

Article

Screen-Printed Sensors Coated with Polyaniline/Molecularly Imprinted Polymer Membranes for the Potentiometric Determination of 2,4-Dichlorophenoxyacetic Acid Herbicide in Wastewater and Agricultural Soil

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Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) is a widely used herbicide worldwide. However, its residues in agricultural products are extremely harmful to human health and to the environment in soil and water. Previous methods for determining 2,4-D in water and soil samples are expensive, cumbersome, and not highly selective. In this study, we developed a novel disposal sensor based on screen-printed electrodes (SPEs) for detecting 2,4-D in wastewater and soil samples. The SPEs were modified with conductive polyaniline (PANI) layer and polyvinyl chloride (PVC) membrane loaded with molecularly printed polymer (MIP). The MIP particles were prepared using 2,4-D as template, methacrylic acid (MAA) as monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker, and benzoyl peroxide as initiator. The best sensor shows a dynamic concentration range of 10^{-2} to 10^{-7} M 2,4-D, a detection limit (LOD) of 3.6×10^{-7} M, Nernst slope (response) of 29.9 mV/decade, and high selectivity over other interfering species previously reported in the literature. The sensors also achieved a short response time of 25 s, high reversibility, and a lifetime of over 2 weeks. The developed sensors were successfully used for determining 2,4-D in real wastewater and soil samples with high accuracy and precision.

Keywords: disposal sensors; dichlorophenoxy acetic acid; molecularly imprinted polymer; screen-printed electrodes; wastewater soil; herbicide



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1. Introduction

Herbicides are phytotoxic chemical compounds used to kill or inhibit the growth of various weeds, but their use poses several risks to humans, animals, and the environment [1]. Among the various herbicides, 2,4-dichlorophenoxyacetic acid (known by the ISO name 2,4-D) is widely used in agriculture, commerce, and residential areas because of its high insecticidal activity. The 2,4-D is one of the world's oldest and most widely used herbicides. It has been commercially available since 1945 and currently, more than 1500 products (e.g., mecoprop and dicamba) contain 2,4-D as an herbicide active ingredient and plant growth regulator. The residues of 2,4-D in agricultural and food products are extremely harmful to humans, animals, fish, and other aquatic life. With prolonged exposure to 2,4-D, toxic effects on the kidneys and liver have been observed [2]. The acid and salt forms of 2,4-D can cause eye irritation; People should not swim in pools containing 2,4-D for 24 h to

avoid eye irritation. The median lethal dose of 2,4-D is 639 mg/kg, as determined in acute toxicity studies on rats [3].

So far, various methods have been developed to determine 2,4-D in wastewater and soil samples using complicated instruments such as capillary electrophoresis (CE) [4,5], and high-performance liquid chromatography (HPLC) [6]. However, those analytical methods need expensive equipment, lengthy pretreatment and preparation steps of the sample, and highly skilled technical personnel. In contrast, electrochemical sensors are widely used for detecting 2,4-D with good sensitivity in concentration ranges from 1.0 to 10 μ M [7]. Among the various electrochemical sensors, potentiometric sensors based on screen-printed electrodes (disposable electrodes, SPEs can be used as suitable control sensors because they offer high sensitivity and selectivity at wide linear concentrations [8,9]. The screen-printed electrodes (three-electrode system) can be printed on plastic or ceramic and fabricated with a variety of silver and carbon inks. However, they can also be based on other metals such as palladium, platinum, gold, or copper. One of the main advantages is the ability to modify the screen-printed electrodes and change the composition of their inks by adding various nanoparticles, enzymes, and recognition elements, which is useful for developing a variety of electrochemical analyses [10,11].

Molecularly imprinted polymers (MIPs) are the most promising synthetic molecular recognition systems with high binding capacity, long-term stability, and high selectivity [12]. They are prepared from the targeted analyte as a molecular template, which can be removed and keep binding cavities that retain the shape, size, and tendency to bind with analyte molecules [12]. MIPs are polymeric structures designed to act like natural antibodies. They combine the high specificity and affinity of related natural compounds with the low cost, robustness, and long-term stability of synthetic polymers. Successful preparation of MIP requires optimization of monomer selection, temperature, solvents, and reaction time [10]. A critical step is the selection of the monomer and cross-linkers for good sensing performance, which depends on the functionality and chemical structure of the analyte molecules [10,13]. Assimilation of MIP with the SPEs increases the selectivity of MIPs, which can be used as recognition elements for antigens, hormones, drugs, herbicides, and pesticides [14,15].

In this work, a novel potentiometric sensor based on screen-printed carbon paste electrodes was developed for the detection of 2,4-D in wastewater and soil samples with high sensitivity and selectivity. The carbon paste of the screen-printed electrodes was coated with a 2,4-D selective membrane consisting of PVC as the polymer matrix, o-nitrophenyl octyl ether (o-NPOE) or dioctyl phthalate (DOP) as the plasticizer, MIP as the recognition element, TDMAC or aliquat as the anion exchanger, and PANI as a conductive precoating layer. The MIP particles were prepared using 2,4-D as a template, methacrylic acid (MAA) as a monomer, and ethylene glycol dimethacrylate (EGDMA) as a crosslinker. The water layer (Morf) test was used to investigate the probability of water penetration between two layers and its effect on the stability of the reading, response time, lifetime, and selectivity of the sensor [16,17]. The pH effect was studied in detail and related to the sensitivity and detection limit of the sensor. Interestingly, the sensors were used to determine 2,4-D in soil and wastewater samples with high sensitivity, selectivity, accuracy, and precision. The sensor showed high selectivity to the previously reported interfering species. The sensors offer several advantages such as low manufacturing cost, design flexibility, high sensitivity, and high reproducibility.

2. Experimental

2.1. Chemicals and Reagents

2,4-Dichlorophenoxyacetic acid (2,4-D, $\geq 95\%$, Figure 1), methacrylic acid (MAA, 99%), acetonitrile (99%), ethylene glycol dimethacrylate (EGDMA, 98%), ethanol (C_2H_5OH , 99%), methanol (CH_3OH , 99%), acetic acid (CH_3COOH , 99%), and polyvinyl chloride (PVC), trisaminomethane (Tris, 99%), Trioctylmethylammonium chloride (Aliquat 336, 98%), tetrahydrofuran (THF, 98%), o-nitrophenyl octyl ether (o-NPOE, 97%), Dioctyl Phtha-

late (DOP, 99%) and benzoyl peroxide (BP, 95%) were obtained from Sigma Aldrich (St. Louis, MO, USA). Tris base buffer (0.1 mM, pH = 7.0) was prepared by dissolving Tris in deionized water and adjusting the pH with HCl (30%) to the exact volume. The interfering species (salicylate, succinate, formate, acetate, urea, trichloroacetic acid, nitrate, sulfate, chloride, orthophosphoric acid, 2,4-dinitrophenylhydrazine, 2,4-dichlorophenylcarbazole) were supplied as analytical grades from ADWIC (Cairo, Egypt). The standard solutions (10^{-2} M 2,4-D) were prepared using Tris buffer (0.1 mM, pH = 7.0) and then more dilute solutions (10^{-3} – 10^{-8} M 2,4-D) were obtained by the appropriate dilutions.

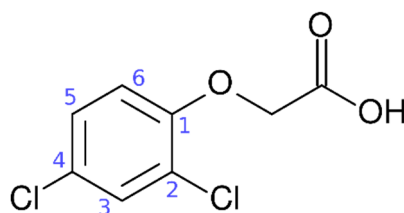


Figure 1. Chemical structure and functionality of the 2,4-dichlorophenoxy acetic acid (2,4-D).

2.2. Preparation and Characterization of MIPs

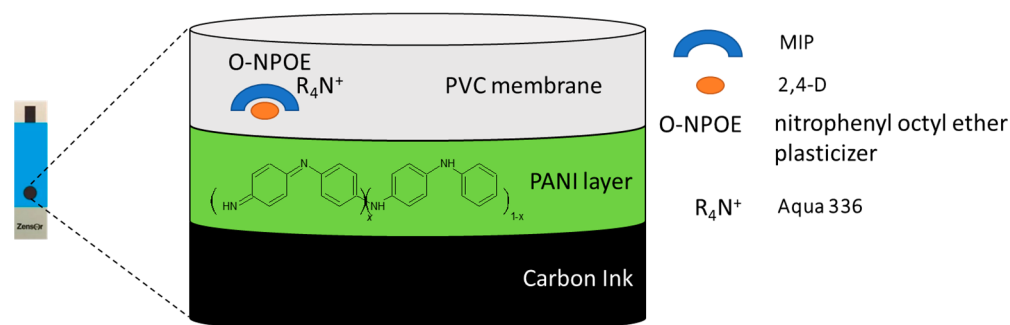
MPs were synthesized by mixing 1.0 mM 2,4-D (template analyte), 3.0 mmol MAA (monomer), and 15 mL acetonitrile as a solvent in a screw-capped glass tube. The mixture was sonicated for one hour before the crosslinker (3.0 mM EGDMA) and initiator (60 mg benzoyl peroxide) were added to the reaction mixture. The oxygen was then removed from the mixture with N_2 gas for five minutes, and the mixture was placed in an oil bath to start polymerization at 70 °C for 24 h. The formed polymer was collected and washed with 100 mL of methanol/acetic acid (4:1) circularly to remove the unreacted substances and by-products. The synthesized polymer was dried at 25 °C for 24 h. Non-imprinted polymers (NIP) were prepared using the same procedure of MIP but without the addition of 2,4-D (template molecule). The bonding structure of MIP and NIP was imaged by Fourier transform infrared spectrophotometer (FTIR, Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA), using solid KBr for sample preparation; the spectra were recorded at 4 cm^{-1} resolutions. Morphological characterization of the as-prepared MIP and NIP particles was examined using a scanning electron microscope (SEM, JEOL JSM-5510LV, Akishima, Tokyo) after coating with Au/Pd.

2.3. Preparation of Screen-Printed Electrode

PANI solution is prepared by chemical polymerization of aniline hydrochloride in presence of ammonium persulfate to form a green solution of PANI [16,17]. The 2,4 D -selective membrane was prepared by mixing 66 mg PVC with different amounts of recognition element (MIP), anion exchangers (TDMAC or aliquat), plasticizers (o-NPOE or DOP), and without and with PANI as a conductive precoat (Table 1). The mixture was prepared using 1.5 mL of THF as a solvent and poured into a glass ring (2.5 cm diameter). The carbon ink surface of the screen-printed electrode (Zensor[®] SE 100, Etterbeek, Belgium) was modified by two layers (1) 10 μ L of PANI solution and then leaving the film to dry at ambient temperature; (2) 10 μ L of the PVC membrane cocktail applied on the PANI layer and leaving the layer to dry at ambient temperature (see Figure 2). The screen-printed electrodes were soaked in 10^{-2} M 2,4-D solution for 2 h before use, and stored in a solution of similar concentration when not in use.

Table 1. FTIR band of 2,4-D, MAA, EGDMA, NIP, and MIP before and after washing.

Band Assignment	2,4-D	MMA	EGDMA	2,4-D@MIP	MIP	NIP
OH-stretching	-	-	-	3443	disappeared	disappeared
C _{Ar} -Cl stretching	693	-	-	689	disappeared	disappeared
C _{Ar} =C _{Ar}	1435, 1475	1637	1637	1630	-	-
C=O	1732	1718	1720	1729	-	-
C-O-C symmetric	1089	-	-	1090	disappeared	disappeared

**Figure 2.** Configuration of the SPE (sensor 4) for the detection of 2,4-D: (bottom layer) screen-printed electrodes (SPEs) based on conductive carbon ink; (middle layer) conductive films of polyaniline (PANI); (top layer) polyvinyl chloride (PVC)/o-nitrophenyl octyl ether (o-NPOE)/molecularly printed polymer (MIP).

2.4. Potential Measurements

All potentiometric measurements were recorded using a pH/mV meter (Orion model 720/SA, Cambridge, MA, USA). The modified SPE was used in conjunction with an Ag/AgCl reference electrode (Orion double-junction, Model 90-20) with cell arrangement as follows: SPEs//test solution//10% *w/v* KNO₃ salt bridge//Ag/AgCl/3.0 M KCl). A ross glass electrode (Orion 81-02) was used for pH measurements. All spectral measurements were performed under standard conditions using a UV-Vis spectrophotometer (Jenway model 1601, Cole-Parmer Ltd., Vernon Hills, IL, USA). Calibration was performed by immersing the SPE against the Ag/AgCl reference electrode in 50-mL beakers containing 10-mL of 2,4-D standard solutions (from 1.0×10^{-8} to 1.0×10^{-2} M). Potential values were recorded from the low to high concentrations of 2,4-D, and the potential was plotted against the logarithm of the 2,4-D concentrations ($\log [2,4-D]$). Potential measurements were recorded after potential stabilization to ± 1 mV. Before each measurement, the SPEs were conditioned in Tris buffer solution (0.1 M, pH = 7), which was adapted for 2 h.

2.5. Water Layer Test and pH Effect

The Morf test or water layer test was used to test the potential drift and the possibility of the formation of a water layer between the conductive layer PANI and the detecting PVC/MIP layer [13,18]. The electrode potential was recorded in a solution containing 10^{-3} M 2,4-D for 30 min, then in a solution containing 10^{-4} M Cl[−] (interfering ion) for 30 min, and then it was changed back to the 10^{-3} M 2,4-D solution [13]. The pH effect (from pH = 2 to pH = 10) on the response (potential, mV) at 25 °C for 10^{-3} M 2,4-D.

2.6. Selectivity against Interfering Ions

The selectivity coefficients were evaluated using the separated solutions method (SSM) [19]. The potential of the cell is measured with two separate solutions, of the 2,4-D (10^{-3} – 10^{-8} M 0.1 M Tris buffer) and the interfering ion (10^{-3} – 10^{-8} M in 0.1 M Tris

buffer), of the same activity $a_A = a_B$. The selectivity coefficients were calculated using the following equation:

$$\log K_{A,B}^{Pot} = [(E_B - E_A)/S] + \log [a_A/a_B^{Z_A/Z_B}] \quad (1)$$

where $K_{A,B}^{Pot}$ is the selectivity coefficient, E_A and E_B are the potentials of 2,4-D and the interfering ion of the charges Z_A and Z_B , respectively, and S is the slope of the best sensor [19].

2.7. Analytical Applications and Sample Analysis

The best SPEs were used for the determination of 2,4-D by the direct calibration curve method [13,18]. About 500 g of an agricultural soil sample was collected from orchards and sprayed with herbicide solutions. After one day, the soil sample was washed several times with Tris buffer and filtered with normal filter paper, and the clear solution was collected to measure the herbicide content. The 2,4-D content in the soil sample was determined using the best sensor, and the results were related to those obtained by a spectroscopic method [20]. The recovery values were calculated using the calibration curve. Statistical calculations were performed to ensure the accuracy and precision of the measurements. The 2,4-D concentration was determined using a calibration curve generated with the best sensor before the soil samples were analyzed.

3. Results and Discussion

3.1. Molecularly Imprinted Polymers Characterization

The MIP was prepared using MAA as a functional monomer and EGDMA as a cross-linker, 2,4-D as a template, which results in strong H-bonding between the carboxyl and chlorides of the template with the carboxylic group of MAA, in addition to the host-guest interaction due to the fitting between the herbicide host molecule and the cavity within the MIP molecular guest [13]. SEM images show that the synthesized MIP and NIP are microspherical shape particles with different diameters of 0.6–3 μm . The imprinted NIP particles (average size 2.8 μm , Figure 3a–c) were less aggregated than MIP particles (average size 0.8 μm), which was probably due to the presence of the 2,4 D during polymerization that facilitated the agglomeration of the forming spheres through hydrogen bonding interaction, Figure 3d–f).

Figure 4 shows the FTIR spectra of MIP and NIP and 2,4-D. The IR spectrum of 2,4-D shows a strong peak at 1732 cm^{-1} corresponding to the C=O group. The peaks at 1089 and 1311 cm^{-1} correspond to the symmetric and antisymmetric vibrations of C-O-C, respectively. The peak at 1234 cm^{-1} corresponds to the O-H deformation, which is coupled with the C-O stretching vibrations [13,21]. The stretching vibrations of CH_2 (alkanes) and C=C (alkenes) are indicated by the peaks at 1435 and 1475 cm^{-1} , respectively [13], while the peak at 693 cm^{-1} is indicative of C-Cl stretching [22]. It has been reported in the literature that, for the typical IR spectrum of MAA (monomer), the IR peak appears at around 1637 cm^{-1} is due to the stretching C=C vibration, while the IR peak appears at 1718 cm^{-1} corresponds to the stretching C=O vibration. In the IR spectrum of the EGDMA (cross-linker), the peaks that appear at 1150 , 1637 , and 1720 cm^{-1} correspond to the stretching C-O, C=C, and C=O vibrations, respectively. In the IR spectrum of NIP, the peak of C=C (stretching vibration, 1637 cm^{-1}) was significantly reduced, and a peak for C-O appeared at 1169 cm^{-1} . These results confirm the polymerization reaction between EGDMA and MAA, which are in agreement with [13,23]. The peak at 1729 cm^{-1} and the broad peak of 3443 cm^{-1} are due to C=O and O-H groups, respectively. These two peaks prove that the MIP involved these groups, which could interact with the 2,4-D, Figure 4. In the IR spectrum of MIP, the fingerprint bands of 2,4-D (693 , 1089 , and 890 cm^{-1}) disappear after washing, which confirms its removal completely from the MIP particles, Figure 4. However, the other bands are the same in the case of MIP and NIP which ensures that the polymers have the same backbone. The H-bonding between the MIP and 2,4-D was confirmed by the band broadening at 3443 cm^{-1} , the shift of C=O, C-O-C, and $\text{C}_{Ar}-\text{Cl}$

bands to longer wavenumber, Table 1 [13,23]. Hydrogen bonding interaction between pesticide and polymer is confirmed by shift in C=O and C-O-C symmetric bands.

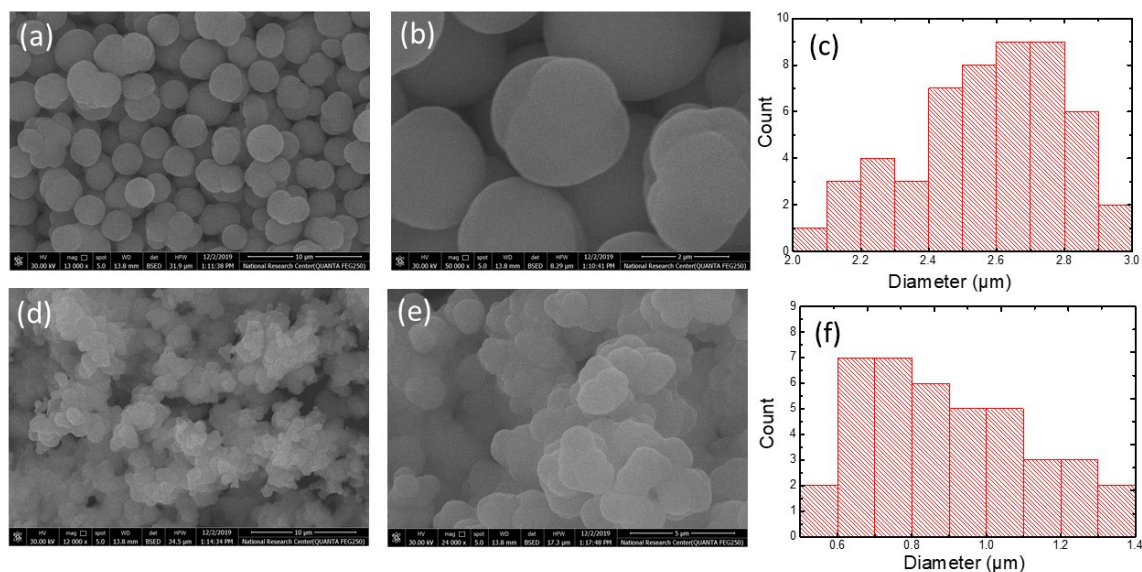


Figure 3. Particle size analysis of NIP and MIP particles: (a,b) SEM image of NIP; (c) particle size distribution of NIP; (d,e) SEM image of MIP; and (f) particle size distribution of MIP; the as-prepared MIP and NIP particles was examined after coating with Au/Pd.

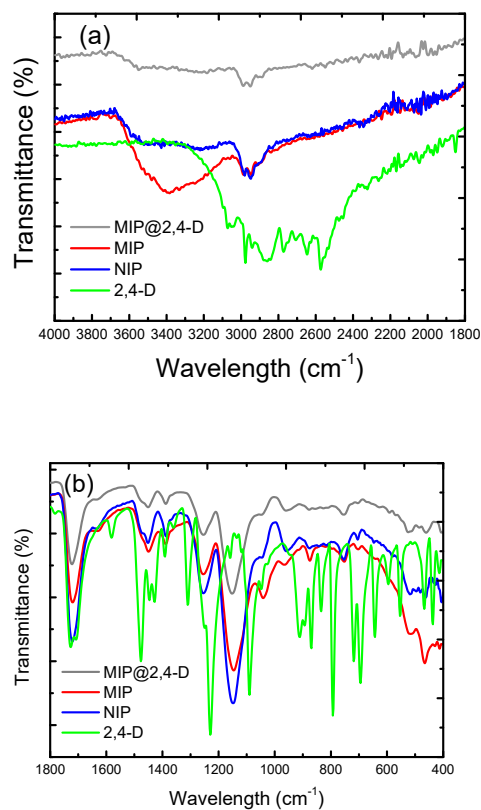


Figure 4. IR spectrum showing the bonding structure of molecularly imprinted polymers (MIP@2,4-D) before removal of the 2,4-D, MIP after the removal of the 2,4-D by washing, non-imprinted polymers (NIP), and the 2,4-D template analyte: (a) IR spectrum from 4000 to 2000 cm^{-1} ; and (b) IR spectrum from 2000 to 400 cm^{-1} .

3.2. Characteristics of the Modified Screen-Printed Electrodes

Different SPEs were prepared for the detection of 2,4-D using different amounts of MIP as an ionophore, TDMAC or Aliquat 336 as an anion exchanger, o-NPOE or DOP as a plasticizer, and PANI as a modifier, as in Table 2. Sensor 1 based on DOP as a plasticizer showed a sub-Nernst response (anionic slopes of 26.4 mV/decade) to 2,4-D in water over a small linear dynamic range (LDR) of 10^{-4} to 10^{-5} M with a limit of detection (LOD) of 1.0×10^{-5} M, Figure 5. Changing the ion exchanger to Aliquate 336 in Sensor 2, caused a comparable slope within wider LDR and the same LOD of 1.0×10^{-5} M. In sensor 3, o-NPOE was used as a plasticizer of a higher dielectric constant; it exhibited a slight improvement in the limit of detection compared with DOP used in sensor 2.

Table 2. Composition of the different SPEs and their response characteristics.

No.	Solution	Membrane Composition (wt %)				Response Characteristics		
		Recognition Element	Anion Exchanger	Plasticizer	Modifier	Slope	LDR, M	LOD, M
Sensor 1	Water	0.006 mg MIP	1 mg TDMAC	137 mg DOP	-	26.4	10^{-4} – 10^{-5}	1.0×10^{-5}
Sensor 2	Water	0.006 mg MIP	1 mg Aliquate	137 mg DOP	-	26.0	10^{-2} – 10^{-5}	1.0×10^{-5}
Sensor 3	Water	0.006 mg MIP	6 mg Aliquate	135 mg o-NPOE	-	24.2	10^{-2} – 10^{-5}	9.1×10^{-6}
Sensor 4	Water	0.006 mg MIP	6 mg Aliquate	135 mg o-NPOE	PANI	29.9	10^{-2} – 10^{-6}	8.8×10^{-7}
Sensor 4	Tris	0.006 mg MIP	6 mg Aliquate	135 mg o-NPOE	PANI	32.9	10^{-2} – 10^{-7}	6.1×10^{-8}
Sensor 5	Tris	0.006 mg NIP	6 mg Aliquate	135 mg o-NPOE	PANI	25.8	10^{-3} – 10^{-4}	1.0×10^{-4}

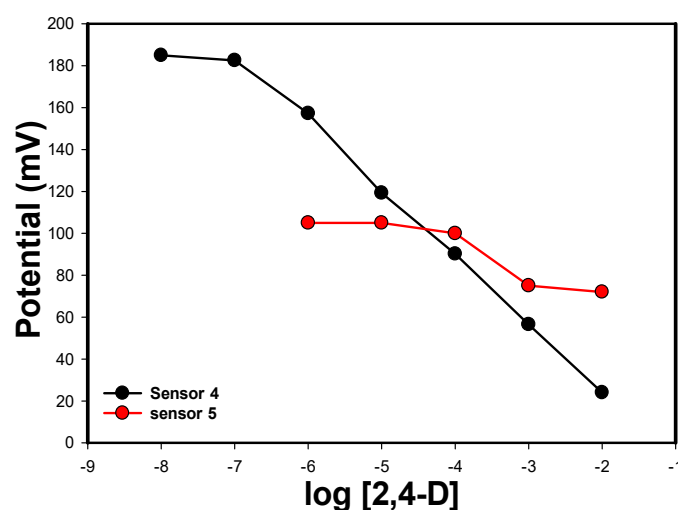


Figure 5. The calibration curve (potential vs. concentration logarithm) of the SPE (sensor 4 and sensor 5) coated with PANI conductive layers and measured in Tris buffer (pH = 7.0) at 25 °C.

Sensor 4 was fabricated using a drop-cast PAN layer as electrically conductive support followed by deposition (drop-casting) of the selective PVC membrane based on o-NPOE as a plasticizer to improve sensor performance; it showed a Nernst response of 29.9 mV/decade over LDR of 10^{-2} – 10^{-6} M, and an improved detection limit of 8.8×10^{-7} M. This good potentiometric response characteristics can be attributed to the high dielectric constant of the plasticizer (o-NPOE), in addition to the presence of the conductive layer that facilitates the electron transfer and improves the response [24,25].

All previous measurements were performed using water solutions of 2,4-D. Thus, to further optimized the sensor performance, sensor 4 was tested in buffered solutions (Tris buffer, pH = 7.0). The results showed that changing the testing solution from pure water to buffered solutions improved the slope to the super-Nernstian value of 32.9 mV/decade

and enhanced the detection limit as low as 6.1×10^{-8} M. This improvement in sensitivity is due to the high solubility of 2,4-D in the Tris buffer solution (pH = 7.0) [26].

Figure 5 shows the difference in a dynamic concentration range, detection limit, and slope between MIP (sensor 4) and NIP (sensor 5). In sensor 5, MIP is replaced with NIP and tested in Tris-buffered 2,4-D solutions. Sensor 5 (based on NIP) showed a slope of 25.8 mV/decade with a high detection limit of 1.0×10^{-4} M in a very small linear dynamic range of 10^{-3} – 10^{-4} M 2,4-D. This confirms the specific host-guest interactions between the binding sites of MIP cavities and 2,4-D, compared to a low binding affinity of NIP. The very small linear dynamic range observed for sensor 5 is due to the limited ability of NIP particles to form hydrogen bonds with 2,4-D in addition to the existence of the o-NPOE (ion exchanger) in the PVC membrane.

3.3. Effect of pH

The effect of pH on the potential of sensor 4 showed a variation of the potential with pH change, Figure 6. Potential is stable in pH ranges of 2–3 and 7–8.3, with a continuous decrease of the potential with the pH change from 2 to 10, which is due to the increase of the number of hydroxyl ions (OH^-); this confirms the anionic response of the sensor which is due to the ability of MIP to form hydrogen bonding with hydroxide ion, in addition to the possibility of the ion exchange between the hydroxide anion and the chloride ion of the Aliquate 336 ion-exchanger. 2,4-D is generally present in the environment as an anion based on its pKa value of 2.73. Understandably, the degree of coking of 2,4-D acid increases at higher pH values of 7–9 [26]. According to the MSDS of these compounds, 2,4-D has a solubility of 29.9 mg/L at pH = 5, 44.5 at pH = 7, and 43.1 at pH = 9. Therefore, the test solutions should be buffered with Tris buffer (pH = 7–8) to control the presence of 2,4-D species.

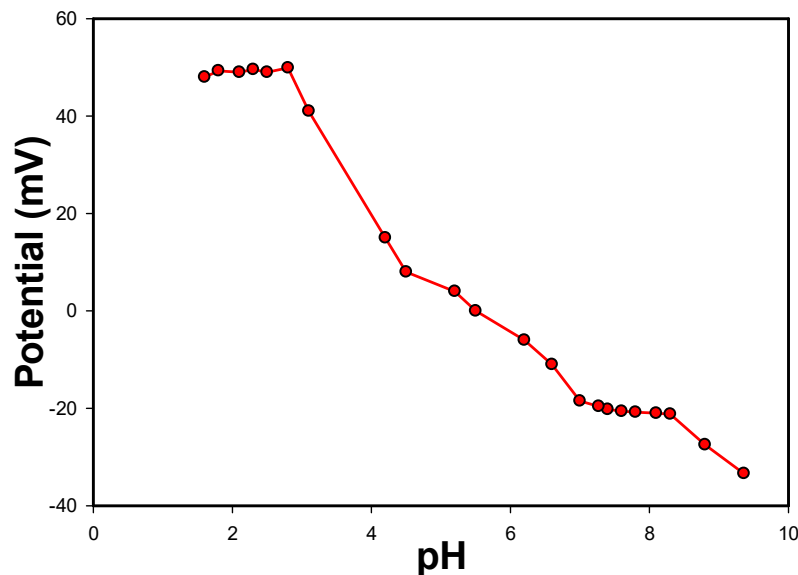


Figure 6. pH effect on the measured potential response (potential, mV) of sensor 4 at 25 °C for 10^{-3} M 2,4-D.

3.4. Stability of Sensor Response

The Morf test is a crucial step to verify the solid contact between the three layers of SPE (carbon ink, PANI layer, PVC membrane) in sensors 3 and 4. The Morf water layer test was used to estimate the drift in response when switching between the analyte and interfering ion. This drift is due to the possibility of the formation of a thin aqueous layer between the carbon ink, PANI layer, and PVC membrane. The composition of this aqueous layer varies based on the sensor composition. As shown in Figure 7, the highest potential drift was observed in sensor 3 (without PANI), followed by a lower drift in sensor

4 (with PANI), which means that a larger water layer can be formed between the carbon ink and the PVC membrane in sensor 3 (without PANI). In sensor 4, the water layer is lesser compared to sensor 3. This is attributed to the higher lipophilic nature of PANI, which delays the formation of a thicker water layer in sensor 4 between the carbon ink and the PVC membrane. In all cases, the lower drift does not significantly affect the sensor performance, which is confirmed by the high signal stability, short response time, and long lifetime of sensor 4. The conductive PANI layer induces ion-to-electron transduction (i.e., coupling of ion and electron transfer in the membrane) because of its mixed ionic and redox sensitivity. Due to electronic conductivity, PANI is expected to facilitate electron transfer between the PCV and carbon ink (SPE) interface. The interfacial potential at the PVC/PANI interface can also be explained by ion partitioning between two phases.

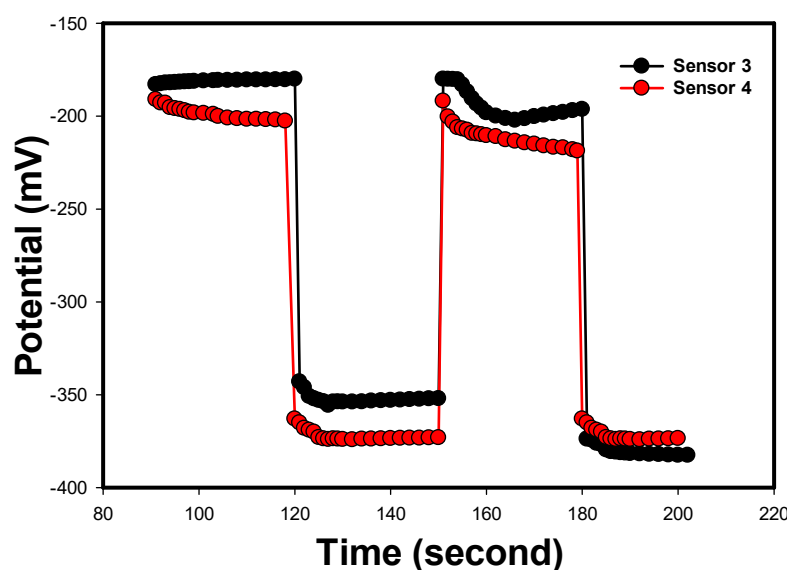


Figure 7. Water-layer tests of sensors 4 and 5 were measured in Tris buffer using solutions of 10^{-3} M 2,4-D and 10^{-3} M NaCl at 25 °C.

3.5. Reversibility, Response, Soaking, and Lifetimes

Soaking is the process of activating the electrode surface by immersing the sensor in a solution containing the primary ion to be measured. This process results in the formation of a layer on the electrode surface that acts as an interface between the solid-state sensor and the aqueous solution. In this layer, the binding and interaction between the MIP and the 2,4 D take place. It has been shown that 2 h is sufficient for activating the electrode surface and verifying the best results when measuring low concentrations. Thus, the screen-printed electrodes were soaked in 10^{-2} M 2,4-D solution for 2 h before use, and stored in a solution of similar concentration when not in use.

Response time can be defined as the time required to reach 90% of the equilibrium potential [9]. It is measured by immersing sensor 4 in various concentrations of 2,4-D (10^{-2} – 10^{-7} M). As can be seen in Figure 8, sensor 4 showed short response times of <20 s, which is confirmed by the plateau region for all measured concentrations except 10^{-7} M. The fast response time, especially at low concentrations, can be attributed to the effect of the small water layer effect at the carbon paste/PANI/PVC interface, which enables fast ion exchange kinetics [14]. The stability of the potential reading indicates fast ion transfer and good host-guest interaction through hydrogen bonding between the MIP and 2,4 D.

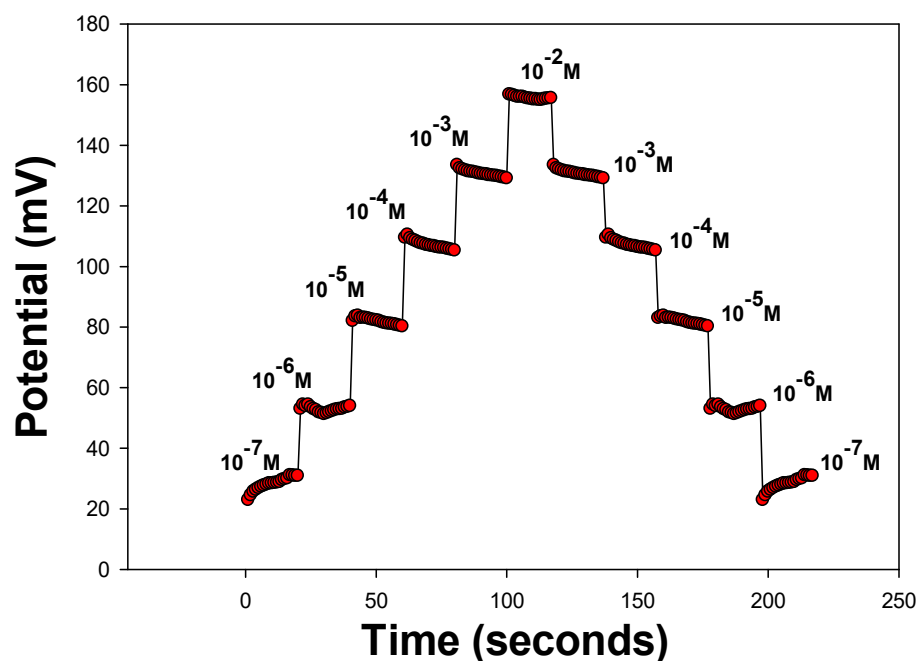


Figure 8. Dynamic response of sensor 4 over a wide concentration range of 2,4-D from 10^{-7} to 10^{-2} M at 25°C prepared in Tris buffer ($\text{pH} = 7.0$).

Reversibility is tested by measurements from low to high concentrations of 2,4-D, followed by measurements in the reverse direction [9]. As can be seen in Figure 8, sensor 4 is highly reversible, which means that the memory effect is minimal in this case. This is to be expected since the interaction between the MIP and the 2,4 D depends on weak hydrogen bonding, which can be overcome by simply washing the electrode [27]. This is an advantage because it facilitates the measurement of different concentrations in routine work without further precautions by simply washing the electrode with distilled water between measurements. The electrode can be used for about two months.

3.6. Interfering Species

Selectivity of the interfering species sensors depends not only on the MIP particles, but also strongly on the other PVC membrane components, the concentration of the analyte and the interfering species, the total ionic strength of the solution, and the temperature [8,27]. Selectivity of sensor 4 (based on PVC/MIP) was estimated for the interfering species (i.e., salicylate, succinate, formate, acetate, urea, trifluoroacetate, nitrate, sulfate, chloride, orthophosphoric acid, 2,4-dinitrophenylhydrazine, 2,4-dichlorophenylcarbazole) and compared with sensor 5 (based on PVC/NIP). As shown in Figure 9, sensor 4 based on PVC/MIP shows the high selectivity for 2,4-D over the interfering species comparing to sensors 5 based on PVC/NIP. Only trichloroacetic acid showed a slight cationic response with sensor 4, this may be due to ability of trichloroacetic acid to form hydrogen bonds with MIP particles. The selectivity coefficient of sensor 4 and sensor 5 was estimated for the interfering species and summarized in Table 3.

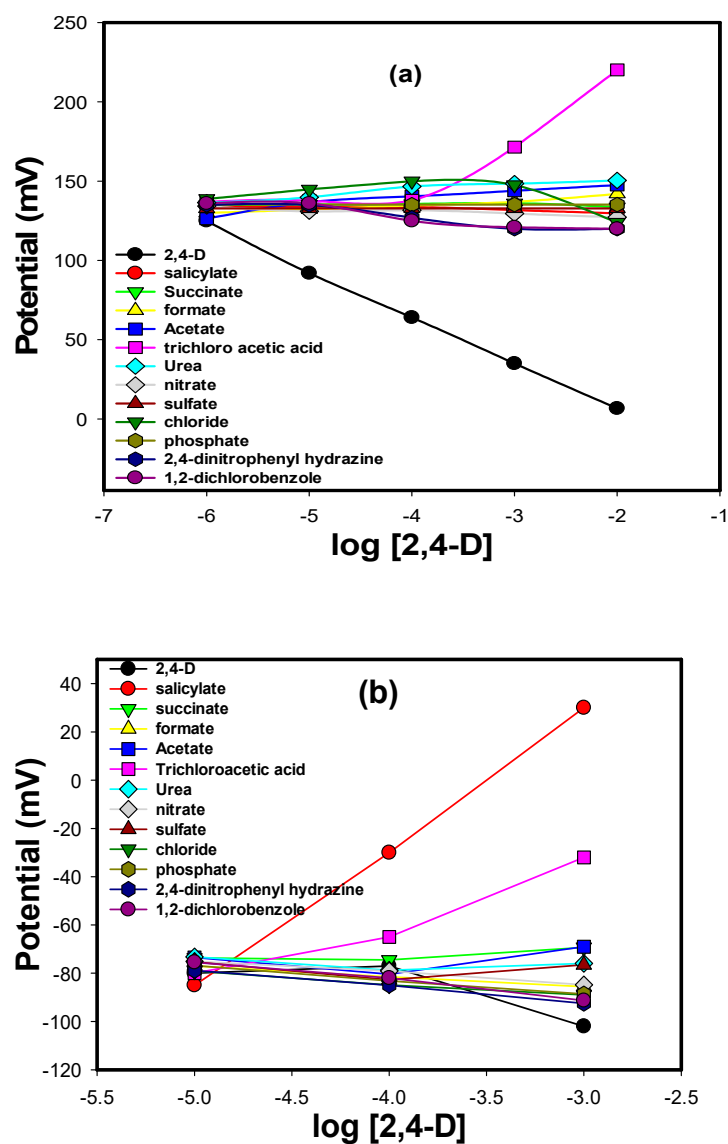


Figure 9. Selectivity of sensors 4 (a), sensor 5 (b) versus different types of interfering species measured in Tris buffer (pH = 7.0) at 25 °C.

Table 3. Selectivity coefficient of MIP and NIPs for 2,4-D and the interfering species.

Selectivity Coefficients	MIP (Sensor 4)	NIP (Sensor 5)
2,4 dinitrophenylhydrazine	−5.30	−1.61
Salicylate	−4.11	−2.50
Formate	−4.53	−1.89
Urea	−4.81	−2.29
Nitrate	−4.04	−1.93
Sulfate	−4.22	−1.76
Chloride	−3.92	−1.78
Phosphate	−4.29	−1.77
1,2 dichlorobenzole	−3.10	−1.66
Trichloroacetic acid	−7.34	−4.60

3.7. Applications

Depending on the type of herbicide and the concentration in the soil, persistent herbicides may act for several months to three or more years before they are completely degraded to inert compounds. In the last decade, considerable attention has been paid to the development of fast, sensitive, specific, and economical sensors for the detection of pesticides to ensure food safety. Screen-printed electrodes are a fast-growing area, enabling the development of potentiometric sensors that can be used as low-cost, rapid, real-time detectors of herbicides at decentralized sites. In this work, the best screen printed electrode (Sensor 4) was tested for measurement of 2,4-D in spiked pure and real samples. Two amounts of 2,4-D were used for spiking of pure water, wastewater and soil samples, Table 4. It can be seen that sensor 4 determined the different amounts of the analyte successfully with good recovery values in the range of 96.58 to 112.83%.

Table 4. Application of sensor (in Tris buffer) for determination of 2,4-D in pure water sample, wastewater sample, and soil sample, with recovery values and values obtained by the reference method [20] for real sample.

Spiked Amount	Pure Sample Sensor 4	Wastewater Sensor 4	Real Soil Sample	
			Sensor 4	Reference Method [20]
2.21 ppm	2.21 (100.10%)	2.13 (96.58%)	2.29 (103.79%)	2.22 ppm
22.10 ppm	22.17 (100.32%)	25.01 (112.83%)	24.37 (110.30%)	22.19 ppm

4. Conclusions

2,4-Dichlorophenoxyacetic acid (2,4-D) is a commonly used herbicide that kills most broadleaf weeds by causing them to grow uncontrollably, while leaving most grasses, such as cereals, turf, and grassland, relatively unaffected. Residues of 2,4-D in soil, water, and food are extremely harmful to human health and the environment. In this study, a rapid, low-cost, disposable screen-printed sensor was developed for determining 2,4-D in wastewater and soil samples. The screen-printed electrodes are coated with a conductive polyaniline (PANI) as a conductive layer and a polyvinyl chloride (PVC)/molecularly printed polymer (MIP) as a 2,4-D capture layer. The MIP (recognition element) was prepared using 2,4-D as template, methacrylic acid (MAA) as monomer, ethylene glycol dimethacrylate (EGDMA) as crosslinker, and benzoyl peroxide as initiator. The developed sensors showed high sensitivity (10^{-2} to 10^{-7} M), low detection limit of 3.6×10^{-7} M, fast response time (25 s) and high selectivity for 2,4-D over all expected interfering ions (salicylate, succinate, formate, acetate, urea, trifluoroacetate, nitrate, sulfate, chloride, orthophosphoric acid). The developed sensors were successfully used for the determination of 2,4-D in waters extracted from soil samples contaminated with 2,4-D with high sensitivity and selectivity.

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References

1. World Health Organization. *The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009*; World Health Organization: Geneva, Switzerland, 2020.
2. Tayeb, W.; Nakbi, A.; Trabelsi, M.; Miled, A.; Hammami, M. Biochemical and histological evaluation of kidney damage after sub-acute exposure to 2,4-dichlorophenoxyacetic herbicide in rats: Involvement of oxidative stress. *Toxicol. Mech. Methods* **2012**, *22*, 696–704. [\[CrossRef\]](#) [\[PubMed\]](#)
3. By, A.; Edwards, D. *Reregistration Eligibility Decision for 2,4-D, EPA 738-R-05-002*; National Service Center for Environmental Publications (NSCEP): Cincinnati, OH, USA, 2005.
4. Zhu, L.; Kee Lee, H. Field-Amplified Sample Injection Combined with Water Removal by Electroosmotic Flow Pump in Acidic Buffer for Analysis of Phenoxy Acid Herbicides by Capillary Electrophoresis. *Anal. Chem.* **2001**, *73*, 3065–3072. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Shin, H.S. Determination of Phenoxy Acid Pesticides in Frog and Fish Tissues by Gas Chromatography-Mass Spectrometry. *Chromatographia* **2006**, *63*, 579–583. [\[CrossRef\]](#)
6. Wu, J.; Kim, H.E.; Hian, K.L. Automated dynamic liquid–liquid–liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection for the determination of phenoxy acid herbicides in environmental waters. *J. Chromatogr. A* **2005**, *1082*, 121–127. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Xie, C.; Gao, S.; Guo, Q.; Xu, K. Electrochemical sensor for 2,4-dichlorophenoxy acetic acid using molecularly imprinted polypyrrole membrane as recognition element. *Microchim. Acta* **2010**, *169*, 145–152. [\[CrossRef\]](#)
8. Bakker, E.; Bühlmann, P.; Pretsch, E. Carrier-based ion-selective electrodes and bulk optodes. 1. General characteristics. *Chem. Rev.* **1997**, *97*, 3083–3132. [\[CrossRef\]](#)
9. Labib, M.; Sargent, E.H.; Kelley, S.O. Electrochemical Methods for the Analysis of Clinically Relevant Biomolecules. *Chem. Rev.* **2016**, *116*, 9001–9090. [\[CrossRef\]](#)
10. Belbruno, J.J. Molecularly Imprinted Polymers. *Chem. Rev.* **2019**, *119*, 94–119. [\[CrossRef\]](#)
11. Hayat, A.; Marty, J.L. Disposable screen printed electrochemical sensors: Tools for environmental monitoring. *Sensors* **2014**, *14*, 10432–10453. [\[CrossRef\]](#)
12. Wang, J.; Liang, R.; Qin, W. Molecularly imprinted polymer-based potentiometric sensors. *TrAC—Trends Anal. Chem.* **2020**, *130*, 115980. [\[CrossRef\]](#)
13. El-Beshlawy, M.; Abdel-Haleem, F.; Barhoum, A. Molecularly Imprinted Polymer-Based Potentiometric Biosensor for Nanomolar Determination of Pioglitazone Hydrochloride in Pharmaceutical Formulations. *Electroanalysis* **2021**, *33*, 1244–1254. [\[CrossRef\]](#)
14. Kröger, S.; Turner, A.P.F.; Mosbach, K.; Haupt, K. Imprinted Polymer-Based Sensor System for Herbicides Using Differential-Pulse Voltammetry on Screen-Printed Electrodes. *Anal. Chem.* **1999**, *71*, 3698–3702. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Kamel, A.H.; Amr, A.E.G.E.; Abdalla, N.S.; El-naggar, M.; Al-omar, M.A.; Almeshia, A.A. Modified Screen-Printed Potentiometric Sensors based on Man-Tailored Biomimetics for Diquat Herbicide Determination. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1138. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Pringsheim, E.; Terpetschnig, E.; Wolfbeis, O.S. Optical sensing of pH using thin films of substituted polyanilines. *Anal. Chim. Acta* **1997**, *357*, 247–252. [\[CrossRef\]](#)
17. Abdel-Haleem, F.M.; Saad, M.; Barhoum, A.; Bechelany, M.; Rizk, M.S. PVC membrane, coated-wire, and carbon-paste ion-selective electrodes for potentiometric determination of galantamine hydrobromide in physiological fluids. *Mater. Sci. Eng. C* **2018**, *89*, 140–148. [\[CrossRef\]](#)
18. Abdel-Haleem, F.M.; Mahmoud, S.; Abdel-Ghani, N.E.T.; El Nashar, R.M.; Bechelany, M.; Barhoum, A. Polyvinyl chloride modified carbon paste electrodes for sensitive determination of levofloxacin drug in serum, urine, and pharmaceutical formulations. *Sensors* **2021**, *21*, 3150. [\[CrossRef\]](#)
19. Kamel, A.H.; Amr, A.E.-G.E.; Abdalla, N.S.; El-Naggar, M.; Al-Omar, M.A.; Alkahtani, H.M.; Sayed, A.Y.A. Novel Solid-State Potentiometric Sensors Using Polyaniline (PANI) as A Solid-Contact Transducer for Flucarbazone Herbicide Assessment. *Polymers* **2019**, *11*, 1796. [\[CrossRef\]](#)
20. Chen, X.; Zhang, H.; Wan, Y.; Chen, X.; Li, Y. Determination of 2,4-Dichlorophenoxyacetic acid (2,4-D) in rat serum for pharmacokinetic studies with a simple HPLC method. *PLoS ONE* **2018**, *13*, e0191149. [\[CrossRef\]](#)
21. Abdel-Haleem, F.M.; Rizk, M.S.; El-Beshlawy, M.M. Molecularly-imprinted polymer-base bulk optode for the determination of ivabradine hydrochloride in Procoralan®. *RSC Adv.* **2022**, *12*, 17645–17654. [\[CrossRef\]](#)
22. Abdel-Haleem, F.M.; Salah, A.; Rizk, M.S.; Moustafa, H.; Bechelany, M.; Barhoum, A. Carbon-based Nanosensors for Salicylate Determination in Pharmaceutical Preparations. *Electroanalysis* **2019**, *31*, 778–789. [\[CrossRef\]](#)
23. Abdel-Haleem, F.M.; Gamal, E.; Rizk, M.S.; Madbouly, A.; El Nashar, R.M.; Anis, B.; Elnabawy, H.M.; Khalil, A.S.G.G.; Barhoum, A. Molecularly Imprinted Electrochemical Sensor-Based Fe₂O₃@MWCNTs for Ivabradine Drug Determination in Pharmaceutical Formulation, Serum, and Urine Samples. *Front. Bioeng. Biotechnol.* **2021**, *9*, 648704. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Malhotra, B.; Dhand, C.; Lakshminarayanan, R.; Dwivedi, N.; Mishra, S.; Solanki, P.; Venkatesh, M.; Beuerman, R.W.; Ramakrishna, S. Polyaniline-based biosensors. *Nanobiosensors Dis. Diagnosis* **2015**, *4*, 25–46. [\[CrossRef\]](#)
25. Dhand, C.; Das, M.; Datta, M.; Malhotra, B.D. Recent advances in polyaniline based biosensors. *Biosens. Bioelectron.* **2011**, *26*, 2811–2821. [\[CrossRef\]](#) [\[PubMed\]](#)

26. World Health Organization Food and Agriculture Organization of the United Nations. *Pesticide residues in food 2006 Evaluations Part I-Residues*; World Health Organization Food and Agriculture Organization of the United Nations: Rome, Italy, 2006.
27. Nagy, G.; Nagy, L. Potentiometric Sensors. In *Agricultural and Food Electroanalysis*; Wiley Online Library: Hoboken, NJ, USA, 2015. [[CrossRef](#)]

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