

Electrochemical Determination of Glyphosate in Waters Using Electrogenerated Copper Ions

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Carbon electrodes with in situ electrodeposited copper are used to the determination of Glyphosate (N-(phosphonomethyl) glycine) in drinking waters. The oxidation signal to form Cu(II) ions is enhanced after the addition of glyphosate due to the formation of a complex, this effect being used to develop an analytical method simpler, cheaper and faster than those conventionally used. Glassy carbon and carbon paste electrodes are investigated, concluding that the last is more suitable for routine analysis.

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

1. INTRODUCTION

The systemic, nonselective and broad-spectrum herbicide Glyphosate, N-(phosphonomethyl) glycine, CAS-RN 1071-83-6, is widely used in agriculture worldwide [1]. The action mechanism of glyphosate involves the inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate synthase, which acts on the biosynthesis of amino acids, mainly those with aromatic rests in the molecules, fundamental to the development of plants, such as tryptophan, tyrosine and phenylalanine [2].

Most of the papers concerning glyphosate determination employ high performance liquid chromatography [3] (HPLC), ion exchange chromatography coupled to a pulsed amperometric detector [4] (PAD), ion chromatography [5] (IC) or gas chromatography coupled to a mass spectrometer [6-9] (GC-MS), all of which are expensive and require long analysis time. Additionally, they have no chromophore, making the simple ultraviolet (UV) detection impossible, but indirect UV-visible absorption, widely used with CE has been employed [10].

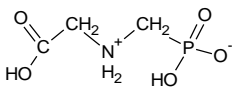
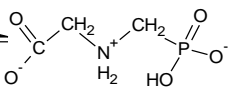
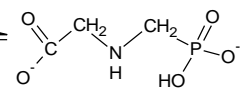
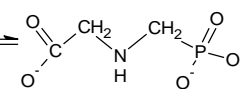
The advantages presented by the electrochemical methods are their relatively low cost, high sensitivity, easy operation, potentiality for miniaturization and automation, etc. Glyphosate itself is not electroactive at accessible potentials and indirect procedures were used such as modification of platinum electrode surfaces by silver carbonate sol [11]. Derivatization was also used, by quantitative hydrolysis of the amino groups to N-nitroso groups, which are easily reduced on mercury electrodes and can be determined by voltammetric techniques [12, 13]. Copper electrodes have also been used to the electrochemical detection of glyphosate because their electrocatalytic possibilities [14-22], making use of the coordination compounds formed between the functional groups of glyphosate (amino-, carboxyl- and phosphonate-) and copper ions in solutions of pH near the neutrality [16-18].

Recently [23], a copper phthalocyanine/multiwalled carbon nanotube film-modified glassy carbon electrode has been used for the determination of by differential pulse and cyclic voltammetry, showing the formation of complexes with copper ions as those shown in table 1.

In a previous paper [24] carbon electrodes with in situ electrodeposited copper were used to the determination of aminomethylphosphonic acid (AMPA), the major metabolite of the herbicide Glyphosate (N-(phosphonomethyl) glycine) in drinking waters.

The aim of this paper was to present an electrochemical method for the quantification of glyphosate in a simple, rapid and inexpensive way.

Table 1. Dissociation equilibria of Glyphosate with their associated pK values [25] and stability constants of the Cu(II) complexes with the different species [28]

Reactions		\rightleftharpoons		\rightleftharpoons		\rightleftharpoons	
pK	-2.23		5.46		10.14		
logK(CuL)	--		18.1		17.5		12.7

2. MATERIALS AND METHODS

All chemicals used in this work were Merck analytical grade reagents and use without further purification, with the exception of glyphosate that was from Sigma. The solutions were prepared using water purified in a Milli-Q system. All experiments were carried out in 0.1 M phosphate buffer and 0.4

mM Cu^{2+} at room temperature and pH 6.5. All potentials were measured against a Metrohm 6.0733.100 Ag/AgCl/KCl 3M.

Measurements were made on a CHI650A electrochemical workstation from IJCambria. The glassy carbon electrode of 38.5 mm^2 area was polished with a silicon carbide paper, followed by diamond ($0.25 \text{ }\mu\text{m}$) slurry and alumina (0.3 and $0.05 \text{ }\mu\text{m}$) slurries. Residual polishing material was removed from the surface by sonication of the electrode in water bath for 30 minutes. An electrode prepared by this procedure will be considered as *untreated glassy carbon*. The electrochemical pretreatment involved five cyclic voltammetry between $+2$ and -2 V on the untreated electrode in a saturated sodium chloride solution. This procedure was done daily prior to the use of the electrode. This will be considered as *activated glassy carbon*.

The solution to be analyzed was first de-aerated by a stream of purified nitrogen for 15 min. The constant stirring necessary for the electrodeposition of the copper was attained by a magnetic stirrer. The deposition was carried out for 120 s at -0.8 V . After that cyclic voltammograms was recorded between the deposition potential and 0.8 V at a scan rate of $0.1 \text{ V}\cdot\text{s}^{-1}$ in solutions with and without glyphosate.

The carbon paste electrode was prepared by hand-mixing 0.50 g of carbon powder and $750 \text{ }\mu\text{L}$ of paraffin (liquid for spectroscopy) from Merck. The paste was carefully mixed and homogenized in a glass mortar with a further sonication for 15 minutes. The resulting paste was kept at room temperature. The paste was packed firmly into a glass tube with an 8 mm diameter. Electrical contact was established through a metal screw connected in the inner hole of the tube. The electrode surface was cut and smoothed by rubbing on a piece of paper just prior to use and also to regenerate the surface. For the electrochemical pretreatment, five cyclic voltammograms were made between -0.4 and $+1.4 \text{ V}$ in a 0.1M phosphoric acid solution.

3. RESULTS AND DISCUSSION

3.1. Glassy carbon electrode

In order to evaluate the reproducibility of the GCE several measurements were made. A 50 mL solution containing Cu^{2+} 0.4 mM and phosphate buffer 0.1M at pH 6.5 was transferred into a voltammetric cell. The Cu was accumulated on the activated glassy carbon electrode at a constant potential of -0.80 V for 180 s while the solution was stirred. After each measurement the glassy carbon electrode was electrochemically activated as described in the experimental section. Figure 1A shows the comparison between typical cyclic voltammograms for Cu^{2+} recorded after electrochemically treating the glassy carbon electrode and deposition of Cu, in separate experiments. As it can be seen, there is a good reproducibility between different measurements.

In addition, the effect of the accumulation time on the oxidation peak current was examined, finding that the peak current increased with the accumulation time, as shown in figure 1B. In order to decrease the time of the overall experiment, which implies the recording of various samples, an accumulation time of 180 s was chosen.

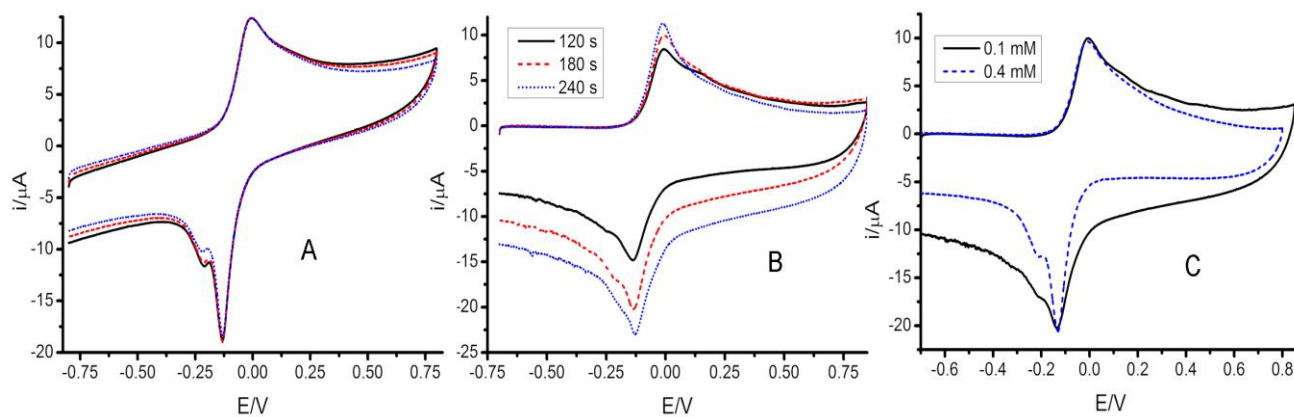


Figure 1. Cyclic Voltammograms on Glassy Carbon Electrode in 0.1 M phosphate buffer at pH 6.5. $E_{\text{deposition}} = -0.80 \text{ V}$; $v = 0.1 \text{ V s}^{-1}$. (A) Reproducibility of the GCE. Copper concentration: 0.4 mM; $t_{\text{dep}} = 180 \text{ s}$. (B) Dependence of the GCE response on the time of deposition. Copper concentration: 0.4 mM. (C) Dependence of the GCE response on the copper concentration in 0.1 M phosphate buffer at pH 6.5; $t_{\text{dep}} = 180 \text{ s}$.

Figure 1C shows the effect of Cu^{2+} concentration on the cyclic voltammograms at constant scan rate. The voltammogram was virtually unchanged above concentrations of 0.4 mM, this being the value selected as optimal concentration for this work.

Figure 2 shows the effect of scan rate of the voltammograms of Cu. When the scan rate is low, the electrochemical processes are mainly diffusion controlled, as can be inferred from the linear behavior of the peak current with the square root of the scan rate. The positive deviations from this straight line at high scan rate values indicate that adsorption processes occurring at the electrode surface are involved in the overall reduction. So, a scan rate of $0.1 \text{ V}\cdot\text{s}^{-1}$ was selected for the study, to ensure that the transport is mainly by diffusion.

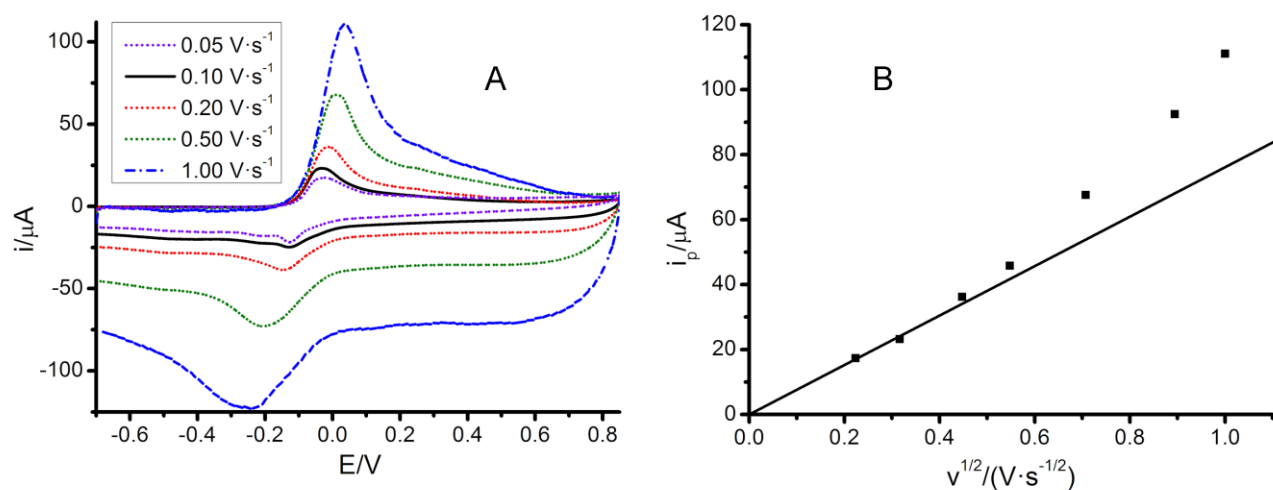


Figure 2. (A) Cyclic voltammograms of 0.4 mM copper on GCE in 0.1 M phosphate buffer at pH 6.5; $E_{\text{deposition}} = -0.80 \text{ V}$; $t_{\text{dep}} = 180 \text{ s}$; $v = 0.1 \text{ V s}^{-1}$. (B) Plot of the oxidation peak current vs. $v^{1/2}$.

Figure 3 shows the effect of glyphosate concentration on the voltammogram of the copper oxidation/reduction process. The increase in the anodic peak for the transformation of Cu^0 to Cu^{2+} upon the addition of glyphosate indicates the formation of a complex between the glyphosate and the copper ions²¹⁻²⁴. The cathodic scan suffers a complex variation with the glyphosate concentration that will be not evaluated in this work, since only the oxidation peak is important for the analytic determination.

The values of the anodic peak current, after subtraction of the current of the oxidation peak in the absence of glyphosate, $I_P - I_P(\text{Cu})$ in the figure, are proportional to the glyphosate concentration from c.a. 0.06 mM until 2.5-3 mM. Thus, this procedure can be used to determine glyphosate in the abovementioned concentration range.

When the glassy carbon electrode is used for several sets of experiments, the oxidation peak signal is 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.

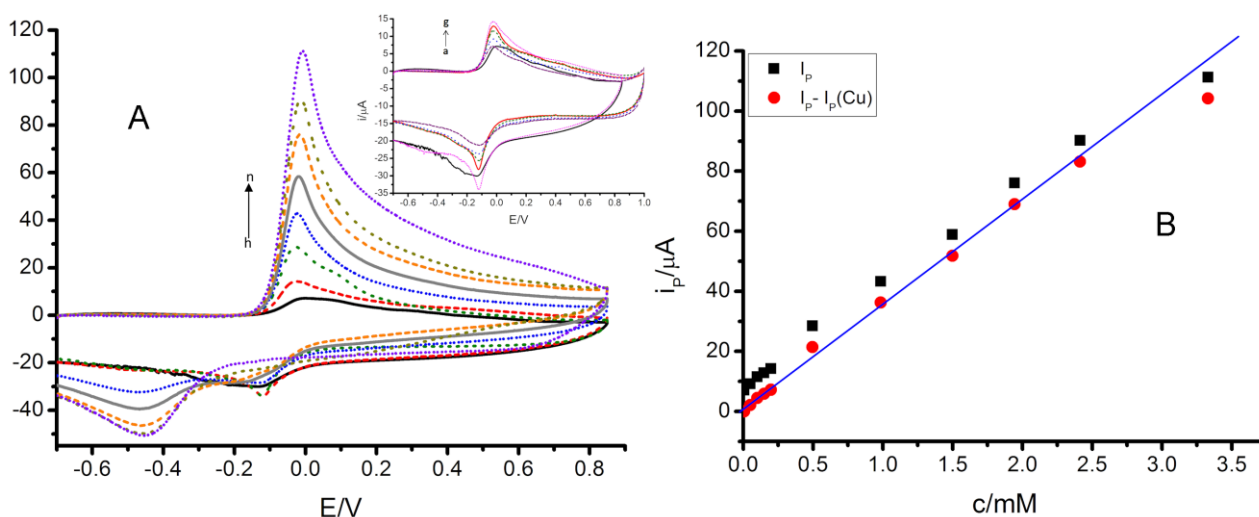


Figure 3. (A) CVs on GCE of copper 0.4 mM in 0.1 M phosphate buffer at pH 6.5; $E_{\text{deposition}} = -0.80$ V; $t_{\text{dep}} = 180$ s; $v = 0.1$ V s^{-1} in the presence of 0, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.3 mM glyphosate (from h to n). Inset: 0, 0.00625, 0.05, 0.1, 0.15 and 0.2 mM glyphosate (from a to g). (B) Plot of the oxidation peak current vs. concentration (●) after subtraction of the current of the oxidation peak in the absence of glyphosate and (■) without subtraction.

The modified glassy carbon electrodes have been attempted to clean up by different methods. First, 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. Other classical and to so classical cleanup methods have been essayed, but 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 frequent change of glassy carbon electrodes.

3.2. Carbon paste electrode

Experiences were made with a carbon paste electrode (see experimental section) due to the simplicity of surface renewability. Before each measurement the electrode was activated through different steps of deposition and oxidation of copper on its surface. The deposition was made at constant potential of -0.8V for 60 s while the solution was stirred. The oxidation was made at constant potential of $+1.2\text{V}$ for 180 s while the solution was stirred. After that, a film of copper is deposited on the surface of the electrode and a cyclic voltammogram is recorded.

Measurements were recorded in order to evaluate the reproducibility of the CPE in the same conditions. The reproducibility between different measurements was very good, though slightly lower than for two successive measurements on GCE. In this case the reproducibility was independent of the number of uses of the electrode because the surface is renewed in each measurement and only a calibration has to be made after the filling of the paste electrode, if the operator changes.

Figure 4 shows the effect of glyphosate concentration on the copper voltammogram. There are two anodic peaks, attributed to the transformations of Cu to Cu^+ and of Cu^+ to Cu^{2+} , which increase upon the addition of glyphosate, this indicating the formation of complexes between the glyphosate and the copper ions [25-28]. The cathodic peaks are moved to more negative potentials when the concentration is increased.

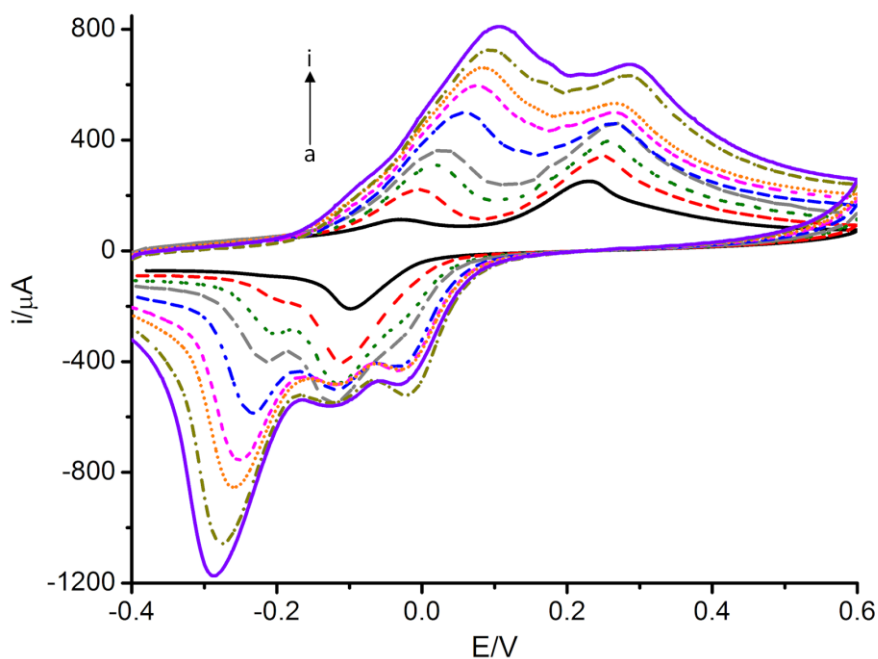


Figure 4. Cyclic voltammograms of 1 mM copper in CPE in 0.1 M phosphate buffer at pH 6.5; $E_{\text{deposition}} = -0.80\text{V}$; $t_{\text{dep}} = 60\text{ s}$; $v = 0.1\text{ V s}^{-1}$ in the presence of 0, 0.075, 0.15, 0.2, 0.3, 0.37, 0.45, 0.5 and 0.59 mM glyphosate (from a to i).

Since the area of the carbon paste electrode is much greater than the area of the glassy carbon electrode, the values of the currents recorded are much greater also. For this reason, and perhaps also for the different morphology of the electrode surface, the differences between the voltammograms in the presence and in the absence of glyphosate can be measured accurately at lower concentrations. So, lower levels of glyphosate can be determined by using this electrode. Nevertheless, the deviations from linearity occur also at lower concentrations, probably due to the most important effect of the adsorption processes in this electrode. So, the values of the anodic peak current, after subtraction of the current of the two oxidation peaks in the absence of glyphosate are proportional to the glyphosate concentration from c.a. 5 μM until 50-70 μM . These values are in the range of other determinations using more expensive and/or more complex electrochemical determination approaches [11, 23].

When the drinking water samples with added glyphosate in the above range were analyzed, the recoveries ranged between 96% and 104%.

In figure 5 are shown the calibration plots for the drinking water of the Córdoba City reinforced with variable amounts of glyphosate.

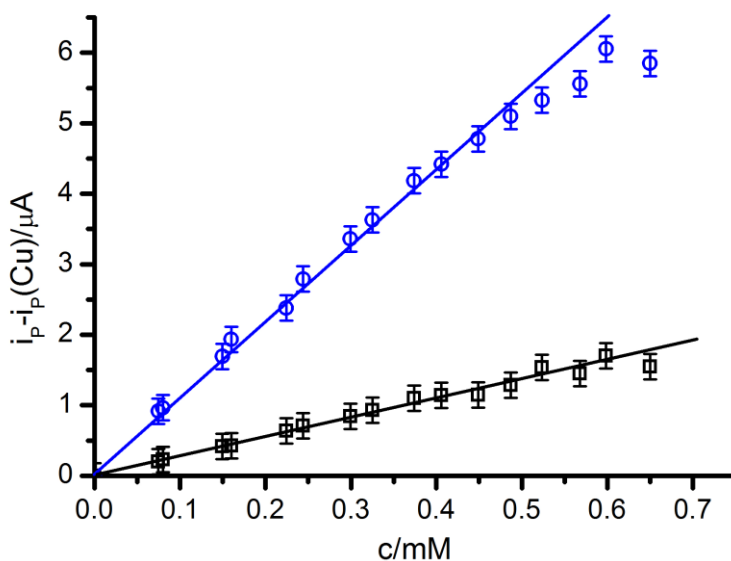


Figure 5. Plot of the oxidation peak current vs. glyphosate concentration after subtraction of the current of the oxidation peak in the absence of glyphosate for drinking water with added glyphosate. (○) peak at 0.1 V and (□) peak at 0.2 V. Experimental conditions as in figure 4.

4. CONCLUSIONS

Carbon electrodes with *in situ* electrodeposited copper can be used to the determination of Glyphosate (N-(phosphonomethyl) glycine) in waters through an analytical method simpler, cheaper and faster than those conventionally used.

The enhancement of the oxidation signal of copper after the addition of glyphosate is due to the formation of a complex.

Glassy carbon and carbon paste electrodes are investigated, concluding that the last is more suitable for routine analysis.

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