Determination of Glyphosate in Water Samples by Alternating-Current Oscillopolarographic Titration

Junping XIAO,** Qingxiang ZHOU,*† Bo YANG,* and Zhenzhen WANG*

*Henan Key Laboratory for Environmental Pollution Control, School of Chemistry and Environmental Sciences, Henan Normal University, Xinxiang 453007, P. R. China

**Department of Chemistry, University of Science and Technology Beijing, Beijing 100083, P. R. China

This paper describes a novel, simple and sensitive method for the determination of glyphosate in water samples by alternating-current oscillopolarographic titration. Under an acidic condition, glyphosate can be transferred into a nitroso-compound by reacting with NaNO_2 . The titration end-point was obtained by the formation of a sharp cut in the oscillopolarographic with infinitesimal NaNO_2 on double platinum electrodes. The results showed that glyphosate had an excellent linear relationship over the range of 7.16×10^{-6} – 4.14×10^{-3} mol L^{-1} , the RSD of the proposed method was lower than 3%, and the spiked recoveries of three real water samples were in the range of 97.7–100.2%.

(Received June 18, 2004; Accepted July 28, 2004)

Introduction

Glyphosate is a well-known herbicide, which is widely used for weed and vegetation control owing to its high efficiency and low toxicity. Moreover, it is also often used as the active ingredient in mixed herbicides. Thus, it may be present in the environment with a certain concentration in areas where it has been applied. Although glyphosate was less toxic than some other herbicides in usual use, it still has an important impact on the environment if it is present over a certain concentration; especially, the effluent from a factory producing glyphosate poses a threat to crops because of their containing higher concentrations of glyphosate. Therefore, it is necessary to establish a simple, rapid and sensitive method for monitoring the residue level of glyphosate in the environment.

Because of its own properties, such as high solubility in water, insolubility in organic solvents and its complexing behavior,¹ it has attracted more attention to develop effective methods for glyphosate analysis. Although the gas-chromatographic method is often used in the analysis of pesticides and herbicides due to its stability, easiness and simplicity for operation, it needs a derivative procedure to convert glyphosate into volatile forms, which is tedious and requires more time and labor. Börjesson and Torstensson made use of GC-MS for a final identification and quantification after ligand-exchange, anion-exchange and derivatization.² Liquid chromatography is another alternative for the determination of glyphosate, which might provide a better detection limit due to its high solubility in water.³ Meanwhile, pre- and postcolumn derivatization procedures have been widely used in liquid chromatographic methods for development to improve the detection sensitivity and to increase the absorption. The derivatization reagents are usually 9-fluorenyl-methyloxycarbonyl chloroformate (FMOC-Cl),^{4,5}

1-fluoro-2,4-dinitrobenzene,⁶ *p*-toluenesulfonyl chloride,⁷ *o*-phthalaldehyde and *N,N*-dimethyl-2-mercaptoethylamine hydrochloride,⁸ *o*-phthalaldehyde-mercaptoethanol (OPA-MERC).^{9–11} Few detection methods for glyphosate have been reported without derivatization. Ion chromatography for detecting glyphosate has also been reported.¹² Chang and Liao¹³ developed an electrophoretic method by indirect fluorescence detection for analyzing glyphosate and its metabolites. In addition, LC-electrospray mass spectrometry was reported for the determination of GLYP and AMPA.^{14,15} You and Koropchak¹⁶ have developed a novel method for the direct determination of glyphosate and its metabolite without the need of sample derivatization, preconcentration, and mobile-phase conductivity suppression by ion chromatography with condensation nucleation light scattering detection (IC-CNLS). It is a simple, fast, and ideal detection technology for these compounds. To date, such methods as mentioned above have their merits for glyphosate analysis, yet not all labs can offer such conditions.

Alternating-current oscillopolarographic titration has been a simple, rapid, sensitive and inexpensive analytical tool in recent years,¹⁷ and it has had many applications in analytical, food and environmental fields. NaNO_2 has been reported as a multifunction reagent for titration, and NaNO_2 in minute quantities can exhibit a sharp cut in an alternating-current oscillopolarograph.¹⁸ Moreover, Dong¹⁹ has found that glyphosate can be transferred into a nitroso-compound under acidic conditions. Based on these experimental results, we designed a new, simple, rapid, and sensitive determination method for glyphosate in water samples without using expensive instruments by exploiting the emergence of a sharp cut of NaNO_2 on the platinum electrodes in an alternating-current oscillopolarograph.

The scope of this study was to establish a rapid and sensitive method for the direct analysis of glyphosate with an alternating-current oscillopolarograph. Several parameters governing the emergence of a sharp cut were optimized.

† To whom correspondence should be addressed.
E-mail: zhouqx@henannu.edu.cn

Fig. 1 Oscillopolarogram before titration.

Fig. 2 Oscillopolarogram at the titration end-point.

Experimental

Reagents and chemicals

Glyphosate was supplied from Anyang Forestry Chemical Factory; sodium nitrite, methanol and triethylamine (gradient grade) were purchased from Acros Chemicals. Hydrochloric acid, sodium thiosulfate, aluminium chloride, manganese chloride, magnesium chloride, ferric chloride, potassium dichromate were of analytical grade. If not stated, the other reagents used were also of analytical grade.

By dissolving 0.35 g of glyphosate in deionized water, and then transferring into a 500 ml volumetric flask, a stock solution of glyphosate was obtained with a concentration of 700 ppm. Before preparing a stock solution of sodium nitrite, sodium nitrite was dried to a constant weight at 105°C, and a certain amount of sodium nitrite was weighted and dissolved in deionized water for a concentration of 35.8 mM after the temperature was lowered to room temperature. These stock solutions were stored at 4°C. Standard working solutions at various concentrations were prepared daily by appropriate dilution of aliquots of the stock solution. The standard solution of sodium nitrite was calibrated with sodium thiosulfate solution before use.

Apparatus

A LS-1A alternating-current oscillopolarograph was used throughout the experiment, which was purchased from The Seventh Shandong Telecommunication Factory (Jining, China). A magnetic stirrer was used for complete reactions. The used micro-platinum electrode and platinum sheet electrode in the further experiments were self-made in our laboratory.

Principle

There is a secondary amine group in the molecule of glyphosate, which can cause a nitroization reaction with nitrite under acidic conditions. The procedure can be simply shown as follows:

From the above reaction, because we can find that there is no reactive hydrogen on the nitrogen atom in the final product molecule, this compound is very stable. Therefore, when the end point of titration comes, a very small quantity of sodium nitrite in excessiveness will produce an acute cut on the micro-platinum and platinum sheet electrodes in the alternating-current oscillopolarograph, and the emergence of an acute cut can be used as an indication marker of the end point of titration coming.

Procedure

After 25 ml of deionized water, 1.5 g of potassium bromide and 40 ml of 6 M hydrochloric acid were mixed in a 100 ml

beaker, the platinum electrodes were placed in the beaker and the magnetic stirrer was turned on. The tip of a buret containing sodium nitrite solution was put below the liquid level at a position about two thirds of the height of the liquid. Until the end point of titration came, the tip was not taken out and washed with a small quantity of deionized water. Titration was then carried out very slowly until the emergence of an acute cut. When a glyphosate solution was used instead of deionized water, the process was the same. The quantity of sodium nitrite used in each step was recorded, and the real concentration of glyphosate in the sample could be calculated based on these data. Figures 1 and 2 are the oscillopolarograms of no titration and the titration end-point, respectively.

Results and Discussion

Quantity of potassium bromide

A constant content of hydrochloric acid kept at a concentration of 3 M and deionized water (40 ml and 25 ml, respectively), and the effect of potassium bromide was investigated. The quantity of potassium bromide changed over the range of 0 – 1.5 g. The results are shown in Fig. 3. From Fig. 3, we can see that with an increase of potassium bromide the needed volume of sodium nitrite decreased over the range. Based on a thorough consideration, the best result was obtained when the amount of potassium bromide was 1.5 g. Therefore, 1.5 g of potassium bromide was used in further experiments.

Optimization of the pH

From the mentioned above, glyphosate could react with NaNO_2 under an acidic environment. Thus, the pH value plays an important role in this reaction, and has a direct impact on the reaction rate. An investigation was carried out in order to obtain a reasonable acidic condition. As we know, both sulfuric acid and hydrochloric acid can be used to provide an acidic environment, yet, in fact, the oscillopolarograph of NaNO_2 occurred very slowly, and showed weak sensitivity when sulfuric acid was employed for use in primary experiments. However, hydrochloric acid exhibited a better performance for a rapid emergence of the oscillopolarograph of NaNO_2 . In final, hydrochloric acid was selected for further research. The concentration and volume of hydrochloric acid also had an effect on the result; they were optimized over the range of 0.1 – 6 mol L^{-1} and 30 – 60 ml. The results showed that the reaction rate was very small and the reaction was not complete. Only some part of glyphosate was transmitted into a nitrous compound when the concentration of hydrochloric acid was too small. On the contrary, according to a previous report,¹⁹ NaNO_2 decreased due to volatilization when its concentration was too high, which made the error increase. In order to make the reaction rapid and sensitive, and to obtain better reproducibility, 6 mol L^{-1} hydrochloric acid was chosen in the following work. At the same time, the results exhibited that there was no

Fig. 3 Effect of the amount of KBr on the consumption of NaNO₂.

significant difference over the investigated volume range of 6 mol L⁻¹ hydrochloric acid. From 30 to 60 ml, the result was all excellent, so the final volume of hydrochloric acid was determined to be 40 ml for further study.

Influence of the concentration of NaNO₂

Although the higher was the concentration of NaNO₂, the easier was it to reach the end point of titration, too high a concentration of NaNO₂ resulted in a difficulty to determine the end point of titration exactly, owing to a significant difference in the concentration of the target compound, and a large error would occur if used. Yet, a too low concentration of NaNO₂ could not obtain better results, because it made a sharp cut of the end point of the titration emerge very slowly and consume too much NaNO₂. To investigate the influence of the concentration of NaNO₂, a series of experiments was designed with a glyphosate concentration of 2.662×10^{-4} mol L⁻¹. The results indicated that it was suitable for glyphosate determination when the concentration of NaNO₂ changed over the range of 0.0160–0.0195 mol L⁻¹. Considering all of the results, 0.0179 mol L⁻¹ was employed for use.

Selection of the reaction time

The reaction time is one of the key factors that determine whether an accurate analysis can be obtained or not. It is necessary to investigate the optimal reaction time because a long time will result in a loss of NaNO₂ due to volatilization, which makes the consumption of NaNO₂ increase. Yet, a short reaction time leads to an incomplete reaction. In this work, the reaction time was optimized within the range of 1–10 min with all other conditions being constant. The results indicated that excellent performance had been gained over 3 min. In order to obtain a better accuracy and precision, 5 min was used as the optimal reaction time.

Interference of the concomitant ions and organic compounds

Usually, the presence of concomitant ions or organic compounds affects the validity of analysis with the proposed method. Therefore, it is very important to investigate the interference of any potential concomitant ions or organic compounds. To do this, the concentration of glyphosate was kept at 2.039×10^{-5} mol L⁻¹ and the relatively error was limited to no more than $\pm 2\%$. The results are listed in Table 1. As can be seen, most ions and organic compounds have no significant influence on the analytical results.

Reproducibility and linear range

The reproducibility of the proposed method was studied for

Table 1 Interference of concomitant ions or organic compounds

Fold	Concomitant ion or organic compound	Interference
1.5	Fe ²⁺ , Fe ³⁺ , Cu ²⁺	—
50	Cr ³⁺	—
200	Mn ²⁺	—
250	Zn ²⁺	—
400	Al ³⁺	—
600	Cr ₂ O ₇ ²⁻	—
1000	Mg ²⁺	—
4000	Ca ²⁺ , triethylamine	—
8000	Cl ⁻ , PO ₄ ³⁻	—
1.2×10^{-5}	Methanol	—

—, No interference.

eight replicate experiments for a sample spiked at 2.039×10^{-5} mol L⁻¹ of glyphosate, and the linear range was investigated using a series of standard solutions of glyphosate at different concentrations. The relative standard deviation (RSD) was 2.9% ($n = 8$). A better linear relationship was found over the range of 7.16×10^{-6} – 4.14×10^{-3} mol L⁻¹, and the coefficient of correlation was 99.5%.

Recovery and sample analysis

The proposed method was also applied to real sample analysis. Three water samples, such as tap water, spring water and river water, were collected for validating. Tap water was collected from our lab, and river water and spring water were collected from a nearby river and spring nearby. These samples were analyzed after simply filtering with a 0.45 μ m micropore membrane, but no glyphosate was found. Further, spiked recovery experiments were performed for checking the utility of the proposed method with a concentration of 2.039×10^{-5} mol L⁻¹ of glyphosate. Each sample was analyzed six times in parallel. The results demonstrated that the recoveries of the three water sample were 100.2, 99.4 and 97.7%, respectively. Also, their relative standard deviations were all lower than 3%. Hence, this method can be used in a real environmental water analysis for glyphosate determination.

Conclusion

The determination of glyphosate in water samples is an important task for environmental researchers due to its environmental safety. Many methods mentioned above can be utilized for this goal, but a derivatization step is often used before analysis. This paper describes a novel method for glyphosate determination based on alternating-current oscillopolarographic titration without any derivative process. The affecting factors were investigated and optimized, and the optimal conditions were as follows: the substrate consisted of 40 ml of 6 mol L⁻¹ hydrochloric acid and 1.5 g of KBr, the concentration of NaNO₂ was set as at 0.0179 mol L⁻¹; and 5 min was taken as the reaction time. The experimental results had demonstrated that this method offered excellent recoveries and could be employed for environmental sample analysis. In view of the rapidity, sensitivity, simplicity, environment-friendly nature and so on, the proposed method will be an excellent alternative detection technology for glyphosate analysis, and will be widely used in the field of environmental monitoring.

References

1. "Handbook of Chemical Products", **1996**, Agricultural Chemical, Chemical Industry Press of China, 363.
 2. E. Börjesson and L. Torstensson, *J. Chromatogr., A*, **2000**, 886, 207.
 3. M. J. Lovdahl and D. J. Pietrzyk, *J. Chromatogr.*, **1992**, 602, 197.
 4. E. A. Hogendoorn, F. M. Ossendrijver, E. Dijkman, and R. A. Baumann, *J. Chromatogr., A*, **1999**, 833, 67.
 5. C. J. Miles, L. R. Wallace, and H. A. Moye, *J. Assoc. Off. Anal. Chem.*, **1986**, 3, 458.
 6. L. N. Lundgen, *J. Agric. Food Chem.*, **1986**, 34, 535.
 7. S. Kawai and M. Tomita, *J. Chromatogr.*, **1991**, 540, 411.
 8. J. Patsias, A. Papadopoulou, and E. Papadoulou-Mourkidou, *J. Chromatogr., A*, **2001**, 932, 83.
 9. M. E. Oppenhuizen and J. E. Cowell, *J. Assoc. Off. Anal. Chem.*, **1991**, 74, 317.
 10. Technical Note 109, Dionex, Sunnyvale, CA, **1995**.
 11. M. P. Abdullah, J. Daud, K. S. Hong, and C. H. Yew, *J. Chromatogr., A*, **1995**, 697, 363.
 12. Y. Zhu, F. Zhang, C. Tong, and W. Liu, *J. Chromatogr., A*, **1999**, 850, 297.
 13. S. Y. Chang and C. H. Liao, *J. Chromatogr., A*, **2002**, 959, 309.
 14. K. Bauer, T. P. Knepper, A. Maes, V. Schatz, and M. Voihsel, *J. Chromatogr., A*, **1999**, 837, 117.
 15. R. J. Vreeken, P. Speksnijder, I. Bobeldijk-Pastorova, and Th. H. M. Noij, *J. Chromatogr., A*, **1998**, 794, 187.
 16. J. You and J. A. Koropchak, *J. Chromatogr., A*, **2003**, 989, 231.
 17. Y. F. Zhan and Y. Q. Zhao, *Fenxi Kexue Xuebao*, **1999**, 15(4), 338.
 18. Y. F. Zhan, *Fenxi Huaxue*, **1992**, 20(4), 493.
 19. W. G. Dong, X. C. Chen, and J. Liu, *PTCA (Chemical Analysis)*, **1997**, 33(8), 347.
-