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Square-Wave Voltammetric Determination of Nanomolar Levels of Linuron in Environmental Water Samples Using a Glassy Carbon Electrode Modified with Platinum Nanoparticles within a Dihexadecyl Phosphate Film

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A new sensitive method for linuron determination using a glassy carbon electrode modified with platinum nanoparticles within a dihexadecyl phosphate film (PtNPs-DHP/GCE) and square-wave voltammetry was proposed. The PtNPs-DHP/GCE was characterised by scanning electron microscopy and the diameter of the Pt nanoparticles was between 13 and 34 nm. The electrochemical behaviour of linuron was studied using cyclic voltammetry and an irreversible anodic peak was obtained at a potential of 1.2 V in 0.1 mol L⁻¹ phosphate buffer (pH 3.0) solution. The analytical curve, obtained by square-wave voltammetry after accumulation, was linear in the linuron concentration range from 1.0 to 74.0 nmol L⁻¹, with a detection limit of 0.61 nmol L⁻¹. This sensitive analytical method was successfully applied for linuron determination in environmental water samples with results that showed good agreement with those obtained using a comparative HPLC method.

Introduction
Herbicides are the largest group of chemicals used as plant protection agents, and of these, phenylurea is used on pre- and post-emergent crops. Phenylurea can persist in water for a few days or weeks depending on conditions such as temperature and/or pH while degradation via microbial activity is very slow and accumulation to toxic levels can occur. Biological contamination by phenylurea was studied in adults rats, where the presence of phenylurea caused the development of tumours. Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] is one of the most important commercial phenylureas that is widely used for the selective control of broadleaf weeds and grasses in fruit or field crops, cereals, and shelter belts. A wide range of analytical methods have been applied to the analysis of linuron that are mostly based on chromatographic techniques. Nevertheless, several methods are prone to drawbacks such as high cost, complicated and lengthy procedures, and unsuitability for practical use.

Electrochemical techniques, such as voltammetry, are a promising alternative to classical approaches due to their relatively low operating cost, good miniaturisation potential, and rapid and sensitive detection procedures suitable for faster analyses. Some methods for detecting linuron by voltammetric techniques have been previously reported. Hernandez et al. used differential pulse voltammetry and a carbon-paste electrode modified with 20% w/w sepiolite. Gonzalez de la Huebra et al. determined linuron in soil extracts using a carbon fibre microelectrode. More recently, Đorđević et al. developed procedures used to detect linuron in spiked river water samples and a commercial formulation using a tricresyl phosphate-based carbon paste electrode, a home-made non-modified glassy carbon electrode, and a modified boron-doped glassy carbon electrode. de Lima et al. employed a carbon paste electrode for electrochemical determination of linuron concentrations in water and vegetable extracts.

The modification of electrode surfaces with metal and metal oxide nanoparticles has been used extensively in the development of sensors as nano-sized transition metal particles have unique properties that differ from those of the bulk metal. The addition of nanoparticles to the surface of conductive substrates is expected to have advantages over conventional electrodes by providing large surface areas, high mass transport, enhanced
signal-to-noise ratios, as well as excellent catalytic and electrocatalytic activities. However, the catalytic properties of these metal particles strongly depend on the size, shape, and catalyst support. In the literature, the electrocatalytic performance of platinum nanoparticles (PtNPs) has been used for the sensing of biological molecules, oxidation of methanol, formic acid, and the reduction of oxygen.

It is worth mentioning that the immobilisation of nanoparticles on substrates such as graphite, carbon nanotubes, glassy carbon, and other metals for sensing purposes has been studied extensively in recent years. Our research group had studied and applied different kinds of surfactants and polyelectrolytes for nanomaterial immobilisation. Dihexadecyl phosphate (DHP) is a hydrophobic surfactant that has two long hydrocarbon chains linked to a phosphate group, which self-assembles into multiple bilayer structures similar to lipid bilayers and exhibit characteristic of gel-to-liquid crystal phase transition.

The aim of this study was the preparation, characterisation, and testing of a new sensor based on a glassy carbon electrode modified with PtNPs within a dihexadecyl phosphate film (PtNPs-DHP/GCE) in order to investigate the electrochemical behaviour of linuron as well as its detection in environmental water samples and commercial samples.

Experimental
Reagents and Solutions
All reagents were of analytical grade and the solutions were prepared with water purified with a Milli-Q system (Millipore) (resistivity >18 MΩ cm). The reagents used were potassium chloride (Merck), hydrochloric acid (Merck), sulfuric acid (Sigma–Aldrich), potassium hexacyanoferrate(III) (Synth), potassium hexacyanoferrate(II) (Synth), dihexadecyl phosphate (Aldrich), sodium phosphate dibasic (Synth), monobasic sodium phosphate (Synth), boric acid (Merck), phosphoric acid (Mallinkrodt), linuron (Aldrich), and ethanol (Mallinkrodt). Acetic acid, sodium acetate, platinum(iv) chloride and sodium citrate were obtained from Sigma–Aldrich. H₃PO₄ (85 %) and NaH₂PO₄ that were used to prepare the supporting electrolyte were purchased from Merck. All other chemicals were of analytical grade.

A 0.1 mol L⁻¹ phosphate buffer solution (pH 3.0) was used as the supporting electrolyte for linuron determination and a 0.1 mol L⁻¹ linuron stock solution was prepared daily in this solution. Standard linuron solutions were prepared from the stock solution in 0.1 mol L⁻¹ phosphate buffer solution (pH 3.0) and bubbled with ultrapure N₂ gas to prevent chemical oxidation.

Apparatus
Voltammetric measurements were carried out using an Autolab Ecochemie PGSTAT20 (Utrecht, Netherlands) potentiostat/galvanostat controlled with the GPES 4.9 software. All the electrochemical experiments were conducted at 25.0 ± 0.5 °C in a three-electrode single-compartment glass cell using a glassy carbon electrode (diameter = 3 mm) modified with PtNPs within a dihexadecyl phosphate film (PtNPs-DHP/GCE) as the working electrode, Pt foil as an auxiliary electrode, and an Ag/AgCl (saturated KCl) reference electrode.

The pH was determined using a Denver Instrument pH meter (UB10 Ultra Basic) employing a combined glass electrode with an Ag/AgCl (saturated KCl) external reference electrode.

The comparative method employed for linuron determination was HPLC using a LC-10AT Shimadzu system with an UV–visible detector set at 250 nm (SPD-M10AVP) and a Shim-Pack CLC-ODS (4.6 mm i.d. × 150 mm length, 5 μm particle size) chromatographic column. The mobile phase consisted of methanol/water/acetonitrile (40: 40: 20, v/v/v) at a flow rate of 1.0 mL min⁻¹ with an injection volume of 20 μL.

Field-emission gun scanning electron microscopy (FEG-SEM) and energy-dispersive X-ray spectroscopy (EDX) analyses were conducted on a Supra 35-VP equipment (Carl Zeiss, Germany) with an electron beam energy of 25 keV. A disk cut from a glassy carbon electrode (GCE) was used for immobilising the dispersion of PtNPs-DHP using the same casting method employed for the electrochemical analysis electrode.

PtNPs Synthesis
The PtNPs were synthesised by the Turkevich method through the citrate-mediated reduction of platinum chloride (PtCl₄). The PtNPs dispersion was obtained by adding 4.0 mL of 0.5 mmol L⁻¹ platinum chloride to 200 mL of water at 85°C with stirring then adding 2.0 mL of 0.3 mol L⁻¹ sodium citrate solution with stirring for 7 min. The solution was placed in an ice-bath to cool to room temperature and was stored in an amber flask at room temperature.

Preparation of PtNPs-DHP Film
The GCE was mechanically polished with sandpaper (P4000), rinsed with water and acetone then air-dried. The PtNPs-DHP film was prepared by dispersing 1.0 mg DHP in 1.0 mL of PtNPs by ultrasonication for 15 min, then adding 10 μL to the GCE surface using the casting method, and then allowed to dry for 24 h. The study of the amount of deposited platinum was carried out previously and 10 μL was the best one.

Analytical Procedure
Linuron determination was performed using square-wave voltammetry (SWV). The SWV parameters used and the optimal chemical conditions (supporting electrolyte and pH) were also evaluated. After optimising the parameters, SW voltammograms for linuron were obtained within the potential range from 0.0 to +1.5 V versus Ag/AgCl (saturated KCl) with an accumulation time of 270 s at open circuit potential, a frequency of 50 Hz, pulse amplitude of 40 mV, and potential increment of 7.5 mV. All measurements were carried out in triplicate (n = 3) for each concentration.

Environmental water samples (A1 and A2) were collected: A1 was collected from the Ribeirão Pirai river (GPS Latitude – 23°26’201’S and Longitude – 47°05’790’W) and A2 was collected from the Jundiaí river (GPS Latitude – 23°2’055’S and Longitude – 47°29’176’W). A 20 μL aliquot of the 1.0 × 10⁻⁶ mol L⁻¹ linuron standard solution was added for every 2 mL of sample. The commercial sample A3 (linuron concentration of 450 g L⁻¹) was purchased from a local market and properly diluted.

Results and Discussion
Characterisation of PtNPs-DHP Film
First, the particle size, morphology, and elemental compositions of the PtNPs-DHP film (dried over the GCE surface) were characterised by SEM. As shown in Fig. 1, two types of particle structures were observed on the surface, the cubic particles could be from the salt solution used to produce the nanoparticles and the small particles (grey) were the PtNPs. EDX analysis was
performed to identify the elements present on the electrode surface and results showed a high percentage of carbon (94.11%), oxygen (4.55%), platinum (0.29%), chlorine (1.02%), and phosphorus (0.02%); those values were in agreement with the expectations considering the chemicals used in the film preparation. It is important to note the carbon detected by EDX was associated with the glassy carbon used as a substrate.

The proposed electrode was characterised by cyclic voltammetry. Fig. 2a shows the voltammetric behaviour of a polycrystalline bulk platinum electrode (dashed line) and PtNPs-DHP/GCE (solid line). The voltammetric profile was well defined for platinum: hydrogen region (−0.2 V to +0.2 V versus Ag/AgCl (saturated KCl)), electric double layer (+0.2 V to 0.7 V versus Ag/AgCl (saturated KCl)), and platinum oxidation region (0.7 V versus Ag/AgCl (saturated KCl)). The same behaviour was observed for the PtNPs supported on the GCE (solid line).

The electroactive areas of the GCE, DHP/GCE, and PtNPs-DHP/GCE electrodes were estimated through the redox response of the [Fe(CN)]$_6^{4-}$ species (1.0 × 10$^{-3}$ mol L$^{-1}$ in 0.1 mol L$^{-1}$ KCl) according to the Randles–Ševčík equation (Eqn 1):

$$I_{pa} = 2.69 \times 10^5 n^{3/2} A D^{1/2} C^{1/2}$$  \hspace{1cm} (1)

where $I_{pa}$ is the anodic peak current (A), $A$ is the electroactive area (cm$^2$), $D$ is the diffusion coefficient of the [Fe(CN)]$_6^{4-}$ species in solution (6.2 × 10$^{-6}$ cm$^2$ s$^{-1}$), $n$ is the number of electrons transferred in the redox reaction, $v$ is the potential scan rate (V s$^{-1}$), and $C$ is the concentration (mol cm$^{-3}$) of [Fe(CN)]$_6^{4-}$.

The electroactive areas of the GCE, DHP/GCE, and PtNPs-DHP/GCE electrodes were estimated at 0.028, 0.005, and 0.020 cm$^2$, respectively. The DHP/GCE showed a lower electroactive area compared with GCE because there was a blockage of active sites and difficulty in the electron transfer and/or diffusion of electroactive species at the electrode surface, thereby decreasing the current signal. However, when the GCE was modified with PtNPs-DHP, the electroactive area increased by a factor of four with respect to the DHP/GCE. The advantage of using DHP dispersions is due to the greater adherence and stability of the film provided by this surfactant.$^{[25,27]}$ Fig. 2b shows the voltammetric behaviour of PtNPs-DHP/GCE for 1.0 × 10$^{-3}$ mol L$^{-1}$ [Fe(CN)]$_6^{4-}$ in 0.1 mol L$^{-1}$ KCl solution.

**Electrochemical Behaviour of Linuron Herbicide Using a PtNPs-DHP/GCE**

The cyclic voltammetric behaviour of PtNPs-DHP/GCE (solid line) towards linuron was compared with those of bare GCE (dotted line), a Pt electrode (dash dotted line), and a DHP/GCE (dashed line) (Fig. 3). The proposed sensor showed a well-defined analytical signal for the analyte of approximately +1.2 V. Of note was the small anodic current (Fig. 3 inset) used with the GCE and DHP/GCE electrodes. This indicated that the incorporation of PtNPs into the DHP film could provide an increased current signal for linuron determination.

Studies were performed to determine the pH, supporting electrolyte, and adsorption time needed to optimise the analytical signal. Fig. 4a, b shows the effect of pH on the anodic peak...
current and anodic peak potential, respectively. The highest anodic peak current was obtained at pH 3.0 (Fig. 4a). The peak potential shifted linearly (with $R^2 = 0.971$) with increasing pHs and a slope of 0.052 was obtained – a value near the Nernst equation ($0.0592 \times E/pH$) – indicating that an equal number of protons and electrons were involved in the oxidation mechanism. The proposed oxidation mechanism for linuron (Fig. 4c) is based on dimerisation, involving two electrons and two protons, where the dimer is bonded to nitrogen I close to the aromatic ring. This mechanism is similar to that for another pesticide (diuron); both pesticides showed a small difference in structure (methoxy group on nitrogen II) and the oxidation potentials are comparable i.e. $\sim 0.85$ V for diuron and 1.2 V for linuron.

Three different supporting electrolytes were evaluated at pH 3.0 (0.5 mol L$^{-1}$ phosphate buffer, Britton–Robinson buffer, and acetic acid). The phosphate buffer presented a well-defined baseline as well as a better definition for the oxidation peak and was selected for further experiments. It is known that platinum-based electrodes may have adsorptive characteristics. Hence, linuron adsorption onto the sensor surface was examined. The results confirmed adsorption and after the stripping, no anodic current was observed, thus an accumulation time range from 30 to 300 s was studied and the selected value was 270 s.

**Optimisation of SWV Parameters and Analytical Curve for Linuron Using PtNPs-DHP/GCE**

The instrumental parameters for SWV were studied with the aim of obtaining a well-defined peak and high anodic peak current for linuron. The experimental parameters and their corresponding investigated ranges were as follows: frequency, $10 \text{ Hz} \leq f \leq 200 \text{ Hz}$; pulse amplitude, $10 \text{ mV} \leq a \leq 150 \text{ mV}$; and potential increment, $1.0 \text{ mV} \leq \Delta E_s \leq 10 \text{ mV}$). The best results for the SWV parameters were as follows: $f = 50 \text{ Hz}$; $a = 40 \text{ mV}$; and $\Delta E_s = 7.5 \text{ mV}$.

The SW voltammograms obtained after successive additions of the respective standard solution of linuron using the PtNPs-DHP/GCE electrode are shown in Fig. 5. The plot of the anodic current versus linuron concentration was found to
Fig. 5. SW voltammograms for: (1) blank, (2) 1.0, (3) 2.0, (4) 5.0, (5) 7.0, (6) 9.9, (7) 20.0, (8) 29.0, (9) 38.0, (10) 48.0, (11) 56.0, (12) 65.0, and (13) 74.0 nmol L\(^{-1}\) linuron using PtNPs-DHP/GCE under the optimum parameters in 0.1 mol L\(^{-1}\) phosphate buffer. Inset shows analytical curve for linuron.

### Table 1. Effect of several species on the determination of 10 nmol L\(^{-1}\) linuron (standard) in environmental water samples

<table>
<thead>
<tr>
<th>Interferents and Comparative Method</th>
<th>Concentration</th>
<th>Interference [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline ions/alkaline earth</td>
<td>100 nmol L(^{-1})</td>
<td>0.8</td>
</tr>
<tr>
<td>Metallic ions</td>
<td>100 nmol L(^{-1})</td>
<td>-5.2</td>
</tr>
<tr>
<td>Humic acid</td>
<td>0.1 % w v(^{-1})</td>
<td>0.2</td>
</tr>
<tr>
<td>Vermicompost</td>
<td>0.1 % w v(^{-1})</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Table 2. Results obtained in the determination of linuron (mg L\(^{-1}\)) in environmental water samples and commercial samples comparing HPLC (standard method) and SWV (proposed methods)

<table>
<thead>
<tr>
<th>Samples</th>
<th>HPLC</th>
<th>SWV</th>
<th>Relative error(^A) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.49 ± 0.01</td>
<td>2.42 ± 0.02</td>
<td>-2.81</td>
</tr>
<tr>
<td>B</td>
<td>2.54 ± 0.06</td>
<td>2.69 ± 0.02</td>
<td>5.90</td>
</tr>
<tr>
<td>C</td>
<td>2.73 ± 0.04</td>
<td>2.34 ± 0.03</td>
<td>-14.29</td>
</tr>
</tbody>
</table>

\(^A\)100 \times (SWV value - HPLC method)/HPLC method.

Table 3. Comparison of analytical parameters obtained for linuron determination using the proposed method (PtNPs-DHP/GCE) and other methods (various electrodes)

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Detection limit (nmol L(^{-1}))</th>
<th>Concentration range (nmol L(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>92</td>
<td>103–1240</td>
<td>de Lima et al.(^{[11]})</td>
</tr>
<tr>
<td>CM</td>
<td>320</td>
<td>361–5260</td>
<td>Gonzalez de la Huebra et al.(^{[9]})</td>
</tr>
<tr>
<td>CP/Sepiolite</td>
<td>300</td>
<td>–</td>
<td>Hernandez et al.(^{[8]})</td>
</tr>
<tr>
<td>CP/Tricresyl phosphate</td>
<td>1500</td>
<td>5050–177 000</td>
<td>Đordević et al.(^{[5]})</td>
</tr>
<tr>
<td>GC</td>
<td>1000</td>
<td>28000–130 000</td>
<td>Đordević et al.(^{[10]})</td>
</tr>
<tr>
<td>BDGC</td>
<td>600</td>
<td>5000–100 000</td>
<td>Đordević et al.(^{[10]})</td>
</tr>
<tr>
<td>PtNPs-DHP/GCE</td>
<td>0.6</td>
<td>1–74</td>
<td>This work</td>
</tr>
</tbody>
</table>
environmental protection agency (CONAMA)\textsuperscript{[34]} (i.e. $2.00 \times 10^{-7}$ mol L$^{-1}$) and Canadian Water Quality Guidelines\textsuperscript{[35]} (i.e. $2.81 \times 10^{-8}$ mol L$^{-1}$).

**Conclusions**

A modified glassy carbon electrode (GCE), Pt nanoparticles, and dihexadecyl hydrogen phosphate film electrode (PtNPs-DHP/GCE) were assembled using a simple drop coating procedure and used for the electroanalytical determination of linuron at extremely low concentrations by SWV. The proposed method had a lower detection limit (0.6 nmol L$^{-1}$) and a wider linear range ($1.0 - 74.0$ nmol L$^{-1}$) than other methods. The proposed method was used for linuron determination in environmental water samples and the results obtained were in agreement with HPLC (confidence level of 95%). The results of this study indicated that the combination of SWV and PtNPs-DHP/GCE is a simple, rapid, and inexpensive alternative analytical method for linuron determination.

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**References**


\[24\] Conselho Nacional de Meio Ambiente, *RESOLUCÃO N° 20, 1986.*