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Electrochemical Determination of the Glyphosate Metabolite Aminomethylphosphonic Acid (AMPA) in Drinking Waters with an Electrodeposited Copper Electrode

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Carbon electrodes with in situ electrodeposited copper are used to the determination of aminomethylphosphonic acid (AMPA), the major metabolite of the herbicide Glyphosate (N-(phosphonomethyl) glycine) in drinking waters. The oxidation signal to form Cu(II) ions changes after the addition of AMPA due to the formation of a complex, this effect being used to develop an analytical method simpler, inexpensive and faster than those conventionally used. Glassy carbon and carbon paste electrodes are investigated, concluding that the last is more suitable for routine analysis. Samples of drinking water fortified with different AMPA concentrations have been used to test the method.

Keywords: AMPA, Glyphosate, electroanalytical determination, interaction with copper, drinking waters

1. INTRODUCTION

Glyphosate, N-(phosphonomethyl) glycine, CAS-RN 1071-83-6, is a systemic, nonselective and broad-spectrum herbicide for controlling long grasses and broad-leaved weeds. Degradation of glyphosate in plants, water and soil mainly occurs under biological conditions yielding aminomethylphosphonic acid (AMPA) as the major metabolite. The introduction of transgenic plants resistant to glyphosate has greatly increased its use in agricultural fields, this generating concerns regarding its possible health hazards and environmental impacts. The monitoring of glyphosate and

AMPA at residue levels has, therefore, attracted considerable attention [1]. Most methods reported for the determination of glyphosate and AMPA are based on [2] the use of gas chromatography (GC), liquid chromatography (LC) [3] and capillary electrophoresis (CE) [4]. The analytical techniques as well as the sample pretreatment methods employed for analysis of glyphosate and AMPA have been reviewed by Stalikas and Konidari. The difficulties in establishing simple methods for the determination of these compounds at residue levels are mainly due to their high solubility in water and insolubility in organic solvents. Additionally, they have no chromophores, making the simple ultraviolet (UV) detection impossible, but indirect UV-visible absorption, widely used with CE, has been employed [5].

Most methods developed for the analysis of glyphosate and AMPA employ pre- or post-column derivatization procedures to confer volatility, fluorescence or UV absorption properties to the analytes, thus increasing analysis time and cost, as well as analytical uncertainty. Nonderivatization methods, such as enzyme linked inmunosorbent assys (ELISA) [6], electrogenerated chemiluminescence detection, conductivity detection, inductively-coupled plasma-mass spectroscopy (ICP-MS) and integrated pulsed amperometric detection (IPAD) at gold electrode [7] have also been reported for analysis of glyphosate though they are complicated and expensive. It is therefore necessary to develop simple and cheap analytical method for screening of glyphosate and AMPA at residue levels.

The aim of this work was to present a simple, rapid and inexpensive method for the determination of AMPA in waters.

2. MATERIALS AND METHODS

All chemicals were used without further purification and purchased form Merck (analytical grade) with the exception of AMPA that was from Sigma. Milli-Q water purified was used.

In the last years, Cu layers have been investigated as useful electrodes because their electrocatalytic possibilities [8-10]. Since above pH 8 the Cu surface is passivated [8], the measurements were made below pH 7. So, 0.1 M phosphate buffer at pH 6.5 was used as supporting electrolyte. 1.0 mM Cu^{2+} concentration was used in the experiments, which were made at room temperature [11, 12]. All potentials were measured against a Metrohm 6.0733.100 Ag/AgCl/3M KCl electrode. A CHI650A electrochemical workstation from IJCambria was used for measurements.

As working electrodes, two carbon electrodes were used.

a) An IJCambria glassy carbon electrode of 38.5 mm^2 area, polished with silicon carbide paper, followed by a diamond (0.25 μm) slurry and alumina (0.3 and 0.05 μm) slurries. Residual polishing material was removed from the surface by sonication of the electrode in a water bath for 30 minutes to obtain the *untreated glassy carbon electrode*. An electrochemical pretreatment of 5 voltammetric cycles between +2 and –2 V on the untreated electrode in a saturated sodium chloride solution was made daily prior to the use of the electrode, to obtain the *activated glassy carbon electrode*.

The solution to be analyzed was de-aerated with purified nitrogen during 15 min. The electrodeposition of copper was carried out with constant stirring for 120 s at –0.8 V. Cyclic

voltammgrams were recorded between the deposition potential and 0.8 V at a scan rate of 0.1 $V \cdot s^{-1}$ in solutions with and without AMPA.

b) A carbon paste electrode that was prepared by hand-mixing 0.50 g of carbon powder and 750 μL of paraffin (liquid for spectroscopy) from Merck. The paste was carefully mixed and homogenized in a glass mortar, and sonicated during 15 minutes. The paste was packed firmly into a glass tube with 8 mm inner diameter (198.5 mm^2 area). Electrical contact was established via a metal screw connected in the inner hole of the tube. The electrode surface was cut and smoothed by rubbing it on a piece of paper just prior to using. The electrochemical pretreatment involved 5 cyclic voltammograms between –0.4 and +1.4 V in a 0.1M phosphoric acid solution.

3. RESULTS AND DISCUSSION

In order to evaluate the reproducibility of the GCE a Cu layer was accumulated on the activated glassy carbon electrode at a constant potential of –0.80 V for 180 s while the solution was stirred, starting from a 0.4 mM Cu^{2+} solution in 0.1 M phosphate buffer at pH 6. After each measurement the glassy carbon electrode was electrochemically activated as described in the experimental section. This was made repeatedly and the comparison between cyclic voltammograms for the oxidation of Cu to Cu^{2+} and further reduction indicates that there is a very good reproducibility between different measurements.

The effect of the accumulation time on the oxidation peak was examined, finding that the peak current increased with the accumulation time. An accumulation time of 180 s was chosen in order to optimize the time of the experiments.

When the scan rate was kept constant and the Cu^{2+} concentration was changed, the cyclic voltammogram of the Cu layer was virtually unchanged above concentrations of 0.4 mM, this being the value selected as optimal concentration for this work.

Finally, for the experimental parameters optimization, the effect of the scan rate was studied. So, when the scan rate was low, the peak current of the oxidation peaks showed a linear behavior with the square root of the scan rate, this meaning that the electrochemical processes was mainly diffusion controlled. The positive deviations from this straight line at high scan rate values indicate that adsorption processes occurring at the electrode surface were involved in the overall reduction. So, a scan rate of 0.1 $V \cdot s^{-1}$ was selected for the study, to ensure that the transport occurred mainly by diffusion.

Figure 1 shows the effect of AMPA concentration on the voltammogram of the copper oxidation/reduction process.

The addition of AMPA at the concentrations given modifies the voltammetric response of the anodic peak for the transformation of Cu^0 to Cu^{2+}. Nevertheless, the response after the addition increases as the AMPA concentration is increased, as can be seen in Figure 2. This response could be used to quantify AMPA in the above concentration range.

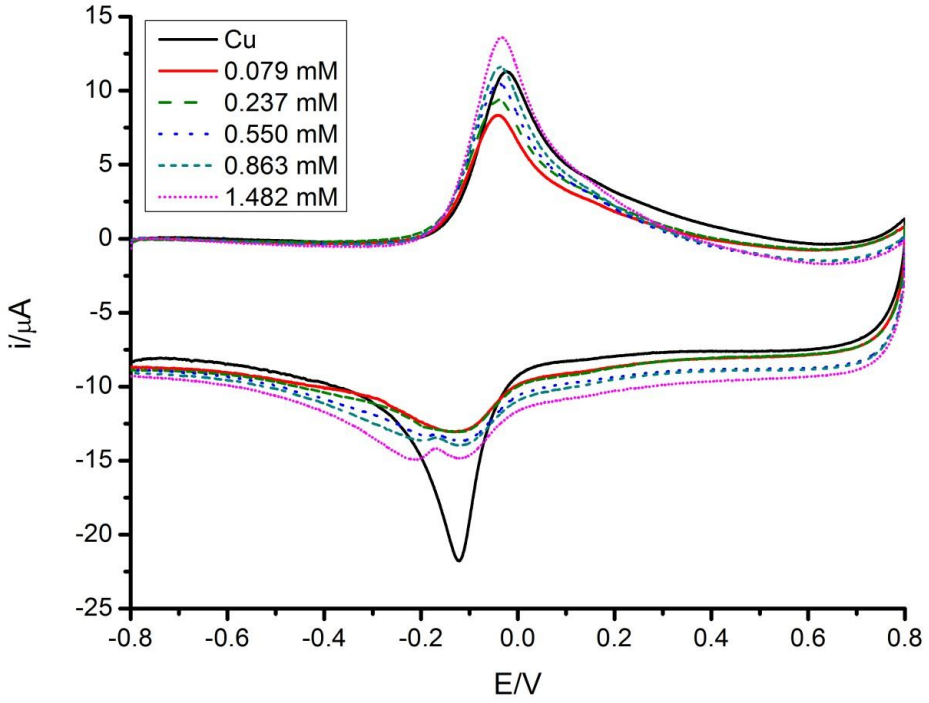

Figure 1. Cyclic Voltammograms on Glassy Carbon Electrode of copper 0.4 mM in 0.1 M phosphate buffer at pH 6.5; $E_{deposition}$ = –0.80 V; t_{dep} = 180 s; v = 0.1 V s^{-1} in the presence of the AMPA concentrations given in the figure.

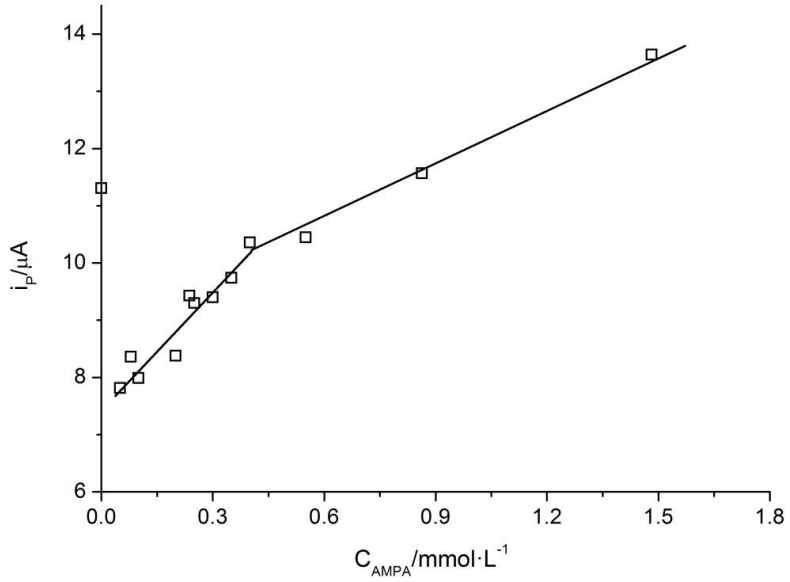

Figure 2. Plot of the oxidation peak current vs. AMPA concentration in the conditions corresponding to figure 1.

This behavior can be explained as follows: The application of the electrodeposition potential causes the formation of a Cu layer in the absence of AMPA. When the electrodeposition is made in the

presence of AMPA, this last molecule changes the morphology of the Cu layer, being adsorbed on the surface and causing a partial passivation of the electrode. When the copper oxidation takes place, the electrogenerated ions form a complex with AMPA molecules of the interface similar to the complex between the glyphosate and the copper ions [13-16]. As the AMPA concentration increases, the amount of adsorbed molecules also increases, and the inhibition due to such molecules increases, but the catalytic response also increases, this contribution being greater than the inhibition effect. The concentration dependence of the coverage must respond to an adsorption isotherm, typically increasing with the bulk concentration at low coverages (i.e. low concentration values of AMPA) and remaining virtually constant at high coverages. The formation of the complex catalyzes the Cu oxidation and the final response increases with the AMPA concentration. From figure 2 it can be assumed that at a concentration value of c.a. 0.45 mM, the maximum coverage of the electrode surface by the AMPA molecules is reached and the increase in the response is only due to the increase in the catalytic wave. The cathodic scan also suffers a variation with the AMPA concentration that will not be evaluated in this work, since only the oxidation peak is important for the analytic determination.

The values of the anodic peak current can be used to determine AMPA in the abovementioned concentration range. Nevertheless, when the glassy carbon electrode was used for several sets of experiments, the oxidation peak signal was modified with respect to the unused glassy carbon electrode. This was attributed to the modification of the glassy carbon structure by the Cu species generated in the oxidation-reduction process. Such modified glassy carbon electrodes have been cleaned up by different methods involving classical and not so classical cleanup methods. For example, an electrochemical clean up in nitric media and a chemical clean up using chromic mixture of different concentrations and treatment time were used, but it was impossible to recover the original surface. It was not possible to obtain reproducible results with those obtained with the unused glassy carbon electrode. For these reasons, a glassy carbon electrode can be used only for a few times, and recalibrations of the electrode must be made periodically. This causes a waste of time as well as an increase in the price of the analysis due to the need for the frequent change of glassy carbon electrodes.

For the above reasons, experiences were made with a carbon paste electrode (see experimental section) to take advantage of the simplicity of its surface renewability. Before each measurement the renewed carbon paste surface was activated through copper deposition and oxidation. The deposition was made at constant potential of –0.8V for 60 s under continuous stirring. The oxidation was made at constant potential of +1.2 V for 180 s, also while the solution was stirred. After that, a film of copper was deposited on the surface of the electrode and a cyclic voltammogram was recorded.

The reproducibility of the CPE between different measurements was very good, though slightly lower than for two successive measurements on GCE. In the case of CPE the reproducibility was independent of the number of uses of the electrode because the surface was renewed after each measurement. So, a calibration is needed to be made after the filling of the electrode, only if the operator changed.

Figure 3 shows the effect of the AMPA concentration on the copper voltammogram, which differ from the results obtained with the GCE. Now, there were two anodic peaks, attributed to the transformations of Cu to Cu^{+} and of Cu^{+} to Cu^{2+}, which increased upon the addition of AMPA, this indicating the formation of complexes between AMPA and copper ions, as in the precedent case. The

cathodic peak was moved slightly to negative potentials when the AMPA concentration was increased. The corresponding calibration curves for the voltammetric signals are shown in figure 4.

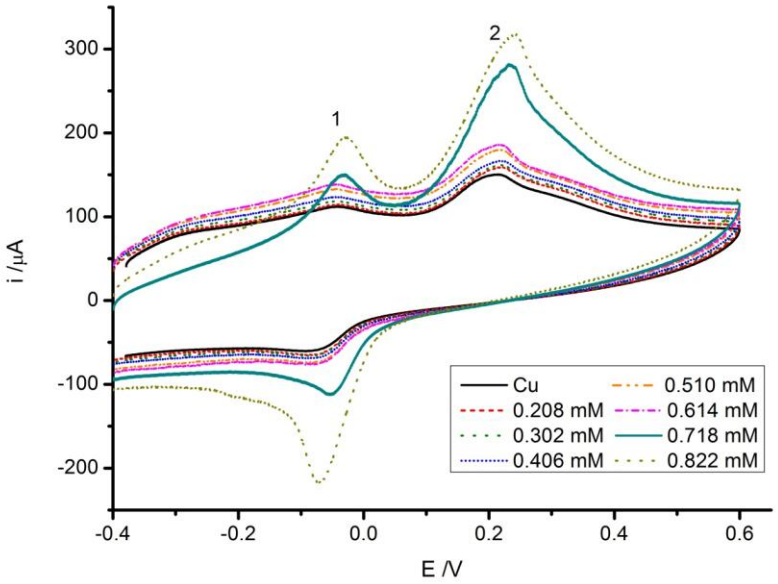

Figure 3. Cyclic Voltammograms on Carbon Paste Electrode of copper 0.4 mM in 0.1 M phosphate buffer at pH 6.5; $E_{deposition}$ = –0.80 V; t_{dep} = 180 s; v = 0.1 V s^{-1} in the presence of the AMPA concentrations given in the figure.

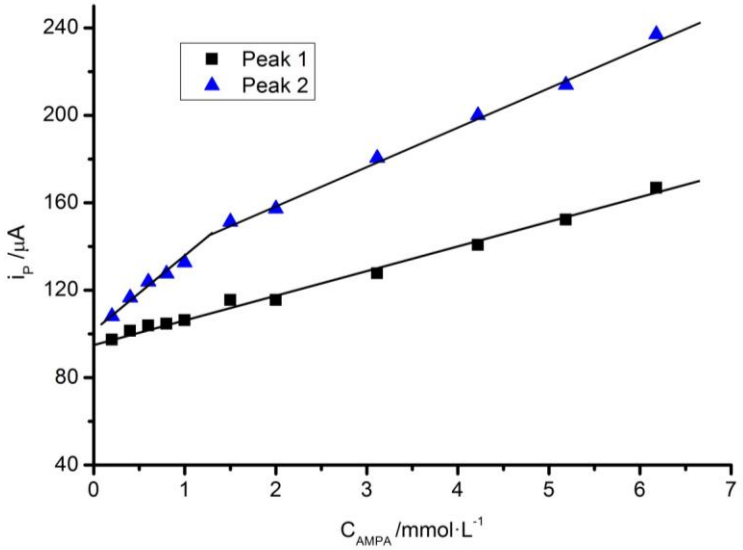

Figure 4. Plot of the oxidation peak currents vs. AMPA concentration in the conditions corresponding to figure 3.

The values of the currents recorded were much greater than those corresponding to the glassy carbon electrode, because the area of the carbon paste electrode was much greater than the area of the

glassy carbon electrode. Nevertheless, possibly due to different morphology and activity of the electrode surface, the differences between the voltammograms in the presence and in the absence of AMPA can be measured accurately at higher concentrations than those accessible with the GCE. So, very low levels of AMPA cannot be determined by using this electrode. The lower concentration that can be measured is c.a. 0.2 mM, whereas by using the GCE this limit was tenfold lower.

The peak 1 shows a linear behavior for all the concentration range studied whereas the peak 2 shows a behavior similar to that observed for GCE (figure 2). This fact can be due to either a best response of the catalytic current of the complex formed with Cu^{+} ions, at the potentials corresponding to the peak 1, with respect to the catalytic current of the complex formed with Cu^{2+} ions, at the potentials corresponding to the peak 2, or to a lower precision in the measurements of the peak 1 intensity.

Several samples of water for human consumption with added AMPA in the above range resulted in recoveries ranging between 97% and 103%, being slightly greater below 0.5 mM (around 105%).

4. CONCLUSIONS

The carbon electrodes with in situ electrodeposited copper used are useful to achieve the determination of aminomethylphosphonic acid (AMPA), the major metabolite of the herbicide Glyphosate (N-(phosphonomethyl) glycine) in drinking waters. The complex formation changes the oxidation signal of Cu to form Cu(II) after the addition of AMPA, this effect being used to develop an analytical method simpler, inexpensive and faster than those conventionally used. It is shown that carbon paste electrodes are more suitable for routine analysis than Glassy carbon electrodes. The method is tested with samples of drinking water fortified with different AMPA concentrations given good recoveries.

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