World Journal of Chemical Education, 2015, Vol. 3, No. 6, 134-140
Available online at http://pubs.sciepub.com/wjce/3/6/2

DOI:10.12691/wjce-3-6-2

Spectroscopic and Electrochemical Investigations of N-(Phosphonomethyl)glycine (glyphosate) and (Aminomethyl)phosphonic Acid (AMPA)

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Abstract N-(Phosphonomethyl)glycin (glyphosate), known by the trade name of Roundup®, is a broad-spectrum systemic herbicide used to kill several types of grass weed. It was first synthesized in 1970 by J. E. Franz, a chemist at the agrochemical corporation Monsanto. Glyphosate's mode of action is to inhibit a plant enzyme involved in the synthesis of some aromatic amino acids. There is some controversy at present about the use of Roundup® because its hazard potential is not clear. In this article, we present some reliable and easily performed spectroscopic and electrochemical measurements to identify glyphosate isolated as well as in some commercial products. The analogous experiments apply to (Aminomethyl)phosphonic acid (AMPA), the hydrolysis product of glyphosate.

Keywords: *Four year undergraduate/beginners of PhD students, analytical, electrochemistry, IR, MS, UVVISspectroscopy, hands-on learning/manipulatives*

Cite This Article: A. Habekost, "Spectroscopic and Electrochemical Investigations of N-(Phosphonomethyl)glycine (glyphosate) and (Aminomethyl)phosphonic Acid (AMPA)." *World Journal of Chemical Education*, vol. 3, no. 6 (2015): 134-140. doi: 10.12691/wjce-3-6-2.

1. Introduction

Glyphosate is one of the most commonly employed herbicides, used on several types of plants, but its hazard potential is still not clear. It is far beyond the scope of this article to report the various arguments for and against the use of glyphosate.

Current methods for analytical measurement of glyphosate and its hydrolysis product AMPA are rather lengthy, expensive, and elaborate, e.g. HPLC, electrophoresis [1] and GC-MSD [2,3]. The latter method requires lowering of the polarity and therefore enhancement of the volatility, which may be done by derivatization of glyphosate and AMPA [2,3], as will be described later.

Kodama et al. [1] used capillary electrophoresis to investigate the contamination of varioustea-based beverages with glyphosate. They created a complex of glyphosate with Cu(II) during the electrophoretic run and measured the retention time as a function of pH.

Chiu et al. [4] used capillary electrophoresis as well, but with electro chemiluminescence detection, for the analysis of both glyphosate and AMPA. The detector was an indium-tin oxide (ITO) working electrode in an alkaline phosphate buffer containing $Ru(bpy)_3^{2+}$. In the presence of glyphosate, the anodic wave of $Ru(bpy)_3^{2+}$ in cyclic voltammetry (CV)increased dramatically while the cathodic one decreased. The luminescence signal increased simultaneously.

Sierra et al. [5] studied the electrooxidation of glyphosate on nickel and copper surfaces. The anodic current peaks in CV increased with the glyphosate concentration. The authors suggested that the detection limit for copper is much lower than for nickel.

Sheals et al. [6,7] successively protonated glyphosate and compared the IR spectra and the extended X-ray absorption (EXAFS) with abinitio calculations. They postulated that the copper ion lies in the center of a (Jahn-Teller distorted) octahedron with all three donator groups of glyphosate. The amine, carboxylate, and phosphonate ligand form two chelate rings with five members each in the equatorial plane.

Three pKavalues for glyphosate are postulated [8]:

pKa1 (2.29) : $HOOCH_3 - N^+H_3 - CH_2 – PO_3H^- \leftrightarrow$
$^-OOCH_3 - N^+H_3 - CH_2 – PO_3H^- + H^+$

pKa2 (5.96) : $^-OOCH_3 - N^+H_3 - CH_2 – PO_3H^- \leftrightarrow$
$OOCH_3 - N^+H_3 - CH_2 – PO_2H^{2-} + H^+$

pKa3 (10.98) : $^-OOCH_3 - N^+H_3 - CH_2 – PO_2H^{2-} \leftrightarrow$
$OOCH_3 - NH_2 - CH_2 – PO_3H^{2-} + H^+$

The analog values for AMPA are [8]:

pKa1 (5.6): $N^+H_3 - CH_2 – PO_3H^- \leftrightarrow$
$NH_2 - CH_2 – PO_3H^- + H^+$

pKa2 (10.2): $NH_2 - CH_2 – PO_2H^- \leftrightarrow$
$NH_2 - CH_2 – PO_3H^{2-} + H^+$

Piccolo et al. [9] described the IRspectra of glyphosate at different pH levels, and suggested that the IRbands vary with the dissociation of the glyphosate acid group.

The authors specifically monitored the P-O stretching of the P-OH and P-O groups of the phosphono radical with IR spectroscopy. The given wave numbers have been helpful in assigning the different bands in our own experiments.

Daniele et al. [10] published thermodynamic (stability constants, heat of formation, entropies of formation, and free enthalpies of formation) and spectrophotometric data (λ_{max} values) of copper (II) complexes of glyphosate in an aqueous solution at different pH levels and temperatures. The data can be explained by assuming the following pH–dependent species: CuLH, CuL^{-}, $CuLH_{1-}{}^{2-}$, $CuL_2{}^{4-}$, and Cu_2L^{+}, where L indicates the glyphosate ligand. The authors saw a slight downward shift in λ_{max} of about 30 nm with increasing pH.

Unfortunately, glyphosate is not electroactive at accessible potentials. Therefore, Pintadoet al. [11] electrodeposited copper onto a carbon electrode to determine the concentration of glyphosatein drinking water. They saw an enhancement of the current in CV on adding glyphosate solution, due to the formation ofa copper-glyphosate complex. The aim of their paper was "to present an electrochemical method for the quantification of glyphosate in a simple, rapid, and inexpensive way." By adding glyphosate to the copper glassy-carbon electrode, the anodic current peak increased linearly with the glyphosate concentration. The copper on the carbon-paste electrode showed a different behavior: Two oxidation peaks were found, which increased with increasing glyphosate concentration due to the formation of Cu^{+}- and Cu^{2+}-glyphosate complexes. The cathodic peaks also increased, and simultaneously the peaks shifted to more negative potentials on increasing the glyphosate concentration.

Dos Santos et al. [12] used square wave voltammetry (SWV) of a hanging mercury-drop electrode to determine the concentration of glyphosate at different levels of pH. The glyphosate formed complexes with copper ions. The current peak in CV was measured as a function of the copper (II) concentration. In addition, they measured the current as a function of the deposition time of the Cu(II)-glyphosate complex adsorbed onto the electrode.

Börjesson and Torstensson [2], following Deyrupet al. [3], developed new methods todetermine the concentration of glyphosate and AMPA with GCMS, such as derivatizing both molecules with a mixture of trifluoroacetic anhydride and trifluoroethanol (volume 2:1).Afterwards, the solution was keptat 100°C for an hour. After cooling to room temperature, the sample was evaporated, redissolved in 1 mL ethylacetate, and then analyzed with GC-MSD. This method is described in details later in this paper.

With such experiments, Börjesson and Torstensson [2] could quantify glyphosate and AMPA near a railway line in Sweden more than a year after expositing the area. Furthermore, the authors estimated the limit of detection of glyphosate in the soil to be approximately 0.003 mg/g.

At first glance, it does not seem to be easy to determine the concentration of glyphosate and its main metabolite AMPA in a simple way, in particular for inexperienced students. But the derivatization method, the formation of copper complexes, and the detection of glyphosate and AMPA with GC-MSD, CV, and UVVIS spectroscopy are practicable ways to improve the students' analytical knowledge. In our experience, advanced students can carry out all of these experiments in about 40 hours.

First, we describe the pH-dependence of glyphosate and AMPA by titration and determine the pK avalues. Then, we describe the production and the investigation of the colored Cu(II)-glyphosate complexes by UVVIS spectroscopy at various levels of pH. These complexes can also be simply identified by IR spectroscopy. We then experimentally determine glyphosate and AMPA with GC-MSD after derivatization with TFAA and TFE. Finally, following Sierra [6,7] and Börjesson [2], we present the results of electrochemical quantification of glyphosate and AMPA as well as for glyphosate in Roundup®.

2. Pedagogical Objectives

Chemistry students need thorough training in analytical theory and practice to improve their chemical knowledge. Therefore, the aim of this article is a versatile experimental investigation of two important environmental chemicals. This includes modern methods such as spectroscopic and electrochemical knowledge in acquisition and interpretation of mass spectra, IR spectra, and cyclic voltammograms. UVVIS spectroscopy and titration are more classical but are still very useful methods. These methods, easy to use and quick to carry out, are the first step in collecting analytical information.

With respect to environmental analysis, the quality of the applied analytical method for the substances under investigation is determined by the recovery rate of the substances in their natural environment. Therefore, one has to first extract the substance, which is normally adsorbed in soil, by the Soxhlet extraction method before the analytical methods can be applied.

We think that the pedagogical benefit of the described procedures is that investigation of the same substances with different analytical methods is enabled. Furthermore, a comparison between the pure substance and the complex mixture of the commercial products may be instructive.

3. Experimental Procedures

Chemicals and instruments:

Glyphosate (Sigma Aldrich, 54521)
AMPA (Sigma Aldrich, 324817)
Potentiostat (μ-Stat 400, DropSens)
Screen-printed electrodes (DS 550)
KOHsolution (10%)
Sodium carbonate (saturated)
Double-distilled water
Copper sheets or CuO powder (Hedinger, Germany)
Bottles with snap-on caps
Photometer (Perkin Elmer Lambda XLS+)
FTIRspectrometer (Bruker, Vector 22)
KBrpellets

3.1. Preparation of Cu-glyphosate and Cu-AMPA

The aim of our experiments in general is to simplify the experimental approach for the detection of glyphosate and AMPA, rather than obtaining preferably low detection limits.

Therefore, we took conventional copper sheets (0.5 g ≈ 0.0077 mol) or CuOpowder (0.62 g ≈ 0.0077 mol), and put them into solutions with different pH levels (5g each

of water and sodium carbonate). We used the following chemical approaches:

1. Cu in water
2. Cu in sodium carbonate
3. CuO in water
4. CuO in sodium carbonate

Solutions both with and without glyphosate (0.02 g ≈ 0.00012 mol) or AMPA (0.015 g ≈ 0.00014 mol) were used. Quantity of copper was significantly higher compared to glyphosate or AMPA.

Figure 1 shows pictures of Cu in water with glyphosate and AMPA respectively, Cu in sodium carbonate with glyphosate and AMPArespectively, and Cu only in sodium carbonate (right) after 12 hours.

It is obvious that only if glyphosate or AMPA are present, the color changes to blue. The color is deeper in sodium carbonate than in water and with glyphosate than with AMPA.

Figure 1. left: Copper sheets in water with glyphosate (left) and with AMPA (right); Right: Copper sheets in sodium carbonate with glyphosate (left), with AMPA (right), and without glyphosate or AMPA (middle)

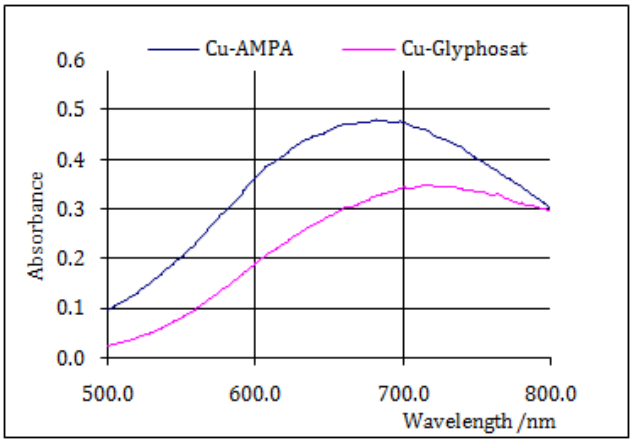

Figure 2. Spectra of Cu-glyphosate and Cu-AMPA

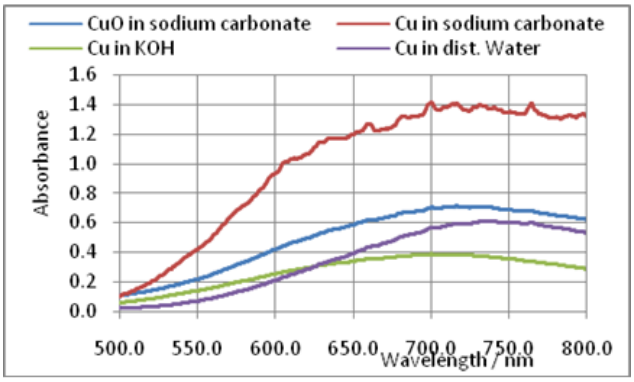

Figure 3. CuO in sodiumcarbonate, Cu in sodiumcarbonate, Cu in KOH, and Cu in H_2O after 12 hours, respectively

In Figure 2, the spectra of glyphosate and AMPA in water are shown. The spectra match those of Daniele and Coutinho [11,13]. This means thatλ_{max} (Cu-AMPA) ≈ 680 nm, and λ_{max} (Cu-glyphosate) ≈ 720 nm.

Figure 3 demonstrates the above-mentioned shift of λ_{max} on increasing the pH by about 30–40 nm (comparing H_2O and KOH as solvents).

Furthermore, the formation of the Cu-glyphosate complex is more pronounced in sodium carbonate than in water.

3.2. Titration

Chemicals and instruments:

Sodium hydroxide (0.1mol/l)
pH electrode
Dosimeter (Metrohm, 554)
Data acquisition system (Sensor Cassy, Leybolddidactric, Germany)
Stirrer
Burette

In the following experiment, we attempt to determine the pK_a values of glyphosate and AMPA (Figure 4). First, we acidify the aqueous solution with 2M hydrochloric acidand titrate with 0.1 M sodium hydroxide solution.

The experimental pKavalues comply with the values mentioned above. The corresponding pKavalues of AMPA are smaller than those of glyphosate.

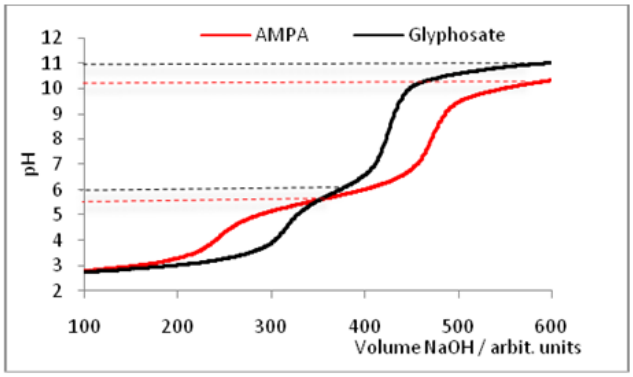

Figure 4. Titration of glyphosate and AMPA

Therefore, one can titrate some commercial products to quickly testwhether they contain glyphosate or not. In Figure 5, one can clearly see that Weed killer® and Roundup®, two tested products, contain glyphosate.

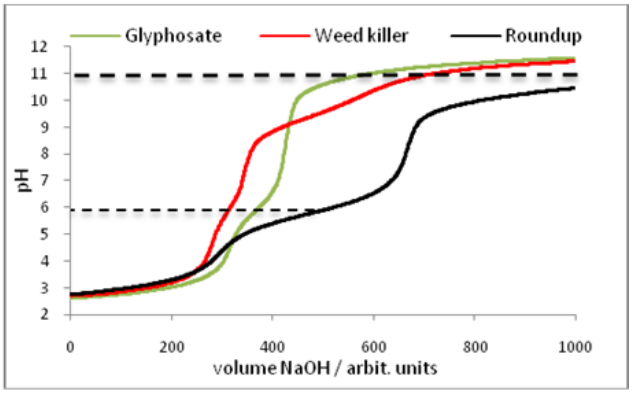

Figure 5. Titration of Weed killer® and Roundup® compared to pure glyphosate

3.3. FTIR Spectra

Figure 6 shows the FTIRspectrum of glyphosate and AMPA in a KBr pellet (300 mg KBr and 1 mg substance; pressure: 1.5 t).

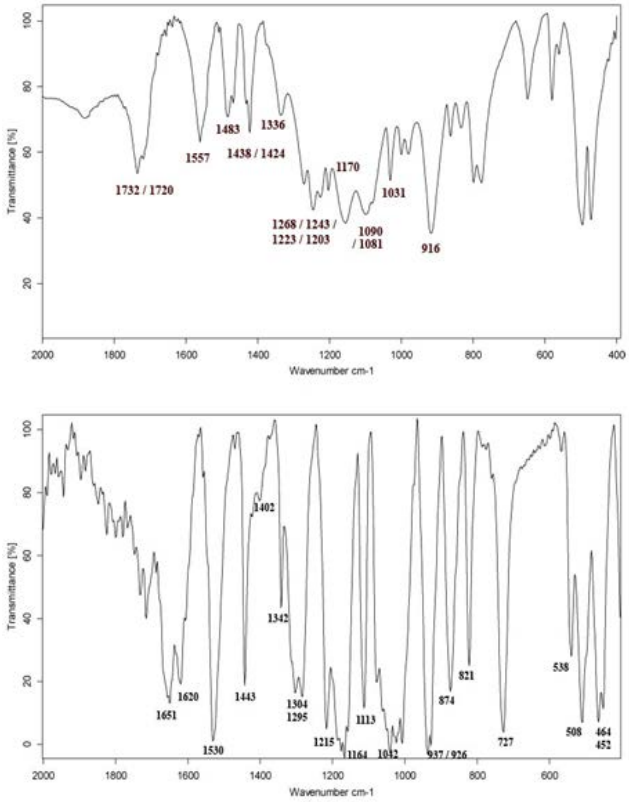

Figure 6. FTIR ofglyphosate (top) and AMPA (bottom)in a KBrpellet.

In Table 1a and Table 1b, we report the assignments of the various bands of glyphosate and AMPA.

Table 1a. IRbands assignments (cm^{-1}) for glyphosate between 2000 and 900 cm^{-1} [6,7]

C=O of free COOH	1732	CH_2 groups	1243
C=O of H-bonded COOH	1720	P-OH	1223
C-O asymmetric vibration	1568		
NH_2 deformations	1557	CO, OH groups	1203
NH_2 deformations	1483	P-OH	1170
CO and OH of free COOH	1434	$P\text{-}O^-$	1090
CO and OH of H-bonded COOH	1424	CCNC skeletal vibrations	1081
CH_2 deformations	1336	CCNC skeletal vibrations	1031
P-O of PO_3H	1268	P-OH	1000
		CCNC	916

Table 1b. Some IRbands assignments (cm^{-1}) for AMPA between 2000 and 400 cm^{-1}[12]

NH_2 deformations	1650, 1620, 1530
CH deformations	1443
P=O	1164
PO_3	1031
P-C	727
HO-P=O and O=P=O deformations	464 / 452

The carboxylate band of glyphosateis detected roughly between 1300 and 1700 cm^{-1},whereas the bands between 950 and 1200 $cm^{-1}$and around 1600 cm^{-1}originate from the phosphonate and the amine group respectively. Complexation of glyphosate can thus be studied by the analysis of these bands. (Note that the glyphosate bands at 1732 cm^{-1} and 1424 cm^{-1}representthe undissociated COOH group).

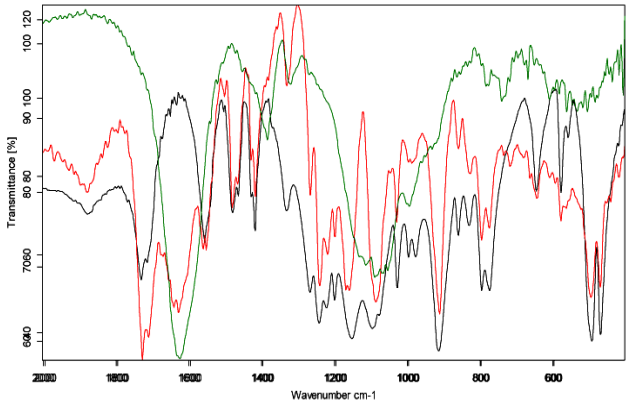

Figure 7. FTIR of glyphosate (black), Cu-glyphosate after 10 minutes (red), and Cu-glyphosate (green line) after 12 hours

Figure 7 shows the increasing complexation of glyphosate with Cu(II). After 10 minutes (red curve), the C=O vibration of the COOHgroup splits to 1602 cm^{-1} and the band at 1424 cm^{-1} shifts to about 1400 cm^{-1}, according to Sheals [6,7]. At the end of the complexation, the shift to lower frequencies is completed. Furthermore, the phosphonate and the amine groups are bonded to Cu(II) as well, causing a shift inthe P-O bands (around 1100 cm^{-1}), whilethe CH deformations (1336 cm^{-1}) are not affected.

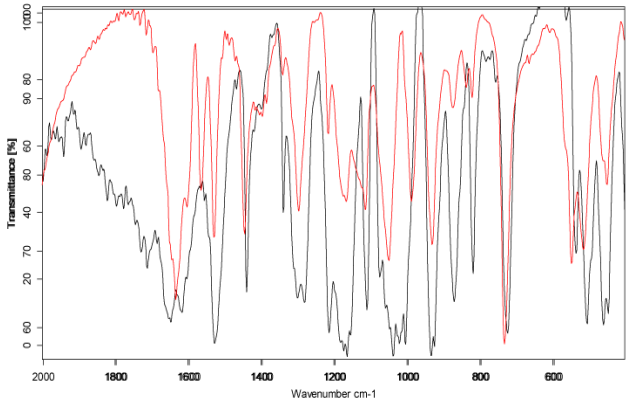

Figure 8. FTIR spectrum of AMPA (black) and Cu-AMPA (red)

The comparison between AMPA (black line in Figure8) and Cu-AMPA (red line in Figure8) shows a shift of the NH_2 deformation band to lower frequency numbers. The P-O frequency numbers (e.g. the PO_3 band)varyin the lower frequencies as well, as expected.

3.4. GC-MSD Measurements

Chemicals and materials:

Trifluoroacetic anhydride (SigmaAldrich, No. 106232)
Trifluoroethanol (SigmaAldrich, No. T 63002)

GC-MSD with autosampler (GC: Hewlett Packard 5890, MSD: Hewlett Packard 5972, autosampler: Hewlett Packard 6890)
Column: RTX-35
Carrier gas: He 5.0
50-mL round-bottom flask
Reflux condenser
Capillary air bleed for solvent evaporation.

Experimental procedure:

10 µg glyphosate was mixed with 200 µg trifluoroacetic anhydride (TFAA) and 100 µg trifluoroethanol (TFE) in a 50-mL round-bottom flask. The mixture was heated to 90°C with a controlled heater and refluxed for about an hour.

Afterwards, the mixture was flushed with clean air for two minutes to evaporate the solvents. The residue was then mixed with 10 mL ethyl acetate and directly analyzed with GC-MSD.

The temperature profile of the GC was 100°C in the first minute and a final temperature of 200°C.Temperature increased at a rate of 5°C/minute at an injection volume of 1 µL. The MS detector started after the solvent peakat 2.5 minutes.

The GC-MS detection was performed according to Deyrupet al. [3] and Börjessonet al. [2]. As described above, both research groups dervatized the trifuncional glyphosate and the bifunctional AMPA by simultaneous esterification and acylation of the carboxylate, the phosphonic,and the amino group.

In the GC of glyphosate (Figure 9), one can see a remarkable peak at8minutes, and two other peaks at 5.8 minutes and 7.2 minutes. The first peak corresponds to a contamination of the injection syringe, while the second peak was formed either due tothe production of the glyphosate derivate or the thermal destruction during the GC analysis. The EI-mass spectrum of the peak at 7.2 minutes (molecular peak m/z 464) may be due to the loss of a P-O group

The corresponding EI-mass spectrum ofglyphosate is in accordance to those described inBörjessonand Deyrup [2,3]: The molecular ions of the derivatives of glyphosate (m/z 511) show only low intensities, whereas the peak m/z 411 (loss of TFE) has a strong intensity. Other fragments are: m/z 492 (loss of HF), m/z 411 which is indicative for $C(O)CH_2N[C\text{-}(O)CF_3]CH_2P(O)(OCH_2CF_3)_2$, and m/z 113 which is an unidentifiable fragment.

Similarly, the molecular peak of the AMPA derivate (m/z 371) is not strongly developed.The fragment peaks (m/e 302: loss of CF_3;m/e 246: $(CF_3CH_2O)_2P\text{-}OH$; and m/e 126: $CF_3C(O)NHCH_3$ fragment) are much more intense.

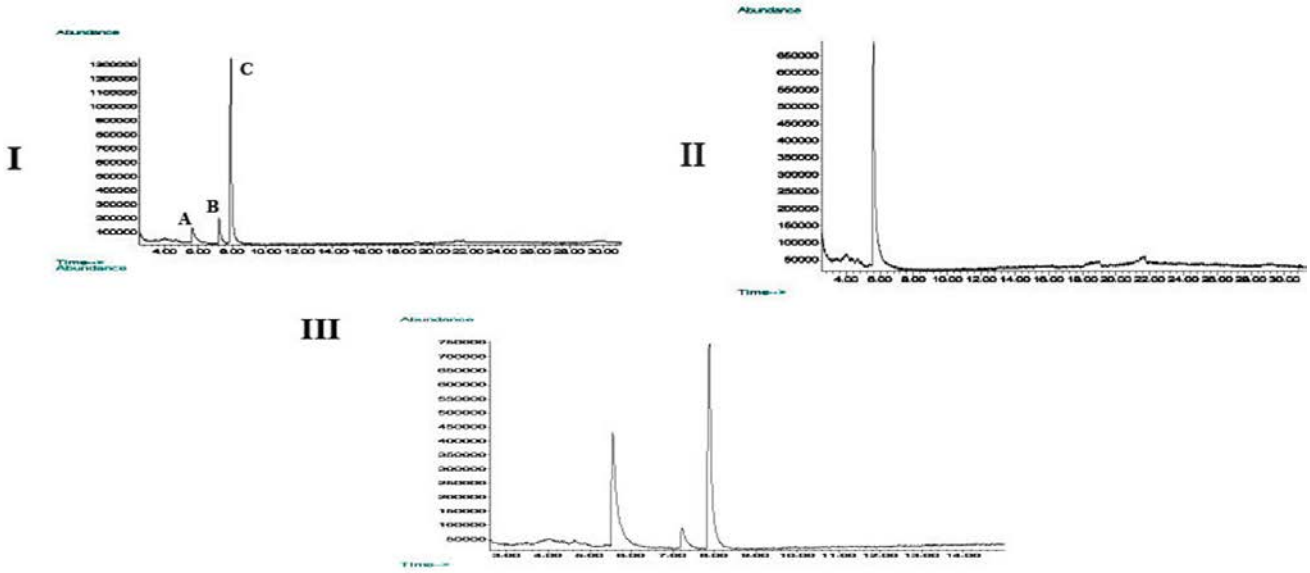

Figure 9. I: GC spectra of the glyphosate derivate C, A: AMPA (contamination of the injection syringe), and B: glyphosate fragment m/z 464; II: GC and EI-mass spectrum of the AMPA derivate; III: GC-MS spectrum of both AMPA and glyphosate derivates simultaneously

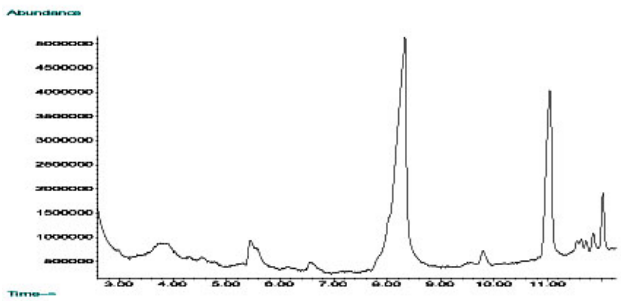

Figure 10. GC-MS spectrum of Roundup®—glyphosate peakC at about eightminutes.

Figure 10 indicates the difference between pure glyphosate (Figure 9, I) and the commercial, glyphosate-containing productcalled Roundup®. We followed the same processing procedure as mentioned above. In case of Roundup®, the GC shows several peaks with retention time above eight minutes (glyphosate), but the glyphosate peak can clearly be identified by its retention time of eight minutes.

3.5. Electrochemical Measurements

Chemicals and materials:

Phosphate buffer (pH 6.9, 0.1 mol)
$CuSO_4*5H_2O$ solution (10 mmol)
Glyphosate (1 mg)
Potentiostat (µStat 400)
Screen-printed electrodes (DS 550. working electrode: Pt; counter electrode: Pt; reference electrode: Ag)

Pipettes

Double-distilled water for purification of the electrodes.

The experimental procedure is the same as given in Pintado [11]. First, we dropped 1 mL of a copper sulfate solution (12.5 mg copper sulfate in 5 mL of a 0.1 mol/l phosphate buffer, pH = 6.9) onto the screen-printed electrodes (DS 550: working electrode: Pt; counter electrode: Pt; reference electrode: Ag). Then, we electrodeposited copperonto the platinum working electrode for 60 seconds at −0.8 V. This was carried out by constant stirring. Afterwards, we took a cyclic voltammogram nine times in a row (scan range: −0.8 V →0.6 V →−0.8 V; scan rate: 0.1 V/s; no stirring) as shown by the red dotted lines in Figure 11. One can see that the anodic current peak (oxidation of copper to Cu(II)) decreases due to the gradual reduction of the copper layer.

We then mixed 1 mL of the copper sulfate solution with 1 mg glyphosate and executed the same procedure. It is obvious that the current peaks drift to lower potentials (of about 0.12–0.2 V) according to Pintado [11], and increase in intensity (compared to the corresponding CVs without glyphosate). We did not attempt to quantify the detection limit of glyphosate, because we only wanted totestthe possibility of easy electrochemical detection of glyphosate.

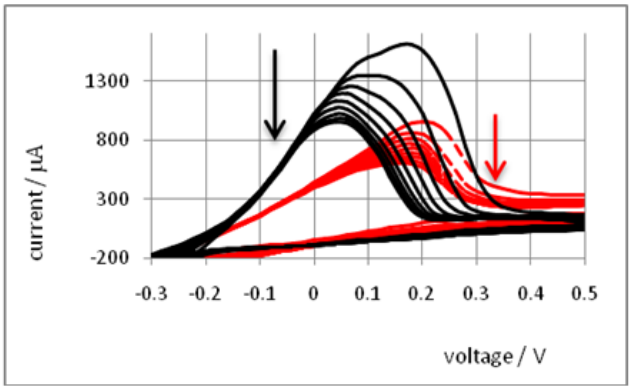

Figure 11. Cyclic voltammograms of copper sulfate after deposition of copper (red, dotted curves) and of copper sulfate / glyphosate mixture (same conditions).Only the anodic curves are shown. The arrows indicate increase of the CV scan.

3.6. Glyphosate from Soil

Chemicals and materials:

Glyphosate
Double-distilled water
Garden soil
Soxhlet extractor
Round-bottom flask
Heater
Reflux cooler
Rotary evaporator

Experimental procedure:

We dissolved 20 mg glyphosate in 5 mL double-distilled water. Of this, 2.5 mL was removed and dried in a drying oven at 70°C. The residue was derivatized as described above and solved in 1 g ethyl acetate. GCMS was recorded as the “reference.”At the same time, the other 2.5 mL solution was mixed with 15 g soil and extracted with 85 mL water in a Soxhlet extractor for two hours. Then, the extract was concentrated with a rotary evaporator, dried in a drying oven at 70°C, and analyzed with GCMS after derivatization, being the “probe.”

Figure 12 shows the extraction equipment:

Figure 12. Extraction equipment: heater, flask, Soxhlet with extraction thimble containing glyphosate and soil, and the reflux cooler.

The quotient of the integrals of the glyphosate GCpeaks with and without soil is: $I_{probe}/ I_{reference} \approx$ 91% recovery rate – quite an acceptable result.

4. Conclusion

In this paper we describe several spectroscopic and electrochemical methodsto identify glyphosate and AMPA as pure substances,in commercial products and in soil. We restrict ourselves to methods that

- show clear results, and
- are easily to perform.

All experiments can be done in about a week (40 hours) in an analytical lab course. With this plethora of experiments, students not only improve their experimental skill, but also learn fundamental theoretical aspects about UVVIS, IR, GCMS, and cyclic voltammetry.

Therefore, we think that the versatile investigation of glyphosate and AMPA is a significant contribution to professional training of students in analytical chemistry.

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