Development of electrochemical sensors for detection of organophosphate pesticides in fruits and vegetables: A review

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Abstract. The use of pesticides for agricultural cultivation may prevent losses due to pest attacks, but excessive and uncontrolled use of pesticides may cause contamination of pesticide residues in the fruits and vegetables. Organophosphate pesticides are highly toxic and their residue in fruits and vegetables may cause neurological disorders in humans. Organophosphate residues may enter the body of animals and humans through oral routes or respiratory systems. Common analytical methods to detect organophosphate require complex sample preparation and sophisticated equipment. Electrochemical detection methods are an alternative in developing organophosphate detection methods in fruits and vegetables. This review provides an overview of the development and performance of electrochemical sensor technology to detect organophosphate residues in fruits and vegetables.

1. Introduction
Pesticides are one of the chemicals that are widely used as a pest control in agricultural cultivation. Organophosphate pesticides (OPs) are one of the most widely used pesticides in the world due to their high effectiveness for insect eradication [1]. Chemical compounds included in the OPs group include chlorpyrifos, diazinon, malathion, and parathion [2]. Excessive and uncontrolled use of pesticides in the agricultural sector causes contamination of pesticide residues in the environment and various food commodities [3]. The presence of OPs in food commodities circulating in traditional markets has been reported by some researchers, including chlorpyrifos residues reported to be contained in cabbage, tomatoes, cayenne pepper, and carrots [4,5]; and diazinon residues that exceed the threshold were found in long beans [6].

Organophosphate compounds affect human health by inhibiting the action of the enzyme acetylcholinesterase (AChE) which acts on the central nervous system [7]. AChE enzyme plays a vital role in central nervous system function and has a major role in the transmission of stimulation between nerve cells. The poisoning of organophosphate compounds permanently inhibits the activity of the AChE enzyme, thereby inhibiting the action of acetylcholine as the main neurotransmitter in the central nervous system [8]. Inhibition of AChE and the action of neurotransmitters causes muscle paralysis, seizures, and organ damage that can lead to death [9,10].

Analytical methods for the detection of OPs such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) are still considered to be the most reliable technique because of their high selectivity and sensitivity [11,12]. However, this method requires trained personnel, requires a long time for preparation and analysis, expensive equipment, and is difficult to apply for on-site detection [13]. Thus, a simple, rapid and inexpensive OPs detection method which can be applied for on-site
detection of food commodities is needed to ensure public safety and health. Electrochemical sensors have been widely developed for the detection of organophosphate residues in food commodities. This method has several advantages, including being relatively inexpensive, can be used for cloudy samples, and easy to develop into portable equipment [14]. The aim of this review is to provide an overview of the development of electrochemical sensors technology to detect OPs residues in fruits and vegetables.

2. Electrochemical sensors

Chemical and biological sensors are devices that utilize a chemically or biologically responsive sensing layer to recognize the target analyte and convert the response into an analytically useful signal. The sensors consist of two main parts, a recognition element (receptor) that is a sensitive sensing layer to stimuli produced by the target analyte and a transduction element (transducer) that functions to convert a change or activity of recognition element into a measurable signal whose magnitude is related to the concentration of target analyte [15]. Transducers in a sensor device can be divided into three types: mass-based, optical, and electrochemical transducer [16]. Electrochemical transducer-based sensors gained interest by many researchers because they have high sensitivity, are inexpensive, rapid detection, and can be developed into portable equipment which makes it possible to develop an analytical instrument for on-site detection [14].

Electrochemical sensors can be classified into four groups based on different parameters for measurement: amperometric, potentiometric, impedimetric, and conductometric [16]. Conductometric sensor analysis is based on measuring the electrical conductivity of the sample solution between two electrodes which is affected by the activity of the receptors [17]. The potentiometric detection method is based on the difference between the working electrode and the reference electrode when the current is close to zero [16]. Among several mechanisms of electrochemical transduction, amperometric and impedimetric sensors to detect organophosphates in fruits and vegetables garnered the largest research interest, while the development of potentiometric and conductometric sensors is very limited. This review presents the results of the latest research on amperometric and impedimetric sensors to detect organophosphate residues in fruits and vegetables.

3. Amperometric sensors for organophosphate detection

Amperometric sensors measure the current as a result of reduction and oxidation reactions between the electrodes and the analytes at a fixed potential. The methods in the amperometric analysis include cyclic voltammetry (CV), squarewave voltammetry (SWV), differential pulse voltammetry (DPV) and chronoamperometry [18]. CV is used to measure the alternating current produced by providing two potential differences in a cycle. DPV and SWV were used to measure the current generated based on the analyte concentration measured by applying a sequence of pulses by increasing the voltage stepwise [19]. Chronoamperometry is an electrochemical analysis in which the voltage applied to the working electrode is increased gradually and the faradaic current that appears at the electrode is measured as a function of time [20].

CV is the simplest method which has a high sensitivity up to micromolar analyte concentration. The SWV and DPV methods have a higher sensitivity than CV, which have detection sensitivity to nanomolar analyte concentrations. In the SWV and DPV methods, the effect of charging current that interferes with faradaic current readings can be minimized. Charging current arises due to the formation of a microscopic double layer, because of the presence of molecules oriented on the surface of the working electrode. The microscopic double layer has properties like a capacitor and produces a charging current [21]. Table 1 shows the developed amperometric sensors for the detection of organophosphates in fruits and vegetables.

The development of detection methods for organophosphate compounds generally utilizes the enzyme acetylcholinesterase (AChE) as a receptor. AChE hydrolyzes acetylthiocholine chloride to produce thiocholine [22]. Thiocholine is an electroactive compound that can be detected by an amperometric method. The presence of organophosphate compounds will inhibit the activity of the AChE and reduce the concentration of thiocholine. Thus, the amperometric signal from the oxidation of
thiocholine compounds can be used to measure the concentration of organophosphates. Mahmoudi et al. [23] reported the use of AChE to detect paraoxon compounds with high sensitivity. The application of this method on spinach and cabbage was able to detect the presence of paraoxon compounds at a concentration of 10 nM.

Xu et al. [24] developed a highly sensitive electrochemical sensor for the detection of chlorpyrifos with a limit of detection (LOD) of 70 pg/mL. The high sensitivity of the method was obtained by exploiting the synergistic effect between copper oxide nanoflowers (CuO NFs) and carboxyl-functionalized single-walled carbon nanotubes (c-SWCNTs). CuO NFs function to increase the electrode surface area and increase electron transfer, while s-SWCNTs function to increase the effectiveness and stability of aptamer immobilization as a receptor on the electrode surface. The real sample analysis of this method on apples and chicory can detect chlorpyrifos with a concentration of 1 ng/mL.

Table 1. Amperometric sensors for organophosphate detection in fruits and vegetables.

| Pesticides                  | Food commodities       | Recognition element                                      | Method  | LOD             | Ref.  |
|-----------------------------|------------------------|----------------------------------------------------------|---------|-----------------|-------|
| Methyl paraoxon, methyl parathion | Tomato, apple, grape, green pepper, tomato | Lab-on-a-glove organophosphate hydrolase                  | CV, SWV | -               | [25]  |
| Malathion                   | Apple, tomato, cucumber, carrot, tomato, lettuce, cucumber, apple, spinach | Gold nanoparticles-chitosan-ionic liquid nanocomposite | CV, SWV | 0.68 nM         | [26]  |
| Malathion                   | Apple, tomato, cucumber, carrot, tomato, lettuce, cucumber, apple, spinach | AChE/HSC-PANI nanocomposite                               | CV, SWV | 0.16 ng/mL      | [27]  |
| Diazinon                    | Cabbage, spinach        | Au-Pt nanoclusters-graphene nanoribbons                   | CV, SWV | 0.002 µM        | [28]  |
| Chlorpyrifos, methyl parathion, fenthion Chlorpyrifos | Apple, celery cabbage, tomato juice | CuO NFs-SWCNTs nanocomposite                              | DPV     | 70 pg/mL        | [24]  |
| Parathion                   | Tomato juice           | Nickel oxide nanoplatelets                                | DPV     | 0.024 µM        | [30]  |
| Chlorpyrifos                | cucumber, capsicum, brinjal | AChE-MOF/µE                                              | DPV     | 6 ng/L          | [31]  |
| Paraoxon                    | Spinach, cabbage       | AChE/Ce/UiO-66/MWCNTs                                     | DPV     | 0.004 nM        | [23]  |
| Methyl parathion            | Lettuce, cabbage       | Lipase@MOF nanofibers                                     | DPV     | 0.067 µM        | [32]  |
| Methyl parathion            | Strawberry and apple juices, orange juice | Semiconducting SWCNT SWCNH-ZE/GCE | SWV     | 0.0375 nM       | [33]  |
| Fenitrothion                | Orange juice           | GCE/VS2QDs-GNP/CMWCNTs/DZBA/BSA aptasensor                | DPV     | 11 fmol/L       | [35]  |
One of the advantages of electrochemical sensors is the ease of development into mini and portable equipment. Mishra et al. [25] developed an electrochemical sensor in gloves that can be used to detect organophosphate compounds on the surface of food products. The use of this method can detect the presence of a solution of methyl parathion (MP) and methyl paraoxon (MPOx) with a concentration of 200 µM on the surface of fruits and vegetables.

Khosropour et al. [35] developed a DPV method using vanadium disulfide quantum dots-graphene nanoplatelets/carboxylated multiwalled carbon nanotubes (VS2QDs-GNP/CMWCNTs) nanocomposites to detect diazinon compounds. The use of nanocomposite has some advantages, that is increasing the quantity of immobilized aptamers as diazinon receptors, increasing conductivity and accelerating electron transfer. This DPV technique has high sensitivity which can detect diazinon with a detection limit of 11 fmol/L and real sample detection on apples and lettuce were able to detect diazinon at a concentration of 1 pmol/L.

4. Impedimetric sensors for organophosphate detection

The principle of impedimetric sensors is based on the changes in conductance and capacitance on the surface of the working electrode which are influenced by the activity of receptors because of the presence of the target analyte [36]. Electrochemical impedance spectroscopy (EIS) is a method that has been widely developed for the detection of organophosphate residues in food commodities. The development of impedimetric sensors for the detection of organophosphates in food commodities is shown in Table 2.

Malvano et al. [37] proposed impedimetric enzyme inhibition-based sensors for the detection of organophosphate. This technique is found to be a rapid method with high sensitivity with an LOD of 2.5 ppb and has the possibility to apply in lettuce samples to detect dichlorvos at a concentration of 25 ppb. A different method to detect organophosphate was reported by Zare et al. [38], which uses the interaction between diazinon and ds-DNA as a sensing mechanism. In this work, they found the LOD was estimated to be 0.3 nmol/L and this method is highly sensitive which has been performed to detect diazinon in lettuce and tomato juice at a concentration of 10 nmol/L.

Highly sensitive detection methods can be achieved by signal amplification. Signals originating from receptor activity are multiplied by converting these signals into other chemical information through the activity of a catalyst [39]. Hou et al. [40] reported the development of an EIS method with signal amplification using an enzyme catalytic process. This method has a very low detection limit of chlorpyrifos, which is 0.07 pg/mL and the applications of this method in mustard greens and lettuce were able to detect chlorpyrifos at a concentration of 1 pg/mL. Different mechanism of signal amplification strategy was reported by Xu et al. [41], which uses autocatalytic target cycling amplification. This mechanism allows one molecule of malathion as a target analyte to continuously produce electrochemical signals by a cascade amplification. This technique produces a highly sensitive method with an LOD of 0.5 ng/L and real sample detection on cauliflower and cabbage were able to detect malathion at a concentration of 50 ng/L.

Table 2. Impedimetric sensors for organophosphate detection in fruits and vegetables.

| Pesticides  | Food commodities | Recognition element | LOD        | Ref. |
|------------|------------------|---------------------|------------|------|
| Dichlorvos | Lettuce          | Monoenzymatic AChE  | 2.5 ppb    | [37] |
|            |                  | MWCNT/poly-L-lysine ds-DNA | 0.3 nmol/L | [38] |
| Diazinon   | Lettuce juice, tomato juice | Anti CPF-HRP-AuNP-BSA | 0.070 pg/mL | [40] |
| Chlorpyrifos | Chinese cabbage, lettuce | PDA-AuNP | 0.5 ng/L | [41] |
| Malathion  | Cauliflower, cabbage |                       |            |      |
5. Conclusions

The development of electrochemical sensors to detect the presence of organophosphate residues in food commodities is a field of research that is needed to ensure food safety. Efforts to produce electrochemical sensors that are sensitive, fast, inexpensive, and easy to use have been reported previously. Several strategies can be used to increase the sensitivity of the sensor, including increasing the effectiveness of immobilization and the stability of receptors on the electrode surface, modifying the electrode by using nanomaterials to expand the active surface between the electrode and the substrate, and employing a signal amplification strategy by using a catalyst. However, this strategy still requires improvement because the immobilization of receptors is susceptible to decrease the activity of the receptors, nanomaterial modification is still constrained by the repeated use of modified electrodes, and signal amplification strategies need to pay attention to the stability and solubility of chemical compounds as signal carriers. That is a great challenge in this research field to enhance the sensitivity and reproducibility without decreasing the stability of the sensors. A new design and application of electrochemical sensors which employ a novel nanostructure, immobilization technique or signal amplification strategy is believed to have contributed towards improving sensitivity and stability.

The development of organophosphate detection methods in food commodities that are portable and can be used directly at the sampling site (on-site detection) is still needed to enhance the performance of the sensors. In the future, with increasing awareness of food safety, this technology will be needed by farmers and end-users for ease of checking food product quality. Moreover, the large food processing industries and wholesalers may be needing the sensor for quality assessment and task management. For commercialization, there is still a lot of scope for improvement in fabrication, cost, and feasibility to be used in the field. In addition, the next generation of electrochemical sensors for the detection of organophosphate residues in foods is expected to have a short detection time, low price, and high sensitivity and reproducibility.

References
[1] Aragay G, Pino F and Merkoçi A 2012 Nanomaterials for sensing and destroying pesticides Chem. Rev. 112 10 5317-38
[2] Thanos C A A, Tomuka D and Mallo N T S 2016 Livor mortis pada keracunan insektisida golongan organofosfat di kelinci (Livor mortis on organophosphate insecticide poisoning in rabbits) E-CliniC 4 1 [In Indonesian]
[3] Carvalho F P 2017 Pesticides, environment, and food safety Food Energy Secur. 6 48–60
[4] Saiya A 2017 Analisis residu klorpirifos dalam sayuran kubis dengan metode hplc di beberapa pasar tradisional di Sulawesi Utara (Analysis of clorpirifos residual in cabbage with hplc method in some traditional markets in North Sulawesi) EKSAKTA Berk. Ilm. Bid. MIPA 18 77–85 [In Indonesian]
[5] Saiya A, Gumolung D and Caroles J D S 2018 Analisis residu pestisida dalam tomat, cabai rawit dan wortel dari beberapa pasar tradisional di Sulawesi Utara (Analysis of pesticide residue in tomatoes, Cayenne pepper and carrots from some traditional markets in North Sulawesi) Fuller. J. Chem. 3 63 [In Indonesian]
[6] Harsojo H and Chairul S M 2011 Kandungan mikroba patogen, residu insektisida organofosfat dan logam berat dalam sayuran (Pathogenic microbes, organophosphate insecticide residues and heavy metals content in Vegetables) J. Ecolab. 5 89–95 [In Indonesian]
[7] Santoni G, De Sousa J, De La Mora E, Dias J, Jean L, Sussman J L, Silman I, Renard P Y, Brown R C D, Weik M, Baati R and Nachon F 2018 Structure-based optimization of nonquaternary reactivators of acetylcholinesterase inhibited by organophosphorus nerve agents J. Med. Chem. 61 17 7630-9
[8] Watanabe H, Satake A, Kido Y and Tsuji A 2002 Monoclonal-based enzyme-linked immunosorbent assay and immunochromatographic assay for enrofloxacin in biological matrices Analyst 127 98–103
[9] Gazzi E N, Sorodoc V, Jaba I M, Lionte C, Bologa C, Lupusoru C E, Lupusoru R, Sorodoc L and
Petris O 2015 Profile of adult acute cholinesterase inhibitors substances poisoning - a 30 years analysis Open Med. 10 1
[10] Hassani S, Motmtaz S, Vakhshiteh F, Maghsoudi A S, Ganjali M R, Norouzi P and Abdollahi M 2017 Biosensors and their applications in detection of organophosphorus pesticides in the environment Arch. Toxicol. 91 109–30
[11] Zhang W, Asiri A M, Liu D, Du D and Lin Y 2014 Nanomaterial-based biosensors for environmental and biological monitoring of organophosphorus pesticides and nerve agents TrAC - Trends Anal. Chem. 54 1–10
[12] Tang Q, Wang X, Yu F, Qiao X and Xu Z 2014 Simultaneous determination of ten organophosphate pesticide residues in fruits by gas chromatography coupled with magnetic separation J. Sep. Sci. 37 7 820–7
[13] Jin M, Zhu G, Jin R, Liu S, Shao H, Jin F, Guo Y and Wang J 2013 A sensitive chemiluminescent enzyme immunoassay for carbofuran residue in vegetable, fruit and environmental samples Food Agric. Immunol. 24 345–56
[14] Mubarak A Z, Mani V, Huang C H, Chang P C and Huang S T 2017 Label-free electrochemical detection of neuraminidase activity: A facile whole blood diagnostic probe for infectious diseases Sensors Actuators, B Chem. 252 641–8
[15] Sekhar P K, Brosha E L, Mukundan R and Garzon F H 2010 Chemical sensors for environmental monitoring and homeland security Electrochem. Soc. Interface. 19 4 35
[16] Velusamy V, Arshak K, Korostynska O, Oliwa K and Adley C 2010 An overview of foodborne pathogen detection: In the perspective of biosensors Biotechnol. Adv. 28 232–54
[17] Chen Z G 2008 Conductometric immunoassays for the detection of staphylococcal enterotoxin B based bio-electrolytic reaction on micro-comb electrodes Bioprocess Biosyst. Eng. 31 345–50
[18] Hernandez-Vargas G, Sosa-Hernández J E, Saldarriaga-Hernandez S, Villalba-Rodriguez A M, Parra-Saldivar R and Iqbal H M N 2018 Electrochemical biosensors: A solution to pollution detection with reference to environmental contaminants Biosensors 8 1–21
[19] Pérez-Fernández B, Costa-Garcia A and De La Escosura-Muñiz A 2020 Electrochemical (bio)sensors for pesticides detection using screen-printed electrodes Biosensors 10 4 32
[20] Scott K 2016 Electrochemical principles and characterization of bioelectrochemical systems Microbial Electrochemical and Fuel Cells (Woodhead Publishing) pp 29–66
[21] Miraceski V, Skrzypek S and Stojanov L 2018 Square-wave voltammetry ChemTexts 4 4 1–14
[22] Arduini F, Cinti S, Scognamiglio V and Moscone D 2016 Nanomaterials in electrochemical biosensors for pesticide detection: advances and challenges in food analysis Microchim. Acta 183 2063–83
[23] Mahmoudi E, Fakhri H, Hajian A, Afkhami A and Bagheri H 2019 High-performance electrochemical enzyme sensor for organophosphate pesticide detection using modified metal-organic framework sensing platforms Bioelectrochemistry 130 107348
[24] Xu G, Huo D, Hou C, Zhao Y, Bao J, Yang M and Fa H 2018 A regenerative and selective electrochemical aptasensor based on copper oxide nanoflowers-single walled carbon nanotubes nanocomposite for chlorpyrifos detection Talanta 178 1046–52
[25] Mishra R K, Hubble L J, Martin A, Kumar R, Barfidokht A, Kim J, Musameh M M, Kyratzis I L and Wang J 2017 Wearable Flexible and Stretchable Glove Biosensor for On-Site Detection of Organophosphorus Chemical Threats ACS Sensors 2 553–61
[26] Bolat G and Abaci S 2018 Non-enzymatic electrochemical sensing of malathion pesticide in tomato and apple samples based on gold nanoparticles-chitosan-ionic liquid hybrid nanocomposite Sensors 18 3 773
[27] He L, Cui B, Liu J, Song Y, Wang M, Peng D and Zhang Z 2018 Novel electrochemical biosensor based on core-shell nanostructured composite of hollow carbon spheres and polyaniline for sensitively detecting malathion Sensors Actuators B Chem. 258 813–21
[28] Pajooheshpour N, Rezaei M, Hajian A, Afkhami A, Sillanpää M, Arduini F and Bagheri H 2018
Protein templated Au-Pt nanoclusters-graphene nanoribbons as a high performance sensing layer for the electrochemical determination of diazinon Sensors Actuators B Chem. 275 180–9

[29] Tunesi M M, Kalwar N, Abbas M W, Karakus S, Soomro R A, Kilislioglu A, Abro M I and Hallam K R 2018 Functionalised CuO nanostructures for the detection of organophosphorus pesticides: A non-enzymatic inhibition approach coupled with nano-scale electrode engineering to improve electrode sensitivity Sensors Actuators B Chem. 260 480–9

[30] Khairy M, Ayoub H A and Banks C E 2018 Non-enzymatic electrochemical platform for parathion pesticide sensing based on nanometer-sized nickel oxide modified screen-printed electrodes Food Chem. 255 104–11

[31] Nagabooshanam S, Roy S, Mathur A, Mukherjee I, Krishnamurthy S and Bharadwaj L M 2019 Electrochemical micro analytical device interfaced with Internet of things using using Ultrafast Reprint Sci. Rep. 9 1–9

[32] Wang Z, Ma B, Shen C and Cheong L Z 2019 Direct, selective and ultrasensitive electrochemical biosensing of methyl parathion in vegetables using Burkholderia cepacia lipase@MOF nanofibers-based sensor Talanta 197 356–62

[33] Kumar T H V and Sundramoorthy A K 2019 Electrochemical bio sensor for methyl parathion based on single-walled carbon nanotube/glutaraldehyde crosslinked acetylene clad acetate nanoribbons-modified screen-printed electrodes J. Iran. Chem. Soc. 16 2777–85

[34] Xu G, Hou J, Zhao Y, Bao J, Yang M, Fa H, Yang Y, Li L, Luo D and Hou C 2019 Dual-signal aptamer sensor based on polydimethylamino-gold nanoparticles and exonuclease I for ultrasensitive malathion detection Sensors Actuators B Chem. 287 428–36