Multiresidue analysis of phenylurea herbicides in environmental waters by capillary electrophoresis using electrochemical detection

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Abstract A rapid multiresidue method has been developed for the analysis of seven phenylurea herbicides in the presence of two s-triazines in environmental waters. A simple end-column electrochemical detector was used in combination with a commercially-available capillary electrophoresis instrument with UV detection. The determination of phenylurea pesticides using micellar electrokinetic capillary chromatography with electrochemical detection represents the first such determination that has been reported. In both detection systems, linear ranges were obtained for the seven phenylurea herbicides at concentrations lower than $2.0 \times 10^{-5}$ mol l$^{-1}$, in 0.020 mol l$^{-1}$ phosphoric acid at pH 7.0 and containing 0.020 mol l$^{-1}$ of sodium dodecylsulfate, in order to obtain selectivity in the additional separation by a micellar distribution process. Under these conditions a detection limit lower than $5.0 \times 10^{-6}$ mol l$^{-1}$ (0.25 pmol of pesticide) was achieved for most of them. The pesticides were resolved in less than 30 min.

Keywords Micellar electrokinetic capillary chromatography · Electrochemical detection · Phenylurea herbicides · Environmental waters

Introduction

The use of phenylurea herbicides in agriculture results in a high risk of these herbicides entering the food chain by means of contaminated water and fodder. Their extensive use also causes other problems, including the requirement for increased doses of herbicides as a result of pest adaptation and the introduction of new herbicides with higher activity and specificity. Progressive increases in the production and application of these kinds of herbicides for plant protection has converted the problem of water quality into an international and national issue. Cases of incidental pesticide pollution of water reservoirs have become more numerous in recent years [1].

The focus in pesticide development has shifted from the non-polar, persistent, long-life pesticides to the more polar and degradable short-life pesticides [2]. Due to the inherently higher environmental mobilities of the polar pesticides, it has become necessary to supplement the public health legislation regulating levels allowed in drinking water supplies. All of these changes mean that we need to develop analytical techniques for residue detection in aqueous samples. The recent intensive use of pesticides has increased the agricultural productivity, but it has also generated pesticide residues in natural waters at concentration levels which exceed legal limits. Pesticides with different chemical structures, including triazines and phenylurea compounds, can be found in ground and surface waters.

The 1989 European Community (EC) Water Act states that the maximum admissible concentration of all pesticides in drinking water should be lower than 500 ng l$^{-1}$, and that the maximum individual pesticide concentration is 100 ng l$^{-1}$. Therefore, the detection limits required for the analysis of drinking water need to be at the low-nanogram level [3].

Phenylureas are selective systemic herbicides commonly used in agriculture, alone or in combination, for the pre-emergence treatment of soil. Due to their polar...
nature, the increased possibility of them leaching from the surface to the water supply and water reserves, together with the emergence of potentially toxic degradation and metabolic products, may constitute a risk to human health. Several techniques have been reported for phenylurea determinations [4, 5, 6, 7].

On the other hand, s-triazine herbicides are among the most common pesticides used to control broadleaf and grassy weeds in corn and other crops. Due to their extensive use and their relatively high persistence, chlorotriazines like simazine contaminate the aquatic environment through agricultural runoff, direct applications, and leaching into ground water, in increasing concentrations. Many efforts have been devoted to developing rapid assays for the quantification of triazine herbicides at low levels in water [8].

Nowadays, the application of capillary electrophoresis (CE) to the separation of analytes in different samples has become increasingly widespread because of its minimal sample volume requirement, short analysis time and high separation efficiency. A UV absorbance detector is commonly used, which is standard on commercial CE instruments. For UV absorbance measurements performed in the on-column configuration detector, induced band broadening can be neglected when a portion of the capillary is used for detection. On the other hand, the short optical path length leads to relatively high concentration detection limits. Lower detection limits can be achieved by laser induced fluorescence detection. However, the laser equipment is rather expensive and in most of the cases derivatization is necessary, which complicates the analytical process, particularly if small samples have to be analyzed.

Electrochemical detection (ED) has the advantage that concentration limits are not compromised by miniaturization. Steady-state measurements with perfectly prepared voltammetric microelectrodes may even lead to lower detection limits than the ones that are obtained with macroelectrodes. ED typically operated in the amperometric mode can be coupled with CE to provide high sensitivity and selectivity for the determination of electroactive substances [9, 10, 11]. The required components for amperometric detection are rather simple and inexpensive. Moreover, ED is applicable to a broad range of important analytes, owing to the variety of electrode materials and electrochemical processes that can be used for detection. The two commonly used CE modes for the analysis of herbicides are free capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC), both with a UV detector [4]. In addition, MEKC with ED with a carbon electrode has been applied for the detection, UV and electrochemical, of asulam in environmental water [12].

In this work, MEKC with simultaneous UV and ED has been employed for the separation and detection of different kind of herbicides (Fig. 1). They belong to two herbicide groups (phenylureas and s-triazines), and are the following: fenuron, chlorotoluron, monuron, monolinuron, isoproturon, diuron, linuron, atrazine and simazine. The working electrode was a carbon paste electrode, inserted in a handy, simple and versatile ED cell for a commercially available CE system. This device has many advantages and it has been described in a previous papers [12, 13]. The effects of several important factors were investigated in order to find the optimal conditions. The proposed method has been applied to determining seven phenylurea herbicides in the presence of two triazines in environmental water samples, collected from the Alberche River in Comunidad Autónoma de Madrid (Madrid, Spain).

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**Experimental**

**Reagents**

Fenuron, chlorotoluron, monuron, monolinuron, isoproturon, diuron, linuron, atrazine and simazine grade Pestanal were purchased from Riedel de Haën (Madrid, Spain). The herbicide stock solutions, $1.0 \times 10^{-2}$ mol l$^{-1}$, were prepared by dissolving an appropriate amount of the compound in methanol:water. All stock solutions were kept away from the light and stored under refrigeration. Diluted solutions were prepared daily from the stock solutions. Methanol grade Pestanal, purchased from Riedel de Haën (Madrid, Spain), was used as solvent. All other chemicals used for the buffer and supporting electrolyte preparation were of analytical-reagent grade. Water used for preparing solutions was purified with a Milli-Q Milli-RO water system (Millipore, Spain). Buffers and samples were sonicated for 5 min and microfiltered through a 0.45 µm MFS-13 filter (Advantec MFS, Inc. USA).

**Apparatus and electrodes**

All electrochemical measurements were performed in the three electrode mode using an electrochemical analyzer (BAS 100B) connected to a 386 PC. Data storage and conversion to “txt” files was performed by BASCOM 2.21 software (BAS, West Lafayette, USA). A silver wire and a platinum wire were employed as pseudo-reference and counter electrode respectively. All potentials given in this work were measured with respect to this reference system. All experiments were carried out at room temperature by applying the desired operating potential. The current was allowed to reach a stable baseline prior to amperometric monitoring.

The CE with a dual detection system (UV and electrochemical) has been described previously [12]. CE experiments were carried out with a SpectraPHORESIS 100 (Thermo Quest Corporation, Spain) equipped with a SC100 variable-wavelength UV/vis detector (Thermo Quest Corporation, Spain). Data acquisition and processing were accomplished using a 486/PC equipped
Fig. 1 Structures, names and chemical abstract registry numbers of the herbicides studied.

with two channels and a Chrom-Card software package (Thermo Quest Corporation, Spain). No variation was introduced on the original commercial set-up. A 100-cm fused silica column with a 2-cm Nafion tubing decoupler was used for electrophoresis separation with electrochemical and UV detection (effective length 70 cm). This column had an i.d. of 75 μm and an o.d. of 365 μm, and was supplied by Supelco, cat. No. 77500 (Bellefonte, USA).

Carbon paste electrodes 500 μm in diameter were handmade by mixing graphite powder (Acheson 38#, Fischer Scientific. Code no. G/0900/60) and mineral oil (Aldrich Chemical Co. no. 16/140-3). The ratio of graphite powder to mineral oil was 70:30. The bodies of the CPEs were 20-mm length PTFE tubes with 0.5 mm i.d and 1.6 mm o.d., filled with the carbon paste. Electrical contact was established with a copper wire. Special activation of the paste was not necessary.

Solid phase extraction procedure

Fortified samples were prepared by adding appropriate amounts of standard solutions of herbicide to the water to yield the desired final concentrations ($1.0 \times 10^{-7}$–$4.0 \times 10^{-7}$ mol l$^{-1}$). In all cases, a blank sample was submitted to the same procedure for comparison. Then the water samples were treated with the solid phase extraction technique to preconcentrate and purify it. C18 cartridges were conditioned with 2 ml of methanol and 2 ml distilled water. A total of 100 ml of water samples were passed through the C18 cartridges. Under these conditions, phenylurea herbicides are retained in the adsorbent. Herbicide retrieval was carried out with 2 ml of methanol. The extracts were evaporated to dryness under a nitrogen stream and subsequently diluted to a final volume of 100 μl with methanol:water (18:82); the same percentage of methanol:water used for...
the stock solutions of the herbicides. The reconstituted samples were prepared in triplicate and 100 µl sample microvials were used to introduce the sample into the electrophoretic system.

The water samples were collected from different sites along the Alberche River. All the samples were collected following the EPA’s recommendations. Sample analysis was carried out within 48 h of being taken from the river.

**Results and discussion**

Cyclic and hydrodynamic voltamograms

Cyclic voltammetry is a suitable technique for studying the electrochemical behaviour of electroactive compounds. It can help to select the potential of the working electrode. Figure 2 shows cyclic voltammograms for the seven investigated electroactive phenylurea herbicides in 0.05 mol l⁻¹ phosphate buffer at pH 7.0. It is obvious that the other two s-triazine herbicides were not electroactive, so simultaneous UV and ED was employed in this work in order to observe the important interference of the s-triazines in the UV detection of phenylurea herbicides. According to the cyclic voltammograms, the oxidations of all of the herbicides were irreversible, with anodic peak potentials ranging from 850 to 1,110 mV.

The effect of the scan rate, between 5 and 100 mV s⁻¹, on the analytical signal was investigated for 5.0x10⁻⁴ mol l⁻¹ herbicide concentration in 0.05 mol l⁻¹ phosphate buffer at pH 7.0. A proportional dependence of the peak current on the square root of the scan rate was observed for all phenylurea herbicides (not shown). This fact is indicative of a diffusional process.

The potential applied to the working electrode directly affects the sensitivity and detection limits of this method and it is necessary to determine the hydrodynamic voltamgrams for the electroactive herbicides to obtain the optimum potential. As shown in Fig. 3, the peak current increases rapidly at a potential of 600 mV for chlorotoluron, and 700 mV for the other phenylureas. When the applied potential exceeds 850 mV, the peak currents of all herbicides increase more slowly. Although an applied potential of greater than 950 mV results in higher peak currents, both the baseline noise and the background current increase substantially for potentials higher than 1,000 mV, due to oxidation of the solvent. The high background current leads to an unstable baseline, which is a disadvantage for sensitive and stable detection in ED of the herbicides. The potential applied to the working electrode was maintained at 950 mV; under this condition the background current is not too high and the signal-to-noise ratio is the highest. The reproducibility and stability of the analytical signal was studied for a series of ten 5.0x10⁻⁵ mol l⁻¹ sample injections (a total of 300 min of analysis time), resulting in a relative standard deviation (RSD) of 6.1%. Higher analysis times imply a slight electrode poisoning and a signal decay (20% from the initial signal for analysis times greater than 420 min). The cell provides stable measurements within reasonable long analysis times under the experimental conditions, despite the non-ideal redox behaviour of the electroactive herbicides.

Analytical conditions for separation and quantification

The development of the electrophoretic method was based on the application of the MEKC, useful for the separation of neutral compounds, such as phenylurea herbicides. Sodium dodecyl sulfate (SDS) was chosen as the micelle-forming agent, which is able to form a pseudophase into which the analyte molecules are partitioned. Our work initially focused on the studies of Barroso et al [16], using a mixture of 0.004 mol l⁻¹ sodium tetraborate, 0.012 mol l⁻¹ potassium dihydrogenphosphate and 0.030 mol l⁻¹ SDS at pH 7.0. The potential applied for the separation was 30 kV, resulting in a current of approximately 30 µA. Under these experimental conditions, we do not obtain a useful separation, and the resolutions of the peaks are very poor. In order to obtain an efficient separation for our phenylurea herbicides, different buffer types were tested. The subsequent studies were carried out in the absence of sodium tetraborate, and different concentrations of phosphate buffer (0.005–0.050 mol l⁻¹ phosphate buffer solutions) were tested. Separation was achieved only when the concentration of phosphate buffer solutions was higher than 0.020 mol l⁻¹. Similar studies were carried out for various SDS concentrations; in all cases the separation was efficient when the SDS concentration was higher than 0.020 mol l⁻¹. A mixture of 0.020 mol l⁻¹ potassium dihydrogenphosphate and 0.020 mol l⁻¹ SDS adjusted to pH 7.0 was found to be the optimum running electrolyte composition and was used for the subsequent analysis. After these experiments, studies of the most suitable applied voltage for the herbicide separation revealed that the resolution improved as the voltage is increased from 5 to 20 kV, so a running voltage of 18 kV (current 34 µA) was selected for subsequent studies in order to avoid the possibility of Joule heating and allowing for the fact that the time of analysis was 30 min. At this point in the investigation, it is important to note the impossibility of separating the isoproturon and atrazine. These herbicides appeared at the same migration time and UV detection was impossible. When ED was used for the analysis of these compounds, detection and quantification of isoproturon was possible, due to the fact that atrazine is not electroactive.
It has been demonstrated in previous studies [12, 13] that an electrochemical device used in connection with a commercial CE system can give good analytical results, for electrochemically reversible and irreversible analytes, in a simple way without the need for complicated precision apparatus. No distortion in the
for each herbicide to check the phosphate buffer at pH 7.0 containing hydrodynamic voltammograms for 1.0 mol l\(^{-1}\) SDS. Separation voltage 20.0 kV. Hydrodynamic of mol l\(^{-1}\) to mol l\(^{-1}\) for all herbicides. In mol l\(^{-1}\) · l\(^{-1}\) to the different thermophysical properties of the sample longitudinal diffusion of the sample in the capillary, owing to the different thermophysical properties of the sample and the separation buffer containing SDS, leading to a widening of the size of the injected sample [17]. Moreover, the presence of methanol affects the micelle formation, which naturally has consequences for the final separation.

The hydrodynamic injection volume was optimized with respect to peak height and peak area at two different concentration levels (5.0\(\times\)10\(^{-5}\) and 1.0\(\times\)10\(^{-4}\) mol l\(^{-1}\)) containing 18% (v/v) methanol–water. Although, in some cases, peak area was linear with injection volumes over 75 nl and resolution was maintained, the peak height response did not increase and peaks became broader with a decrease in the number of theoretical plates. An injection volume of 50 nl (1.0 s) provided the best results in the herbicide concentration studies. Moreover, the ED presented a better background signal than the UV detection, so the detection limits for ED will be lower than the detection limits for UV.

Figure 4 shows typical electrophoretic separations for the nine herbicides in both detection systems. It is very important to point out the differences in the migration times of the electroactive herbicides in both electropherograms. In the UV detection, the effective length of the capillary column is 70 cm while the effective length in the ED is the same as the total length of the capillary column, 100 cm. Also, the ED electropherogram shows seven peaks, corresponding to the phenylurea herbicides. In the UV detection electropherogram, we can see eight peaks, corresponding to seven phenylurea and one s-triazine (isoproturon and atrazine appeared at the same migration time).

Calibration curves based on peak area were prepared with introduced volumes of 50 nl and were used to quantify the seven phenylurea herbicides, using only ED. In Table 1 we list the regression equations, correlation coefficients, and detection limits for all of the herbicides. Each point reported is the average of six analyses. The UV detector response at 210 nm and the electrochemical one at +950 mV were both linear in most cases (with exception of isoproturon and simazine) in the sample concentration range 2.0\(\times\)10\(^{-5}\) to 3.0\(\times\)10\(^{-4}\) mol l\(^{-1}\). Also, linearity was maintained at higher concentrations, but this was not considered to be of practical use considering the expected concentration levels for these compounds in environmental water samples. The detection limits, calculated for a signal-to-noise ratio of 3, are listed in Table 1 for all herbicides. In the case of isoproturon, UV detection and quantification was almost impossible, so only the ED can be used for its quantification.

The migration time and the peak response reproducibility were evaluated at a concentration of 5.0\(\times\)10\(^{-5}\) mol l\(^{-1}\) for each herbicide to check the performance of the MEKC system for these phenylureas. The RSD values obtained were below 5.5% of the peak areas in ED, lower than 4.7% of the peak areas in UV detection and below 1.1% of the migration times. The high reproducibility indicates that this method is accurate and rugged.
Application and recovery in environmental water samples

We then demonstrated the applicability of the proposed MEKC method based on ED to the determination of the studied phenylurea in real samples under optimum conditions, was demonstrated by applying it to the environmental water samples from Alberche River (Comunidad Autónoma de Madrid, Spain). The water samples were collected from different sites along the Alberche River. All of the samples were collected following the EPA’s recommendations. The samples were analysed within 48 h of collection from the river.

First, a non-spiked 100 ml aliquot of each sample was analyzed, following the solid phase extraction procedure described in the “Experimental” section, to check for the presence of these herbicides. Qualitative analysis of the concentrated extracts from the Alberche River water samples did not show the presence of these herbicides. Therefore, subsequent water samples (100 ml) were spiked with different quantities of herbicides (1.0x10⁻⁷–4.0x10⁻³ mol l⁻¹). In all cases, a blank sample was submitted to the same procedure for comparison. Then the water samples were treated using the solid phase extraction technique described in the “Experimental” section. Figure 5 shows the electropherograms obtained, with ED, for a river water sample and the same sample spiked with 3.0x10⁻⁷ mol l⁻¹ of the herbicides studied.

Percent recoveries ranged from 85 to 102 for all herbicides, using ED. The RSDs for samples spiked at 1.0x10⁻⁷ mol l⁻¹ were 5.6%.

Conclusions

A new method, based on the ED of phenylurea using MEKC, has been presented. An electrochemical cell, designed to maintain a reliable operation at CE flow rates, was used. The electrochemical device could be easily coupled to commercially-available CE equipment without the need for special modification to its original configuration.

Joint detection with both UV detector and electrochemical detector was performed. The UV detection was performed in its usual configuration, on-column, while

Table 1 Results of regression analysis on calibration curves, and detection limits (working potential was 950 mV using ED)

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Regression equationa</th>
<th>Correlation coefficient (n=10)</th>
<th>Detection limit (mol l⁻¹)</th>
<th>Linear range (mol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenuron</td>
<td>( y = -0.1 \pm 0.3 + (6.5 \pm 0.4) \times 10^4 x )</td>
<td>0.998</td>
<td>3.80x10⁻⁶</td>
<td>1.4x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Monuron</td>
<td>( y = -0.5 \pm 0.1 + (5.1 \pm 0.1) \times 10^4 x )</td>
<td>0.9998</td>
<td>4.85x10⁻⁶</td>
<td>1.6x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Monolinuron</td>
<td>( y = +1.0 \pm 0.2 + (4.0 \pm 0.1) \times 10^5 x )</td>
<td>0.9990</td>
<td>6.10x10⁻⁶</td>
<td>2.0x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Chlortoluron</td>
<td>( y = +0.1 \pm 0.1 + (4.2 \pm 0.2) \times 10^5 x )</td>
<td>0.998</td>
<td>5.64x10⁻⁶</td>
<td>1.9x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>( y = +0.6 \pm 0.4 + (4.4 \pm 0.2) \times 10^5 x )</td>
<td>0.997</td>
<td>5.57x10⁻⁶</td>
<td>1.8x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Diuron</td>
<td>( y = -0.1 \pm 0.5 + (3.8 \pm 0.3) \times 10^5 x )</td>
<td>0.996</td>
<td>6.59x10⁻⁶</td>
<td>2.1x10⁻⁵–3.0x10⁻⁴</td>
</tr>
<tr>
<td>Linuron</td>
<td>( y = +0.7 \pm 0.3 + (1.2 \pm 0.2) \times 10^5 x )</td>
<td>0.994</td>
<td>1.97x10⁻⁵</td>
<td>6.6x10⁻⁵–3.0x10⁻⁴</td>
</tr>
</tbody>
</table>

The detection limits correspond to concentrations that give a signal-to-noise ratio of 3

aWhere \( y \) is peak area (nA min) and \( x \) is the concentration of the herbicide (mol l⁻¹), respectively
the amperometric detection was performed in an end-column configuration. Such a combined method shows very good versatility and selectivity. This permits electroactive and non-electroactive compounds to be detected by the electrochemical and UV detection systems at the same time.

The data reported show that MEKC is suitable for mono and multiresidue analysis of different kinds of herbicides (in this case phenylurea herbicides) in environmental water samples using the ED system. The determination of seven phenylurea herbicides was achieved under the best conditions for their separation and detection. The detection limits for all of the herbicides were better than $5.0 \times 10^{-6}$ mol l$^{-1}$ (0.25 pmol of herbicide) using the ED system. Qualitative analysis of the concentrated extract of water from Alberche River did not show the presence of these herbicides. When the method was applied to river water samples, collected from Alberche River (Comunidad Autónoma de Madrid, Spain) but spiked to different concentration levels of herbicides, very good results were obtained using a standard addition method. Future applications of the electrochemical device used in this paper will be in the field of herbicide determination in environmental samples, using a previous extraction procedure with different cartridge types in order to obtain detection limits ($5.0 \times 10^{-9}$ mol l$^{-1}$) close to the maximum residue levels allowed for the European Union.

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References