathion utilizing aptamer, cationic peptide and unmodified AuNPs. This aptasensor was found to be linear in the range of 0.01 – 0.75 nM with LOD of 1.94 pM (Bala et al., 2017).

While most electrochemical techniques have been employed in developing biosensors, electrochemical impedance spectroscopy (EIS) technique has recently gain an attention owing to its fast, label-free technique to measure the properties of electrode surfaces and bulk electrolytes (Bahadır & Sezgintürk, 2016). Combination of impedimetric technique with aptasensor in this proposed research work, allowing impedance changes following the binding of DNA aptamer to malathion without using enzyme labels. In recent years, label-free electrochemical impedance aptamer sensors have attracted the interest of researchers as an alternative strategy for the rapid and sensitive quantification of target analytes due to the advantages in terms of simplicity, sensitivity as well as simple operation(Malecka et al., 2021; Y. Wang et al., 2021). To best of our knowledge, this research work is the first to investigate the impedimetric aptasensing technique for the determination of malathion for potential application as VX nerve agent detector.

Another challenge in developing biosensor for the determination of malathion is the sensitivity of the aptasensor. The accuracy and sensitivity of the aptasensors are influenced directly by the amount of the immobilized aptamer on the surface of electrode (Oberhaus et al., 2020). The use of nanomaterials can be applied to overcome this challenge, and among of nanomaterials, graphene and gold nanoparticles are widely used because of its superior properties. By having larger surface area, high conductivity, and good biocompatibility (Wu et al., 2014), they can be used as material to fabricate the electrode surface for the development of aptasensor. By combining the unique properties of these hybrid nanomaterials with a specific DNA aptamer, we can amplify redox electrical signal and show high selectivity and sensitivity towards malathion. In this study, we propose DNA aptamer conjugated AuNPs/rGO as a novel molecular recognition sensing for impedimetric determination of malathion. This proposed research will be focused on the investigation of the impedimetric behaviour of DNA aptamer conjugated AuNPs/rGO nanocomposites-modified electrode towards the binding with malathion. Fig. 1 shows the chemical structure of malathion.

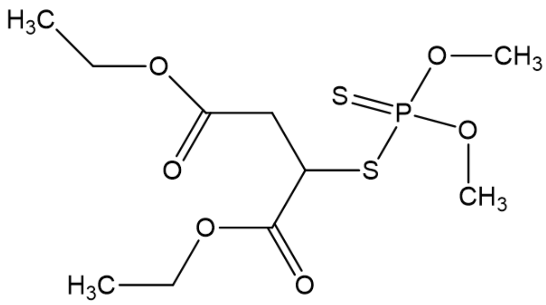

Figure 1. The chemical structure of malathion

Materials and Methodology

Materials

Graphene oxide (GO) (code: 777676) in water suspension (4 mg/ml), hydrogen tetrachloroaurate (III) trihydrate. gold chloroauric acid salt ($HAuCl_4.3H2O$), potassium ferricyanide (III) ($K_3[Fe(CN]_6$), sodium citrate, phosphatebuffered saline (PBS) were purchased from Merck, USA. The aptamer was purchased from Apical Scientific Sdn. Bhd. (Selanogr Malaysia); had the following sequence: *5'-ThioMC6-ATC CGT CAC ACC TGC TCT TAT ACA CAA TTG TTT TTC TCT TAA CTT CTT GAC TGC TGG TGT TGG CTC CCG TAT-3'* (Barahona et al., 2013). The aptamer contained a thiol C-6 modifier on the 5'-end for conjugation to gold nanoparticles attached to the surface of reduced graphene oxide. All other chemicals were of analytical reagent grades. Preparation of AuNPs suspension with an average diameter of 20 nm was prepared according to our previous work (Taufik et al., 2016). All aqueous reagents were prepared in sterilized ultrapure water (Milli Q ultrapure water system (18 M Ω cm^{-1}), Millipore Billerica, MA, USA).

Instrumentations

The electrochemical experiment was carried out using a screen-printed carbon electrode (SPCE) from Rapid Genesis (Malaysia) featuring Carbon as the working and auxiliary electrode and Ag/AgCl as a reference electrode. The electrochemical analysis was performed using a potentiostat/impedance analyzer (STAT-I 400s, Metrohm, Netherlands) controlled by electrochemistry software NOVA version 2.1 (Metrohm). The characterization of the modified electrode surface was performed using ZEISS GeminiSEM 500 Field Emission Scanning Electron Microscope (FESEM).

Preparation and charaterization of DNA aptamer conjugated AuNPs/rGO-modified electrode

The first part of the project was to synthesize materials that will be used for the preparation of sensing platform. In this stage, gold nanoparticles (AuNPs) was synthesized, and transmission electron microscopy (TEM) and UV-absorbance was used for characterisation.

The SPCEs were prepared with 15 µL graphene oxide (2 mg/mL GO) and left overnight to bind, and then electrochemically reduced to rGO-modified SPCE by using cyclic voltammetry (CV) technique in 0.1 M KCl solution, pH 7.4 for 11 cycles with a potential range of -2.0 V to 0.0 V vs. Ag/AgCl at a scan rate of 50 mV/s until a stable CV response was observed at both oxidation and reduction potential. The modified electrode was rinsed with distilled water and dried at room temperature. Following that, AuNPs is drop casted on rGO-modified SPCE for minimum of 2 hours at room temperature. The modified electrode after this step is denoted as AuNPs/rGO-modified SPCE. Several characterizations of the prepared rGO/AuNP-modified SPCE were carried out to determine the successful modification of gold electrode with the hybrid nanomaterials of rGO and AuNP including electrochemical technique, and surface morphology studies.

The thiolated DNA aptamer was then immobilized on the AuNP/rGO-modified electrode via the S-Au bond forming DNA aptamer/AuNP/rGO-modified AuE. Further surface characterization at this stage was carried out to confirm the attachment of DNA aptamer to the AuNP/rGO-modified electrode. CV and EIS were used for the electrochemical behaviour studies, while FTIR will be used for the elemental composition study. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) was used to monitor the electrochemical behaviour of the stepwise modification process. Fig. 2 shows the schematic diagram of stepwise electrode surface modification to produce Apt/rGO/AuNP-SPCE.

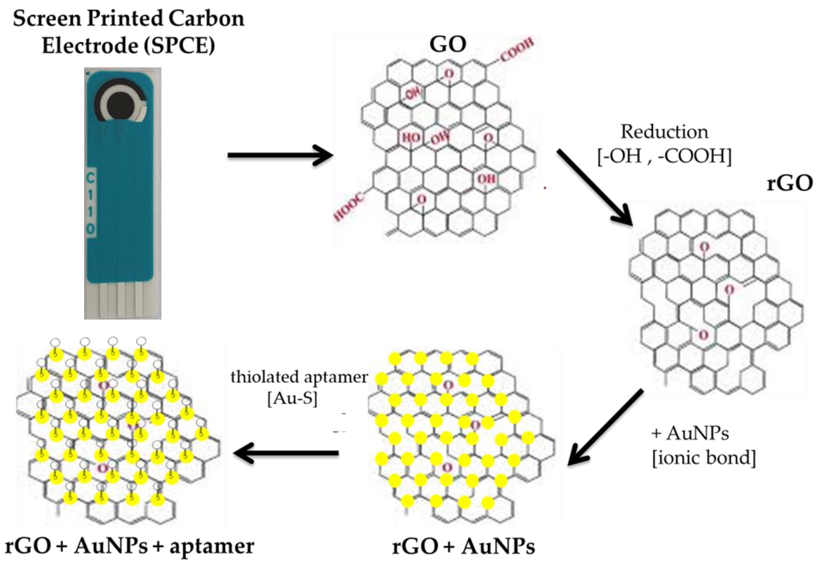

Figure 2. Schematic diagram of the fabrication of sensing interface for detection of malathion.

Electrochemical behaviour of the DNA aptamer/AuNPs/rGO-modified electrode towards the interaction with malathion

The aptamer selection for specific binding to target OP (malathion) has been reported previously where the aptamer was developed using the modified Systematic Evolution of Ligands by Exponential enrichment (SELEX) method primarily developed in 1990 (Ellington & Szostak, 1990). In this stage, the ability for the DNA aptamer/AuNPs/rGO-modified electrode to capture malathion was studied by using the electrochemical impedance spectroscopy (EIS) which it measures the charge-transfer resistance (R_{ct}) value before and after the aptamer-malathion binding in the presence of a redox species to provide the electrochemical response.

Results and Discussion

Surface morphological studies of AuNPs/rGO-modified SPCE

Surface morphology of AuNPs/rGO-modified SPCE was characterized by SEM. Fig. 3 depicts the attachment of AuNPs on the rGO layer with an even distribution of the nanoparticles that has an average diameter of 20 nm.

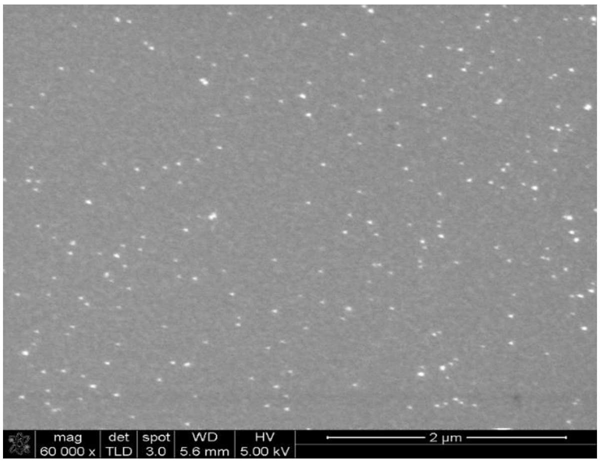

Figure 3. SEM images of AuNPs/rGO-SPCE at 60k magnification.

Electrochemical characterization of different modified electrodes

Electrochemical characterization of the electrodes were carried out by using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Fig. 4 shows the cyclic voltammogram of each modification step in 5 mM ferri/ferrocyanide solution prepared in 0.1 M KCl. In the CV, it was observed that AuNPs/rGO-modified SPCE (yellow curve) recorded the highest anodic and cathodic peak, 248.0667 µA and -250.45 µA at 0.108 V and -0.02 V, respectively as compared to rGO- or GO-modified SPCE. This indicates that the couple of AuNPs to rGO could enhance the electron transfer between the redox species and electrode surface.

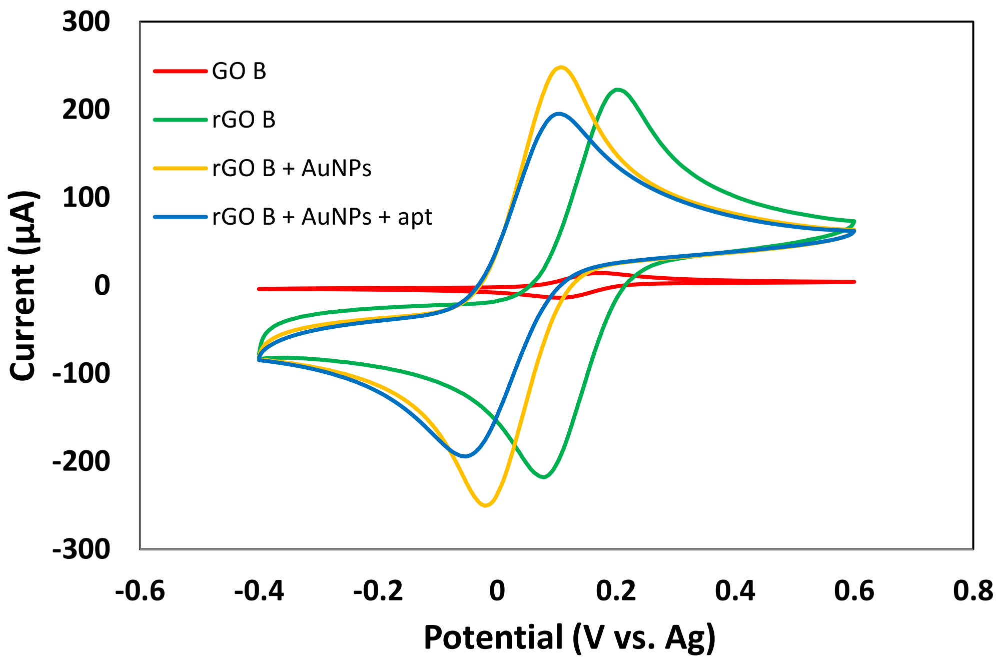

Figure 4. CV of the different modified electrode in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl. Scan rate: 100 mV s^{-1}

Nyquist plot observed from the same modified surfaces was further confirmed the result obtained in CV as shown in Fig. 5. The Nyquist plots including a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear part at lower frequency range representing the diffusion-limited process (Su et al., 2012) [15]. Charge transfer resistance (Rct) of the electrode can be obtained from the diameter of the semicircle. The curves of rGO/SPCE and

AuNPs/rGO-SPCE exhibits smaller radius of semicircles compared to GO/SPCE and bare SPCE, which showed that the result obtained is in a good agreement with CV in Fig. 4.

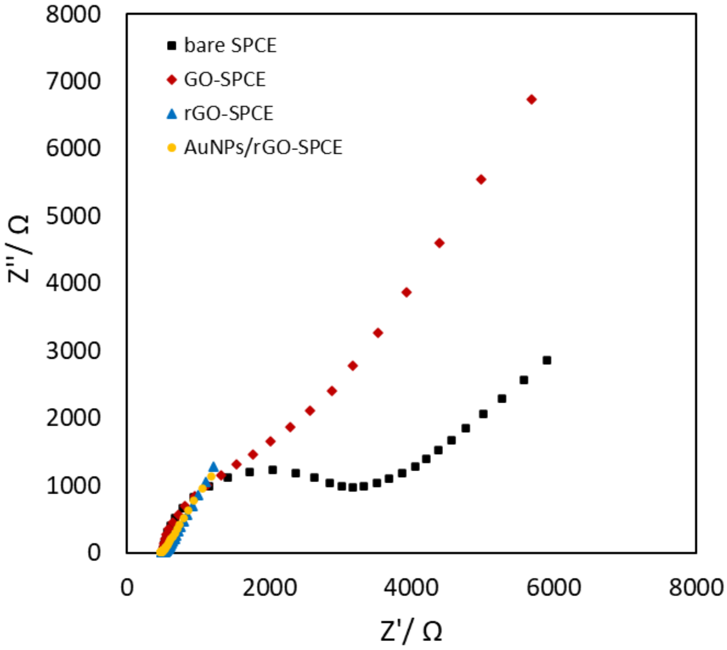

Figure 5. Nyquist plot of different modified surfaces in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl.

Electrochemical behaviour of redox probe on DNA aptamer conjugated AuNPs/rGO-modified SPCE upon binding to malathion

Further electrochemical characterization was carried out to compare the radius of semicircle of DNA aptamer attached to AuNPs/rGO-modified SPCE before and after binding to malathion as shown in Fig. 6. The large increase of semicircle after the attachment of aptamer indicates the successful formation of Apt/AuNPs/rGO-modified SPCE (curve a) which hindering the electron transfer. When the malathion has been captured by the aptamer, the semicircle of the EIS curve significantly increased (curve b), suggesting that the malathion on the modified electrode has block the electron transfer.

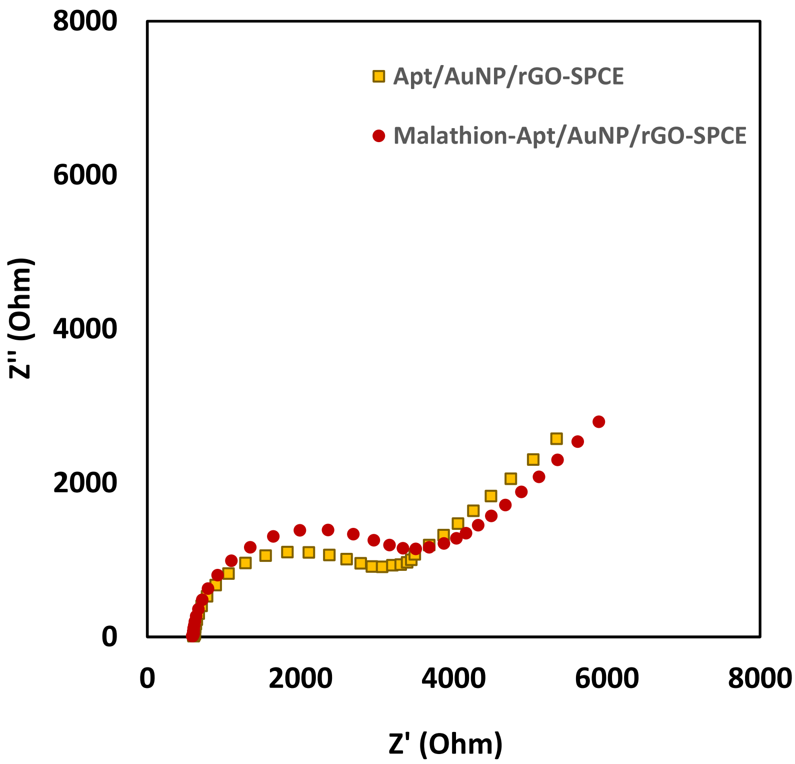

Fig 6. Nyquist plot of Apt/AuNPs/rGO-modified SPCE before (a), and after binding to malathion (b), in 5 mM $[Fe(CN)_6]^{3-/4-}$ in 100 mM KCl. Scan rate: 100 mV s^{-1}.

Conclusion and Future Direction

An aptamer-based functionalized AuNPs/rGO-SPCE was developed for the electrochemical determination of malathion. The selected aptamer exhibits high affinity to malathion, which was covalently bonded to AuNPs via thiolated end of the DNA aptamer. The stepwise modification of SPCE was characterized by CV and EIS, as well as SEM for the morphological studies. Based on the results obtained, AuNPs/rGO-modified SPCE provide rather a good sensing platform which was shown in the increase in electrochemical response, due to the couple of AuNPs and rGO that enhance the electron transfer. The results from EIS also demonstrate that the Apt/AuNPs/rGO-SPCE can bind malathion which is observed from the increase of semicircle in Nyquist plot. To generate a highly sensitive method with low detection limit for the determination of malathion, several parameter studies will be carried out to choose the optimum condition for the construction of aptasensors. These include the aptamer concentration, the reaction time, and the binding time between DNA aptamer and malathion.

Acknowledgement

The authors are grateful to the Ministry of Higher Education Malaysia for the financial support through RACER/1/2019/STG01/UPNM//1 under the Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (FRGS-RACER).

References

Bahadır, E. B., & Sezgintürk, M. K. (2016). A review on impedimetric biosensors. *Artificial Cells, Nanomedicine, and Biotechnology*, *44*(1), 248–262. https://doi.org/10.3109/21691401.2014.942456

Bala, R., Dhingra, S., Kumar, M., Bansal, K., Mittal, S., Sharma, R. K., & Wangoo, N. (2017). Detection of organophosphorus pesticide – Malathion in environmental samples using peptide and aptamer based nanoprobes. *Chemical Engineering Journal*, *311*, 111–116. https://doi.org/https://doi.org/10.1016/j.cej.2016.11.070

Banoub, J., Gentil, E., & Kiceniuk, J. (1995). Analysis of Organophosphorus Pesticide Residues by Low Energy Tandem Mass Spectrometry Using Electrospray Ionization. *International Journal of Environmental Analytical Chemistry*, *61*(2), 143–167. https://doi.org/10.1080/03067319508026244

Barahona, F., Bardliving, C. L., Phifer, A., Bruno, J. G., & Batt, C. A. (2013). An Aptasensor Based on Polymer-Gold Nanoparticle Composite Microspheres for the Detection of Malathion Using Surface-Enhanced Raman Spectroscopy. *Industrial Biotechnology*, *9*(1), 42–50. https://doi.org/10.1089/ind.2012.0029

Cai, Y., Wang, J., Zou, Q., Zhang, W., Chen, S., Liu, W., & Zhang, L. (2021). In situ hydrothermal growth of metal-organic gels coating for efficient solid-phase microextraction of pesticide residues. *Talanta Open*, *4*, 100077. https://doi.org/https://doi.org/10.1016/j.talo.2021.100077

Ellington, A. D., & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature*, *346*(6287), 818–822. https://doi.org/10.1038/346818a0

Hongsibsong, S., Prapamontol, T., Xu, T., Hammock, B. D., Wang, H., Chen, Z.-J., & Xu, Z.-L. (2020). Monitoring of the Organophosphate Pesticide Chlorpyrifos in Vegetable Samples from Local Markets in Northern Thailand by Developed Immunoassay. *International Journal of Environmental Research and Public Health*, *17*(13), 4723. https://doi.org/10.3390/ijerph17134723

Khalifa, M. E., Kenawy, I. M. M., Abou El-Reash, Y. G., & Abdallah, A. B. (2017). Extractive separation of Profenofos as an organophosphorous insecticide from wastewater and plant samples using molecular imprinted cellulose. *Journal of Environmental Chemical Engineering*, *5*(4), 3447–3454. https://doi.org/https://doi.org/10.1016/j.jece.2017.07.012

Liu, M., Khan, A., Wang, Z., Liu, Y., Yang, G., Deng, Y., & He, N. (2019). Aptasensors for pesticide detection. *Biosensors and Bioelectronics*, *130*, 174–184. https://doi.org/https://doi.org/10.1016/j.bios.2019.01.006

Malecka, K., Mikuła, E., & Ferapontova, E. E. (2021). Design Strategies for Electrochemical Aptasensors for Cancer Diagnostic Devices. *Sensors (Basel, Switzerland)*, *21*(3), 736. https://doi.org/10.3390/s21030736

McConnell, E. M., Nguyen, J., & Li, Y. (2020). Aptamer-Based Biosensors for Environmental Monitoring. *Frontiers in Chemistry*, *8*. https://www.frontiersin.org/article/10.3389/fchem.2020.00434

Moore, P. D., Yedjou, C. G., & Tchounwou, P. B. (2010). Malathion-induced oxidative stress, cytotoxicity, and genotoxicity in human liver carcinoma (HepG2) cells. *Environmental Toxicology*, *25*(3), 221–226. https://doi.org/10.1002/tox.20492

Mulchandani, A., & Rajesh. (2011). Microbial Biosensors for Organophosphate Pesticides. *Applied Biochemistry and Biotechnology*, *165*(2), 687–699. https://doi.org/10.1007/s12010-011-9288-x

Oberhaus, F. v, Frense, D., & Beckmann, D. (2020). Immobilization Techniques for Aptamers on Gold Electrodes for the Electrochemical Detection of Proteins: A Review. *Biosensors*, *10*(5), 45. https://doi.org/10.3390/bios10050045

Picó, Y., Blasco, C., & Font, G. (2004). Environmental and food applications of LC–tandem mass spectrometry in pesticide-residue analysis: An overview. *Mass Spectrometry Reviews*, *23*(1), 45–85. https://doi.org/https://doi.org/10.1002/mas.10071

Rapini, R., & Marrazza, G. (2017). Electrochemical aptasensors for contaminants detection in food and environment: Recent advances. *Bioelectrochemistry*, *118*, 47–61. https://doi.org/https://doi.org/10.1016/j.bioelechem.2017.07.004

Sapahin, H. A., Makahleh, A., & Saad, B. (2019). Determination of organophosphorus pesticide residues in vegetables using solid phase micro-extraction coupled with gas chromatography–flame photometric detector. *Arabian Journal of Chemistry*, *12*(8), 1934–1944. https://doi.org/https://doi.org/10.1016/j.arabjc.2014.12.001

Su, W., Lin, M., Lee, H., Cho, M., Choe, W.-S., & Lee, Y. (2012). Determination of endotoxin through an aptamer-based impedance biosensor. *Biosensors & Bioelectronics*, *32*(1), 32–36. https://doi.org/10.1016/j.bios.2011.11.009

Sun, P., Gao, Y. L., Xu, C., & Lian, Y. F. (2018). Determination of six organophosphorus pesticides in water samples by three-dimensional graphene aerogel-based solid-phase extraction combined with gas chromatography/mass spectrometry. *RSC Advances*, *8*(19), 10277–10283. https://doi.org/10.1039/C7RA13316B

Taufik, S., Barfidokht, A., Alam, M. T., Jiang, C., Parker, S. G., & Gooding, J. J. (2016). An antifouling electrode based on electrode–organic layer–nanoparticle constructs: Electrodeposited organic layers versus self-assembled monolayers. *Journal of Electroanalytical Chemistry*, *779*, 229–235. https://doi.org/https://doi.org/10.1016/j.jelechem.2016.01.031

Wang, P., Wan, Y., Ali, A., Deng, S., Su, Y., Fan, C., & Yang, S. (2016). Aptamer-wrapped gold nanoparticles for the colorimetric detection of omethoate. *Science China Chemistry*, *59*(2), 237–242. https://doi.org/10.1007/s11426-015-5488-5

Wang, Y., Chen, L., Xuan, T., Wang, J., & Wang, X. (2021). Label-free Electrochemical Impedance Spectroscopy Aptasensor for Ultrasensitive Detection of Lung Cancer Biomarker Carcinoembryonic Antigen. *Frontiers in Chemistry*, *9*. https://www.frontiersin.org/article/10.3389/fchem.2021.721008

Wu, L., Xiong, E., Zhang, X., Zhang, X., & Chen, J. (2014). Nanomaterials as signal amplification elements in DNA-based electrochemical sensing. *Nano Today*, *9*(2), 197–211. https://doi.org/https://doi.org/10.1016/j.nantod.2014.04.002

Yue, Y., Chen, J., Zhang, M., Yin, Y., & Dong, Y. (2022). Determination of Organophosphorus Pesticides in Vegetables and Fruit by an Indirect Competitive Enzyme-Linked Immunosorbent Assay (ic-ELISA) and a Lateral-Flow Immunochromatographic (LFIC) Strip Assay. *Analytical Letters*, *55*(11), 1701–1718. https://doi.org/10.1080/00032719.2021.2023170