



Review paper

Some progress in developing electrochemical sensors for detection of 2,4-dichlorophenoxyacetic acid based on modified carbon interfaces: a brief review

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Abstract

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-DPAA) is commonly used in agricultural practices. Unfortunately, it has a high toxicity level and is known to be a carcinogenic substance. Therefore, developing an analytical technique capable of detecting this compound is crucial. Electrochemical methods offer a viable solution for the rapid and on-site analysis of 2,4-DPAA residues in real samples. The detection of 2,4-DPAA can be achieved through electrochemical redox electron transfer reactions, making voltammetry an effective approach. Various studies have explored the use of carbon electrodes, such as glassy carbon electrodes (GCE), carbon paste electrodes (CPE), and screen-printed electrodes (SPE), for voltammetric detection of 2,4-DPAA. However, researchers have encountered challenges in detecting 2,4-DPAA using these carbon electrodes. Consequently, modifications have been made to the carbon materials by incorporating chitosan hierarchical porous silica, Fe₃O₄-polyaniline nanocomposites, silver, manganese oxide nanoparticles, alizarin yellow R polymer, hierarchical porous calcium phosphate, and molecularly imprinted polypyrrole with TiO₂ nanotubes. In this comprehensive review, we have examined the effectiveness of each modified electrode, considering factors such as the limit of detection, precise linear range, and recovery rate for detecting 2,4-DPAA in real samples.

Keywords

Herbicide, LOD, sensors, voltammetry, redox reaction, catalysis

Introduction

The traditional method of dealing with agriculture consists of a combination of physical, chemical, and biological methods to increase the yield and fertility of crops [1,2]. Due to the increase in population, crop yields are increased quickly using pesticides, fungicides, herbicides, fertilizers, and so on. One such compound is 2,4-dichlorophenoxyacetic acid (2,4-DPAA). It is an organic compound widely used as an herbicide in agriculture. Herbicides are used on plants to control the growth of unwanted plants like weeds, agricultural pests, and invasive species. Chemical herbicide is more advantageous over mechanical herbicide because it has ease of application and often saves labour costs [3,4].

Citrus and numerous other plants like cereals, sugarcane, soybean, potato, wheat, corn, aquatic weeds, dicot plants, barley, *etc.*, use 2,4-DPAA as a growth regulator [5]. It selectively kills most of the broad-leaf weed, 2,4-DPAA is absorbed from roots and leaves and then translocated to the weeds. They also serve as a natural auxin, causing cell division, cell elongation, and an increase in the ability of the fruit to reproduce independently. They are used when more weeds are germinated and in the crop at the 4-5 leaf stage. The mixture of 2,4-DPAA and picloram is called Tordon or Australian Agent White. During the Vietnam War, 2,4-DPAA was combined with 2,4,5-D, forming the agent orange, which was used for defoliation [6].

2,4-DPAA is used extensively despite its toxicity and carcinogen properties. It was mainly found in environmental samples like water, food, and air. The amount of toxin that penetrates water resources is approximately 91.7 %, which contaminates the water and poses a risk to aquatic life, animal life, vegetation, and human health. The World Health Organization (WHO) recommends that the 2,4-DPAA concentration in drinking water does not exceed $70 \mu\text{g L}^{-1}$. According to the European Commission (EC), the maximum residue level of 2,4-DPAA in milk should not exceed 0.01 mg kg^{-1} . The U.S. Environmental Protection Agency (EPA) has placed 2,4-DPAA in group D of carcinogenicity suspected of endocrine disruption. The toxin present in 2,4-DPAA has low biodegradability in water because it forms oil-soluble amine salts and low-volatile esters. Therefore, it does not dissolve well in water, and due to these formed compounds, it has the potential to contaminate groundwater and is considered the main pollutant in water resources. When 2,4-DPAA meets the surface water, it quickly gets distributed to water bodies, having a half-life of 1-3 weeks. Its residue can still be detected after six months. Some national and international organizations have spotted this problem and minimized the usage of 2,4-DPAA, but the residue of 2,4-DPAA is still found in grains, fruits, vegetables, and water samples. According to the EPA, 2,4-DPAA has already been found in drinking water, surface water, and groundwater [7-9].

2,4-DPAA is applied by spray drift in agricultural land. Since 2,4-DPAA has a low vapor pressure, it is present in the atmosphere particulate phase as spray droplets or may be linked to dust particles. Increased soil moisture, warmth, falling clay content, and soil organic matter cause the volatilization of 2,4-DPAA. Inhalation of this compound causes many breathing issues and affects health. In soil, 2,4-DPAA has a half-life of 1-2 weeks.

2,4-DPAA was detected in the dust on the floor, tables, and lawn applications. A survey was done in some pre-schools in North Carolina, and 2,4-DPAA was detected in solid food, beverages, and air in low concentration. Due to regular exposure, 2,4-DPAA was found in the children's urine. The extensive use and oral exposure of 2,4-DPAA cause acute toxicity, allergic skin reactions, serious eye damage, respiratory irritation, headaches, dizziness, abnormal human behaviour, vomiting, and abdominal pain, and they have carcinogenic properties causing cancer. These herbicides are sold in the form of powder or liquid. 2,4-DPAA in plasma seems to have acute fatal concentrations between 447 and 826 mg L^{-1} . There is no known treatment for poisoning with 2,4-DPAA herbicide which

harms the heart, central and peripheral neurological systems, liver, kidneys, muscles, lungs, and endocrine system [10]. Non-Hodgkin's lymphoma (a blood cancer) and sarcoma (soft-tissue cancer) have been linked by researchers to exposure to 2,4-DPAA [11]. Few studies have shown that regular exposure to 2,4-DPAA harms reproductive health and many developments affect growing children [12]. Therefore, the detection of 2,4-DPAA has a huge importance.

Detection of 2,4-dichlorophenoxy acetic acid

The EPA and the International Agency for Research on Cancer (IACR) have classified this pesticide as a human carcinogen and a hazardous pollutant. It has harmful effects on humans, animals, and the environment. Determining the precise concentration of 2,4-DPAA in environment samples requires an effective, trustworthy, and economical approach. Several techniques are used to detect and determine the 2,4-DPAA such as gas chromatography (GC) [13], high-performance liquid chromatography [14], thin-layer chromatography [15], and mass spectrophotometry [16]. Even though these procedures are highly sensitive and produce precise findings, they have some drawbacks, including high costs, lengthy processing times, and a need for pre-treatment and expertise [17,18].

In recent days, the electrochemical method has received notice because of its simplicity, inexpensive sensitivity, ease of access, ease of sampling, rapid response time, miniature size, and monitoring of samples on location [17,18]. The importance of electrochemical techniques is based on the reaction that occurs on the electrode surface by an electron transfer. 2,4-DPAA is difficult to detect by direct electrochemical method because, under normal conditions, they are inactive. So, it becomes important to modify the electrode or the compound. Modification of the compound is usually done by nitration [18]. Various chemical substances, such as organic compounds, are used to modify the surface of the bare electrode, like nanoparticles, metals, biomaterials, polymers, and composites. There are different methods in electrochemistry to detect the 2,4-DPAA, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), amperometry, and electrochemical impedance spectroscopy (EIS) [19,20].

Cyclic voltammetry

The voltammetry technique that is most frequently employed in the electrochemical detection of electroactive species is the cyclic voltammetric (CV) technique. It is based on the three-electrode system, consisting of the working, reference, and counter electrodes, where the current is measured between the working and counter electrodes. The potential is measured between the reference and working electrode, which both need to be inert and conductive. The shape of the CV curve and peak position is due to the potential between the reference and working electrode, which causes the species to lose or gain electrons. CV method is one of the simplest methods and can detect the analyte concentration in the micromolar range [21].

Differential pulse voltammetry (DPV)

Differential pulse voltammetry (DPV) is another voltammetry method used for the detection of electroactive species. It is utilized to measure the current generated by the analyte concentration determined by using a series of stepwise voltage increases. Before and after each potential pulse is applied, the current is monitored, and the difference between the currents is then carried out. This results in the faradic current being free of the majority of capacitive current, which helps to lower the background current caused by the DC ramp. The main benefit of DPV is that it has a low capacitive current, which results in the high sensitivity of this method, which is also based on the three-electrode system [21].

Amperometric detection

Amperometry is the most used electrochemical method for the detection of herbicides due to its extreme sensitivity and selectivity. The current peak is measured depending on the optimal potential applied in a three-electrode system. The current is obtained due to the oxidation or reduction associated with the electrode and analyte at a fixed potential [22,23].

Impedimetric detection

Impedimetric sensors work by measuring changes in capacitance and conductance on the working electrode surface, which are impacted by the activity of the receptors brought on by the presence of the analyte [24].

Significance of the working electrode

The important component of the electrochemical sensor is the working electrode because, based on the interaction of the working electrode surface and analyte, the current peak is observed due to the redox reaction. In crafting a high-performing electrochemical sensor, the careful choice of working electrode materials is a paramount consideration. The ideal working electrode must satisfy criteria such as affordability, ready accessibility, environmental friendliness, and resistance to fouling [23].

In this review, carbon-based working electrodes are chosen to detect the 2,4-DPAA. The most used carbon electrodes are glassy carbon electrodes (GCE), carbon paste electrodes (CPE), screen-printed electrodes (SPE), boron-doped diamond electrodes (BDDE), and pencil graphite electrodes (PGE), all with certain modifications. The advantages of carbon-based electrodes are cheapness and large active surface area. Hence, carbon-based electrodes are preferred to detect organochlorine herbicides.

Detection of 2,4-DPAA using glassy carbon electrode (GCE)

Zhou *et al.* [25] prepared a modified GCE with the hierarchical porous silica supported with gold (HPSNs-NH₂@Au) and molecularly imprinted polymer (MIPs). Chitosan (CS) was added dropwise on the HPSNs-NH₂@Au, forming a complex of CS-HPSNs-NH₂@Au, because chitosan increases the mechanical strength of the film and prevents the falling of HPSNs-NH₂@Au. Additionally, it offers conductivity properties, effective film-forming capability, and good biocompatibility. The functional monomer of O-phenylenediamine (O-PD), which serves as the template molecule for MIPs, was electropolymerized onto the surface of modified GCE. Then, the 2,4-DPAA was removed, forming the special cavities to recognize 2,4-DPAA. This sensor is used to detect the 2,4-DPAA in milk, tap water, and lake water.

Goswami and Mahanta [17] prepared the environment-friendly electrode by modifying the surface of the GCE using Fe₃O₄-polyaniline nanocomposite (Fe₃O₄-PANI). Spectroscopic, microstructural, and elemental analysis was done to show the presence of Fe₃O₄ nanoparticles along with the polyaniline covering. The synergetic effect of the Fe₃O₄-polyaniline composite caused superior electrochemical behaviour toward the detection of 2,4-DPAA. PANI-based composites are used because of their high surface area, π -conjugated structure, excellent redox properties, and presence of an amine group, while iron oxide nanoparticles, because of their biosensing application due to their advantageous band gap, biocompatibility, nontoxicity, thermal stability, intriguing optical and magnetic properties, large natural abundance, and strong electric conductivity even at ambient temperature. By conducting CV and amperometry studies, the electrochemical response of 2,4-DPAA on Fe₃O₄-PANI-GCE was assessed. At bare GCE, no characteristic peak was observed, but when GCE was modified with the Fe₃O₄-PANI, 2,4-DPAA showed a prominent redox peak. The modified

electrode without 2,4-DPAA showed a peak potential of 0.163 vs. Ag/AgCl and with 2,4-DPAA the peak potential is 0.165 V. When exposed to 2,4-DPAA, the modified GCE demonstrated a redox process with oxidation and reduction peaks at 0.255 V and 0.1 V respectively, as shown in Figure 1a. The amperometric measurement exhibited a linear range from 1.35 to 2.7 μM , the limit of detection was 0.12 μM and sensitivity was 4.62 $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$. It was discovered that this alternation was a straightforward, affordable, and biocompatible non-enzyme sensor for the detection of 2,4-DPAA in an aqueous solution at room temperature. The EIS results also confirmed that lower charge transfer resistance was observed at the modified electrode due to the synergistic effect between Fe_3O_4 and PANI in the nanocomposite.

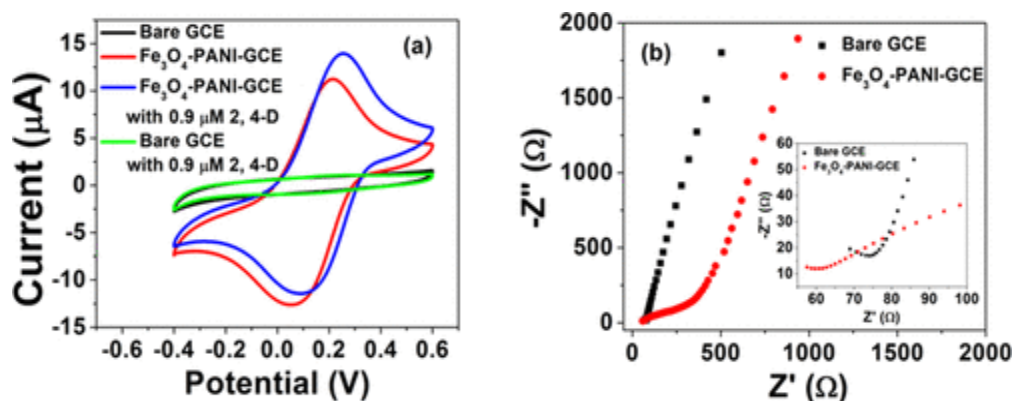


Figure 1. (a) CVs of the bare GCE and Fe_3O_4 -PANI-GCE in the absence and presence of 2,4-D and (b) Nyquist plots for bare GCE and Fe_3O_4 -PANI-GCE (inset shows the magnified Nyquist plots) [17].

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In 2016, a noteworthy study by Liu *et al.* [26] demonstrated the preparation of a modified GCE through the incorporation of hierarchical porous calcium phosphate (hp-CaP). This biocompatible nanomaterial was utilized to immobilize catalase, enabling it to exhibit remarkable bio-electrocatalytic activity for the direct electrochemical reduction of H_2O_2 . The performance of the electrode was evaluated using CV, revealing a low limit of detection (LOD) of 0.015 μM . Moreover, this sensor exhibited the added advantage of reusability, making it a promising candidate for various applications in electrochemical sensing. As such, the findings from this study hold significant relevance in the field of electrochemistry and can potentially contribute to the advancement of bio-electrochemical sensors.

Shi *et al.* [27] demonstrated the detection of 2,4-DPAA using a photoelectrochemical sensor based on GCE modified with molecularly imprinted polypyrrole (PPy) and TiO_2 nanotubes (TiO_2NTs). Despite 2,4-DPAA being a putative endocrine disruptor without inherent electrochemical activity, this study successfully developed a highly selective and sensitive sensor with a low detection limit of 10 nM ($S/N=3$). The incorporation of the PPy-based molecularly imprinted polymer as the recognition element endowed the sensor with remarkable selectivity for 2,4-DPAA determination even in the presence of multiple coexistent pesticides, which showed negligible interference on the photocurrent of 2,4-DPAA at 20-fold excess. The practical application of the sensor was demonstrated through the selective determination of 2,4-DPAA in spiked samples. The unique combination of TiO_2 NTs and PPy resulted in high surface area and multidimensional spaces, contributing to achieving the lower LOD. The photocurrent was further enhanced by the quick electron-transfer rate enabled by the PPy-based molecularly imprinted polymer. CV analysis of drinking water and river water samples using the modified electrode displayed a linear detection range of 0.5 to 13 μM and a limit of detection of 0.01 μM . The sensor exhibited excellent long-term stability since, even after a month of storage, the photocurrent was reduced only by 1.5%. This innovative photoelectrochemical sensor represents a

significant advancement in the monitoring of endocrine disruptors, exhibiting high selectivity, sensitivity, and practical applicability in environmental analysis.

Fathi *et al.* [28] presented a novel approach for the detection of 2,4-DPAA using a modified GCE. The modification involved the integration of silver and manganese oxide nanoparticles, along with the Alizarin yellow R polymer (Ag-MnOXNPs/PAYR) coating. The authors confirmed the successful coating of the complex on the GCE surface through scanning electron microscopy. Electrochemical impedance spectroscopy was employed to analyse the electrical resistance of the modified electrode, contributing to its characterization. Compared to previous works using mono metal nanoparticles and PANI polymer, this study utilized a bimetal nanoparticle with alizarin yellow R polymer, resulting in a substantial increase in anodic peak current for the effective 2,4-DPAA detection. The modified GCE showed a linear detection range of 22 to 11,752 μM and a LOD of 7.33 μM . Additionally, the modified electrode exhibited good stability even after four weeks of storage and maintained a low detection limit, wide linear range, and satisfactory repeatability at pH 6, making it a promising tool for environmental monitoring and analysis. A summary of GCE-based electrochemical sensors developed for the detection of 2,4-DPAA is given in Table 1.

Table 1. Summary of carbon-based electrochemical sensors for detection of 2,4-DPAA

Ref.	Method	Electrode	Modifications	Linear range	Limit of detection	Samples
[25]	CV	GCE	Hierarchical porous silica-supported with gold and molecularly imprinted polymer	--	--	Milk, tap water, and lake water.
[17]	CV and amperometry	GCE	Fe_3O_4 -polyaniline nanocomposite	1.35 - 2.7 μM	0.12 μM	--
[26]	CV	GCE	Hierarchical porous calcium phosphate	0.03 - 3 μM	0.015 μM	--
[27]	CV	GCE	Molecularly imprinted polypyrrole and TiO_2 nanotubes	0.5 - 13 μM	0.01 μM	Drinking water and river water
[28]	EIS	GCE	By the integration of silver and manganese oxide nanoparticles, along with an alizarin yellow R polymer	22 - 11,752 μM	7.33 μM	Water
[29]	CV and amperometry	CPE	A complex of 5,10,15,20-tetrakis (pentafluoro phenyl)-21H,23H-porphyrin iron (III) chloride and multi-walled carbon nanotube	9.9 - 140 $\mu\text{mol L}^{-1}$	2.1 $\mu\text{mol L}^{-1}$	---
[30]	DPV	CPE	Fluorohectorite heterostructure	---	1.22 $\mu\text{g L}^{-1}$	Soil
[31]	SWV	CPE	Graphite polyurethane composite	660 -2620 $\mu\text{g L}^{-1}$	17.6 $\mu\text{g L}^{-1}$	Sandy and clayey soil
[32]	Amperometry	SPE	Alkaline phosphatase (ALP) and Fe_3O_4 nanoparticles within a hybrid sol-gel/chitosan membrane	0.5 - 30 $\mu\text{g L}^{-1}$	0.3 - 0.4 $\mu\text{g L}^{-1}$	--
[33]	Amperometry	SPE	Gold	0.3 - 30 $\mu\text{g L}^{-1}$	0.1 $\mu\text{g L}^{-1}$	Water
[34]	CV and amperometry	SPE	Carbon nano-onions (CNOs) in a cyclodextrin polymer matrix by an immobilized peroxidase enzyme	--	5.1 $\mu\text{g L}^{-1}$	River water and soil samples of the barley crop field
[35]	CV	SPE	Nafion film-coated	0.01 - 100 $\mu\text{g L}^{-1}$	0.01 $\mu\text{g L}^{-1}$	Water
[36]	DPV and CV	BDDE	Cathodically and anodically pre-treated BDDE	2.5 - 22.5 $\mu\text{mol L}^{-1}$	0.12 $\mu\text{mol L}^{-1}$	Water
[37]	SWV	BDDE	BDDE with H_2SO_4 pre-treatment	0.100 - 0.911 $\mu\text{mol L}^{-1}$	34 nmol L^{-1}	Water
[38]	CV and EIS	PGE	Electro-polymerization of MIP	0.06-1.25 $\mu\text{g L}^{-1}$	0.02 $\mu\text{g L}^{-1}$	Packaged drinking water and tap water

Detection of 2,4-DPAA using carbon paste electrode (CPE)

Wong and Sotomayor [29] prepared a CPE modified with a complex of 5,10,15,20-tetrakis(pentafluoro phenyl)-21H,23H-porphyrin iron(III) chloride and multi-walled carbon nanotubes (MWCNT). The research was focused on exploiting carbon nanotubes to develop voltammetric and amperometric sensors for monitoring environmental analytes. The study presented a biomimetic sensor based on the modified CPE incorporating 5,10,15,20-tetrakis(pentafluoro phenyl)-21H,23H-porphyrin iron(III) chloride and multi-wall carbon nanotubes for sensitive 2,4-DPAA detection. The sensor exhibited a linear response range from 9.9 to 140 $\mu\text{mol L}^{-1}$, with a low detection limit of 2.1 $\mu\text{mol L}^{-1}$ and enhanced response due to MWCNT inclusion. The technique is a versatile and cost-effective tool for selective and stable 2,4-DPAA analysis in environmental matrices.

Ozkan *et al.* [30] prepared a mixed-ion, amphiphilic fluorohectorite heterostructure to study its effect as an efficient electrochemical sensor for 2,4-DPAA and 2,4-dichlorophenol. The fluorohectorite heterostructure incorporated at 5 wt.% into a CPE, the clay-modified electrode showed high sensitivity to the 2,4-DPAA. It exhibited a reduced response to herbicides lacking necessary aromatic hydroxyl groups for oxidation. The hetero-structured fluorohectorite's unique ability to achieve electrical neutrality upon oxidation contributed to its superior electrochemical response. The clay-modified CPE offers a cost-effective, sensitive, and practical alternative for detecting environmental pollutants and herbicides.

Andrade *et al.* [31] developed an electrochemical method to detect 2,4-DPAA in the soil sample using graphite polyurethane (GPU) composite electrode or modified CPE. The GPU electrode was made by combining graphite and polyurethane resin in a ratio of 60:40 (w/w). In that very interesting research, the authors compared numerous working electrodes like GCE, CPE, and GPU to check their performance to determine 2,4-DPAA by conducting the square wave voltammetry (SWV) in 0.1 mol L^{-1} Britton-Robison (BR) buffer with pH 2. As shown in Figure 2 (curve f), 2,4-DPAA displayed a well-defined reduction peak on the GPU electrode at -0.54 V vs. Ag/AgCl, indicating a favourable interaction between polyurethane and 2,4-DPAA.

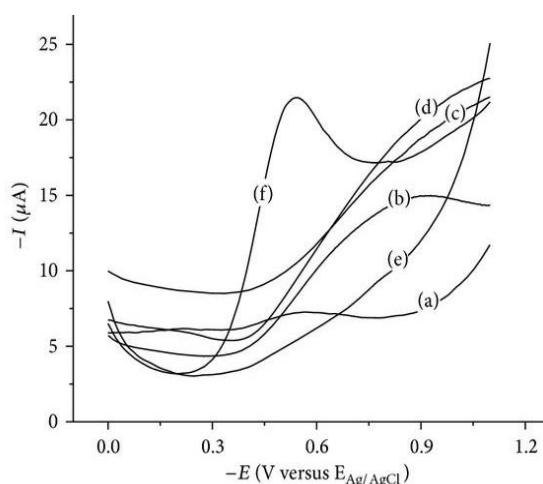


Figure 2. SWV curves in 0.1M BR buffer solution (pH 2) without and with 2,4-DPAA for: (a) and (b) GCE; (c) and (d) CPE; (e) and (f) GPU electrode [31]. Reprinted with the permission of Hindawi

Other electrodes have poor voltammetric profiles and reduction processes. Under optimum conditions, the value of the limit of detection LOD is 17.6 $\mu\text{g L}^{-1}$, the linear range is 0.66 to 2.62 mg L^{-1} and the LOQ is 58.6 $\mu\text{g L}^{-1}$. The average recovery rate from different soil samples was $93.6 \pm 1.2\%$ for the sandy soil and $94.6 \pm 4.2\%$ for the clayey soil. A summary of CPE-based electrochemical sensors developed for the detection of 2,4-DPAA is given in Table 1.

Detection of 2,4-DPAA using screen-printed electrode (SPE)

Loh *et al.* [32] developed an electrochemical biosensor by combining alkaline phosphate (ALP) and Fe_3O_4 nanoparticles within a hybrid sol-gel/chitosan membrane deposited on the surface of a screen-printed electrode (SPE). The biosensor utilized the reaction between ALP and ascorbic acid-2-phosphate (AA2P) for amperometric detection of 2,4-DPAA. ALP inhibited the detection process, and the catalysis of AA2P by ALP led to the production of ascorbic acid. Subsequently, ascorbic acid becomes oxidized to form dihydroascorbic acid with the assistance of Fe_3O_4 nanoparticles, promoting electron transport to the electrode and enhancing sensitivity. The biosensor immobilized with 0.4 wt.% Fe_3O_4 demonstrated the best performance (as shown in Figure 3), with a linear range of 0.5 to 30 $\mu\text{g L}^{-1}$, a limit of detection of 0.3-0.4 $\mu\text{g L}^{-1}$, and approximately 40 % maximum inhibition at 30 $\mu\text{g L}^{-1}$ of 2,4-DPAA, achieving accurate results comparable to the high-performance liquid chromatography (HPLC) reference method.

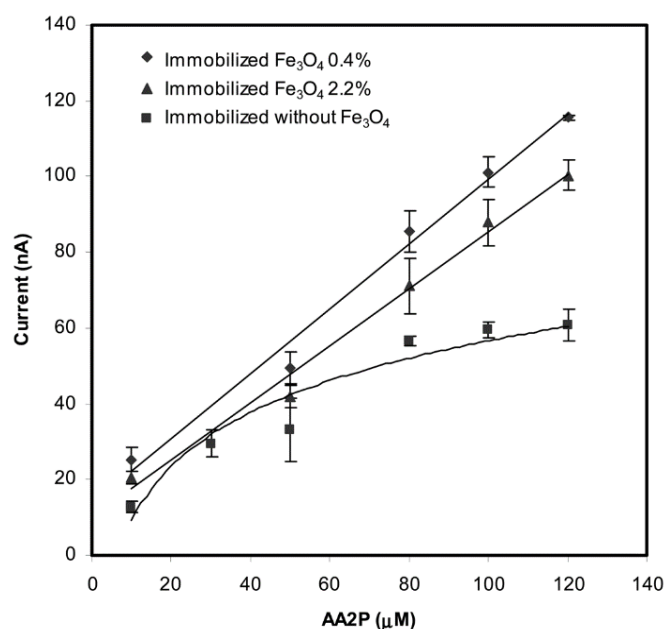


Figure 3. The response of biosensors with immobilized Fe_3O_4 (0.4 and 2.2 wt.%) and without Fe_3O_4 in the presence of changing concentrations of AA2P substrate [32]. Reprinted with permission from MDPI

Skládal and Kaláb [33] developed a multichannel immunochemical sensor based on an electrochemical transducer such as a hydrogen peroxide mediator. The working electrode was made of gold and the counter electrode of silver in the electrochemical system. This sensor is based on the screen-printing technique, and it consists of multichannels and is not reusable. To activate the analyte, modified mixed anhydrides were used. In this project, an indirect competitive assay method was used. For the immobilization of the monoclonal antibodies (Mab) on the nitrocellulose membrane, 2,4-DPAA- peroxidase conjugate was used. The sensor itself served as the site for the immunoreaction. Each well included a variety of materials. After adding a tracer and allowing it to incubate, the free tracer molecules were separated by washing before adding the peroxide substrate. The peroxidase reactions happened during this time. The formed product is benzoquinone, which accumulates in the well and is cathodically reduced when the potential is applied. Based on the relative binding of the tracer and immobilized anti-2,4-DPAA monoclonal antibodies, the calibration curve was plotted. The performance was evaluated by performing amperometric measurements using hydrogen peroxide and hydroquinone as a substrate for peroxidase. A linear range was observed in the range of 0.3 to 30 $\mu\text{g L}^{-1}$ and the LOD was 0.1 $\mu\text{g L}^{-1}$ in the given water

sample. This method uses several channels, allowing multiple analytes to be identified in a single sample, utilizing various biorecognition components in separate channels.

Sok and Fragoso [34] prepared an electrochemical biosensor to detect the 2,4-DPAA in river water and soil samples of the barley crop field using SPE modified with the carbon nano-onions (CNOs) in a cyclodextrin polymer matrix by an immobilized peroxidase enzyme. The presence of this composite enhances the sensitivity, stability, and repeatability of the sensor. Horseradish peroxidase enzyme (HRP) was used as a conjugate to activate CNOs, and they were immobilized on the SPE using β -cyclodextrin (CD) polymer. The effectiveness of the modified SPE was assessed by CV of SPE alone, SPE/CD/CNO/HRP, and SPE/CD/HRP as a control sensor (without CNOs), using $\text{Fe}[(\text{CN})_6]^{3-}$ as an electroactive marker. A greater peak current was observed at SPE/CD/CNO/HRP when compared to SPE/CD/HRP, as shown in Figure 4. This result shows that CNO is useful for the electrochemical detection of 2,4-DPAA using SPE.

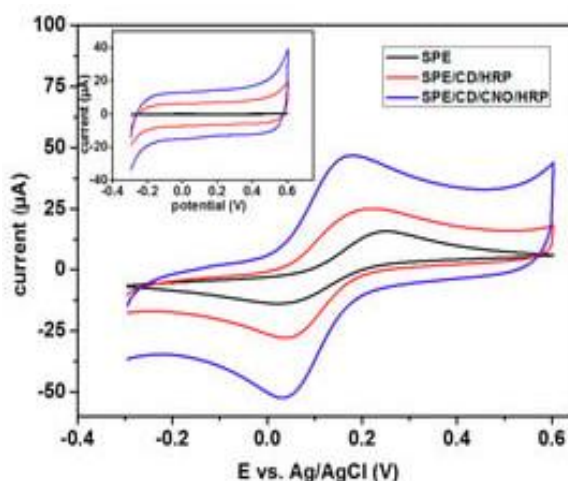


Figure 4. CV (100 mV/s) of SPE and modified SPEs in 0.1 M KCl containing 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ and 0.1M KCl (inset) [34]. Reprinted with permission from MDPI

Dequaire *et al.* [35] presented a novel immunomagnetic electrochemical sensor designed for the sensitive and rapid detection of 2,4-DPAA in water sources. The sensor incorporates a solid phase based on magnetic particles coupled with a Nafion film-coated screen-printed electrode (Nafion-SPE) placed within a polystyrene cylinder. Utilizing a competitive immunoassay approach, the sensor enables the selective binding of 2,4-DPAA and alkaline phosphatase (AP)-labeled 2,4-DPAA to polyclonal anti-2,4-DPAA antibody-coated magnetic beads. By monitoring the AP label activity bound to the beads electrochemically, the concentration of 2,4-DPAA was accurately quantified. This innovative immunomagnetic electrochemical sensor exhibits remarkable sensitivity and achieves a detection limit of 2,4-DPAA in water samples as low as $0.01 \mu\text{g L}^{-1}$. The introduction of a magnet aids in localizing the AP-associated beads on the Nafion-SPE surface, leading to significant amplification of the sensor response. Furthermore, the authors claimed that the device's practicality is enhanced by its disposable nature and low amounts of antibody-coated magnetic beads, making it cost-effective for mass production. Moreover, the adaptability of the sensor to a portable multichannel potentiostat renders it suitable for decentralized environmental applications. In conclusion, this immunomagnetic electrochemical sensor offers a reliable, convenient, and promising tool for accurate 2,4-DPAA detection in water samples, with potential applications in environmental monitoring and various fields of research and diagnostics. A summary of SPE-based electrochemical sensors developed for the detection of 2,4-DPAA is given in Table 1.

Detection of 2,4-DPAA using boron-doped diamond electrode (BDDE)

Boron-doped diamond electrode (BDDE) has enduring stability and extremely low background current inside the acid and alkaline media. Duarte *et al.* [36] used a cathodically pre-treated BDDE for the determination of 2,4-DPAA. The authors evaluated the performance of cathodically and anodically pre-treated BDDE by DPV and CV techniques. BDDE was used to simultaneously determine the diuron (DI), 2,4-DPAA, and tebuthiuron (TB). DPV with optimized parameters was employed for electroanalytical measurement of 2,4-DPAA with different interferents (ametryn, hexazinone, imazethapyr, glyphosate, imazethapyr and picoxystrobin) using BDDE. The linear correlation coefficients for DI and 2,4-DPAA were 0.99 within the concentration range of 1.0 to 9.0 $\mu\text{mol L}^{-1}$, and for TB, the range was 2.5 to 22.5 $\mu\text{mol L}^{-1}$. The method exhibited very low LOD for DI, 2,4-D, and TB at 0.035, 0.12 and 0.34 $\mu\text{mol L}^{-1}$, respectively. The approach was not susceptible to interferences from other pesticides or humic acid substances. Application to the lake and well water samples yielded satisfactory recovery values (96 to 104 %), confirming the feasibility of the proposed method for herbicide determination. The combination of SPE with electroanalytical techniques exhibits a promising tool for developing simple, accurate, and interference-free methods for herbicide analysis in water samples. Overall, this study highlights the promising potential of integrating BDDE with electroanalytical techniques for the development of robust, accurate, and interference-free methods in environmental monitoring and herbicide analysis. By addressing the challenge of multi-residue determination, this research contributes to the advancement of analytical methodologies for water quality assessment, ensuring the protection and preservation of aquatic ecosystems and public health.

Neto *et al.* [37] also used BDDE for voltammetric detection of 2,4-DPAA in environmental water samples. The noteworthy aspect of this study was that researchers gathered samples using a customized unmanned aerial vehicle (UAV) equipped with a micropump and miniature solenoid valve, all powered by an open-source microcontroller. The utilization of UAVs for actual sample collection presents an intriguing approach with potential benefits in contamination reduction. The optimized condition for electrochemical detection of 2,4-DPAA was done using the SWV technique, and the authors obtained a linear analytical curve for 2,4-DPAA within a concentration range from 100 to 911 nmol L^{-1} , with a limit of detection of 34 nmol L^{-1} , using 0.5 mol L^{-1} Na_2SO_4 (pH 2.0, regulated with 0.5 mol L^{-1} H_2SO_4 solution) as the medium. Further, in situ analysis by SWV was compared with the gas chromatography-mass spectrometry (GC-MS) analysis, showing that the results of both methods for detecting 2,4-DPAA match at a 95 % confidence level. This pioneering method using an adapted UAV with described features allows access to challenging areas for water sampling, enabling fast, practical, and safe environmental monitoring. It serves as a valuable screening tool for alerting the presence of 2,4-DPAA. The approach provides a real-time response, making it useful for environmental and sanitary control agencies to monitor and effectively supervise water quality in various locations. This study marks the first-ever application of an adapted UAV for in situ electrochemical analysis of environmental water, exhibiting its potential as an essential tool for environmental monitoring and detection of hazards.

Detection of 2,4-DPAA using pencil graphite electrode (PGE)

Prusty and Bhand [38] developed a capacitive sensor using a pencil graphite electrode (PGE) modified with MIP to detect the 2,4-DPAA in the packaged drinking water and tap water. The modification was done by electro-polymerizing MIP on PGE using a chronopotentiometry method. The characterization of the prepared electrode was done by field emission gun-scanning electron

microscopy, CV, and EIS. PGE was chosen by the authors because it serves as a low-cost substrate that is simple to create without polishing, offers a good repeatable surface, and exhibits strong electrochemical activity. To compare the results of the modified PGE, another type of working electrode was developed, i.e., PGE modified with the non-imprinted polymer (NIP). The values were assessed by performing the CV on both types of modified PGE electrodes. The authors employed the EIS method to gauge alterations in capacitance, detecting significant changes within a linear range spanning from 0.06 to 1.25 $\mu\text{g L}^{-1}$, with an impressive limit of detection as low as 0.02 $\mu\text{g L}^{-1}$. A similar kind of response was seen for NIP electrodes, but a higher current response was seen for the MIP electrode because of the presence of void space within the polymer. The maximum capacitance variation was seen in MIP of 3.15 % for 0.06 $\mu\text{g L}^{-1}$ and in the NIP electrode, 3.18 % was observed for 12.5 $\mu\text{g L}^{-1}$. This shows that the MIP sensor has better sensitivity than NIP sensor. The recovery rate from the drinking and tap water samples is 96 to 110 %.

Conclusion

The development of electrochemical sensors capable of detecting 2,4-dichloro phenoxy acetic acid (2,4-DPAA) residues in real samples is vital to safeguard water and soil quality. Various strategies involving nanoparticles, polymers, enzymes, and molecularly imprinted polymers (MIPs) have been explored to improve the sensitivity and immobilization of electrochemical sensors. Among the carbon-based electrodes, the glassy carbon electrode (GCE) stands out as the most used and has been successfully modified with MIP@TiO₂ to achieve a low limit of detection (LOD) of 0.01 μM and a precise linear range of 0.5 to 13 μM .

Graphite polyurethane-modified carbon paste electrode (CPE) offers another promising option with LOD of 17.6 $\mu\text{g L}^{-1}$ and a linear range of 0.66 to 2.62 mg L^{-1} , enabling the identification of 2,4-DPAA in different soil types with high recovery rates. Additionally, modified screen-printed electrode (SPE) variants, such as those with peroxidase and gold or Nafion membrane, exhibit very low LOD (0.1 $\mu\text{g L}^{-1}$) and precise linear ranges, making them effective for 2,4-DPAA detection in water samples. Although modified boron-doped diamond electrodes (BDDE) and platinum-gold electrodes (PGE) have been utilized for 2,4-DPAA detection, their usage remains relatively limited compared to other carbon-based electrodes.

Overall, this comprehensive review outlines the progress in electrochemical techniques for 2,4-DPAA detection, providing valuable insights into environmentally friendly ways of monitoring this herbicide in real samples. Among the modifications, GCE with MIP@TiO₂ and SPE with enzymes stand out as preferred choices, offering low LOD and precise linear ranges. The reusability and long-term stability of modified GCE make it a promising option for future applications.

As the need for on-site detection of 2,4-DPAA grows with increasing awareness of water and soil quality, the development of electrochemical sensors plays a pivotal role in ensuring the safety of agricultural practices and environmental sustainability. Nevertheless, challenges persist in enhancing sensitivity and reproducibility without compromising electrode stability, warranting continued research efforts in this area. Ultimately, the progress made in electrochemical sensing enables effective identification and monitoring of 2,4-DPAA, benefiting farmers and environmental stakeholders alike.

The modified carbon-based electrodes have been used widely for the detection of 2,4-DPAA because of their high sensitivity, reproducibility, and cost-effectiveness. However, there are still challenges associated with this method. Preparation of the modified layer should be done very precisely because it can be easily damaged, reducing the reusability and leading to a reduction in the analysis of the sample. The analysis of 2,4-DPAA in the real sample may not be accurate if the

sample has a similar compound, causing the interfering matrix for the detection of the analyte. Much research has been going on to overcome these problems and improve the reusability and accuracy of modified electrodes to determine the concentration of 2,4-DPAA in real samples.

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