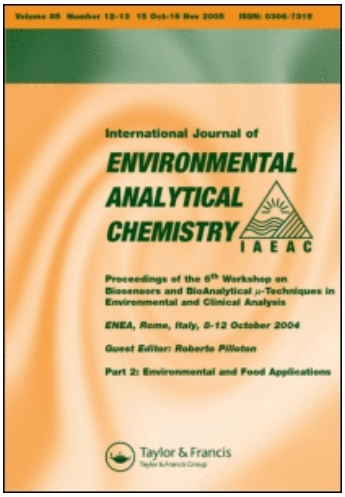

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Paraquat in Waters by Enzymatic Inhibition Using Flow-Injection Analysis

Carmen Lopez-erroz[a]; Pilar Viñas[a]; Natalia Campillo[a]; Manuel Hernandez-cordoba[a]
[a] Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, Murcia, Spain

To cite this Article Lopez-erroz, Carmen , Viñas, Pilar , Campillo, Natalia and Hernandez-cordoba, Manuel(1998) 'Determination of Paraquat in Waters by Enzymatic Inhibition Using Flow-Injection Analysis', International Journal of Environmental Analytical Chemistry, 72: 4, 267 — 274
To link to this Article: DOI: 10.1080/03067319808035898
URL: http://dx.doi.org/10.1080/03067319808035898

Intern. J. Environ. Anal. Chem., Vol. 72(4), pp. 267-274
Reprints available directly from the publisher
Photocopying permitted by license only

DETERMINATION OF PARAQUAT IN WATERS BY ENZYMATIC INHIBITION USING FLOW-INJECTION ANALYSIS

CARMEN LOPEZ-ERROZ, PILAR VIÑAS, NATALIA CAMPILLO and MANUEL HERNANDEZ-CORDOBA*

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, E-30071 Murcia, Spain

(Received 22 November, 1997; In final form 8 June, 1998)

A flow injection method based on the inhibition of the hydrolysis of acetylthiocholine to thiocholine catalyzed by acetylcholinesterase is proposed for the determination of the herbicide paraquat. The liberated thiocholine is monitored spectrophotometrically using 5,5'-dithiobis(2-nitrobenzoic acid). The calibration graph based on peak area is linear in the range 0.2–1.5 μg/ml with a relative standard deviation of ±4.1% The method is applied to the determination of paraquat in a commercial product and in several water samples.

Keywords: Flow injection; paraquat; acetylthiocholine; acetylcholinesterase; waters

INTRODUCTION

Enzymes are valuable analytical tools and offer sensitive and specific methods for the quantitation of many substances[1]. The use of enzymes in flow injection systems (FI) is widespread because they allow automation using closed or open systems and immobilized enzymes[2]. Ellman's reaction [3] is based on the acetylcholinesterase (AChE)-catalyzed hydrolysis of acetylthiocholine (ATC) to thiocholine, which reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to give a product which is measured spectrophotometrically. This reaction has been automated using FI methods[4–6]. The determination of enzyme inhibitors results in very sensitive methods. Thus, some pesticides and heavy metal ions have been determined because of their effect on the enzyme inactivation using FI[7–9].

* Corresponding author. FAX: +34–968–364148. E-mail: hcordoba@fcu.um.es

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride or methyl viologen) is a herbicide which is often used as a selective weedkiller[10]. It is toxic to man, causing lung, heart, kidney and liver disorders, thus deserving particular attention its determination in the environment. Several analytical techniques have been applied to the analysis of paraquat, most of which are based on liquid chromatography[11–13], although spectrophotometric[14], voltammetric[15] and FI[16–17] techniques have also been proposed.

In the present study, the ability of paraquat to inhibit the catalytic effect of AChE was studied using Ellman's reaction and automation by FI. The method was applied to the determination of the pesticide in several water samples of different origin. Other pesticides did not interfere with the method.

EXPERIMENTAL

Instrumentation

A Pye Unicam (Cambridge, UK) PU8625 UV/visible spectrophotometric detector set at a wavelength of 410 nm and a PC integration pack (Kontron, Zurich, Switzerland) to record and integrate the peaks were used. The flow injection system consisted of a Gilson (Worthington, OH) Minipuls HP4 peristaltic pump, an Omnifit (Cambridge, UK) injection valve, a Hellma (Jamaica, NY) 178.012-QS photometric flow cell, 0.5 mm i.d. PTFE tubing and various end fittings and connectors (Omnifit). Thermostating of the reactor coil was carried out using a laboratory-made electronic device.

Reagents

High quality water obtained using a Milli-Q system (Millipore, Milford, MA, USA) was used throughout. All the chemicals used were obtained from Sigma (St. Louis, MO). The 0.01 M acetylthiocholine iodide reagent was prepared in water and the 0.01 M DTNB solution was prepared in 0.05 M phosphate buffer (pH 7.2). Both reagent solutions were kept in dark bottles at 4°C. The stock solution of AChE from *Electric Eel* (500 units) was dissolved in 10 ml of 0.05 M phosphate buffer (pH 7.2) and stored in 1-ml aliquots at −30°C. Appropriate diluted solutions of the enzyme were prepared daily and maintained in an ice bath while they were in use. No problems due to instability were noted throughout the working day. A 300 μg/ml stock solution of the inhibitor paraquat (Serva,

Heidelberg) was prepared in water and also kept at 4°C; working standard solutions were prepared by dilution with water just before use.

Analytical procedures

A schematic diagram of the three channel flow manifold is shown in Figure 1. Paraquat (200 µl) was injected into channel I, through which flowed the 0.05 M phosphate buffer (pH 7.5); channel II contained a solution prepared by mixing 1 mM DTNB and 1 mM ATC, while a solution containing the AChE enzyme flowed through channel III. Solutions II and III were kept in an ice bath during the measurements in order to avoid instability problems. The three solutions merged in a 4-way connector and the resulting solution flowed through a thermostated reaction coil (3 m × 0.5 mm I.D.) at 37°C before passing into the flow cell for the absorbance (410 nm) to be recorded. All streams were pumped at the same flow rate by means of a peristaltic pump with a total flow rate of 1.0 ml/min. Calibration graphs were obtained by plotting the area of the negative peaks obtained when injecting the herbicide against the paraquat concentration.

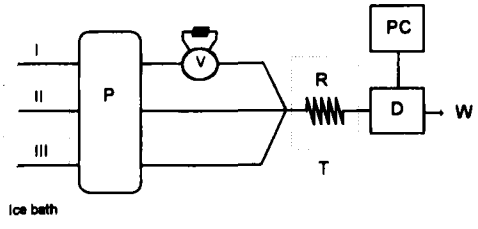

FIGURE 1 Flow-injection manifold for the determination of paraquat. (P) peristaltic pump, total flow-rate 1 ml/min; (V) injection valve, sample-loop 200 µl; (R) reactor coil, 3 m long and 0.5 mm I.D.; (T) thermostat at 37°C; (D) spectrophotometer at 410 nm; (PC) personal computer; (W) waste

For the determination of the herbicide in waters, an aliquot was filtered through a 0.2 µm nylon Millipore chromatographic filter and analyzed by the FI method.

RESULTS AND DISCUSSION

Paraquat determination was based on inhibition of the ATC hydrolysis reaction catalyzed by the enzyme AChE. The optimal conditions for the inhibited reaction would also be influenced by those values adequate for the catalyzed reaction.

Consequently, several preliminary experiments were carried out to establish the optimal values for the chemical and FI variables for both the catalyzed and the inhibited reactions. The catalyzed reaction was followed by measuring the absorbance of the reaction between the ATC and DTNB in the presence of the AChE enzyme, whereas the inhibited reaction was followed by measuring the area of the negative peak obtained when the paraquat was injected into the FI system.

Optimization of the chemical variables

The chemical variables were optimized to obtain maximum signals for both the catalyzed and the inhibited reactions, thus giving maximum sensitivity to the chemical analytical procedure. Since the pH of the reaction strongly affected both reactions, all the reagent solutions were prepared with different pH buffers. Figure 2 shows that the signals from both the catalyzed reaction (dotted line) and the inhibited reaction (solid line) increased when the acidity of the medium decreased. Consequently, a pH value of 7.5 was selected. This pH was obtained by using a potassium phosphate buffer, the concentration of which was varied from 0.005 to 0.5 M and the peak area was observed to decrease with increasing the concentration of the buffer. The 0.05 M value giving maximum signal from the catalyzed reaction was selected. On the other hand, higher values led to very broadening peaks.

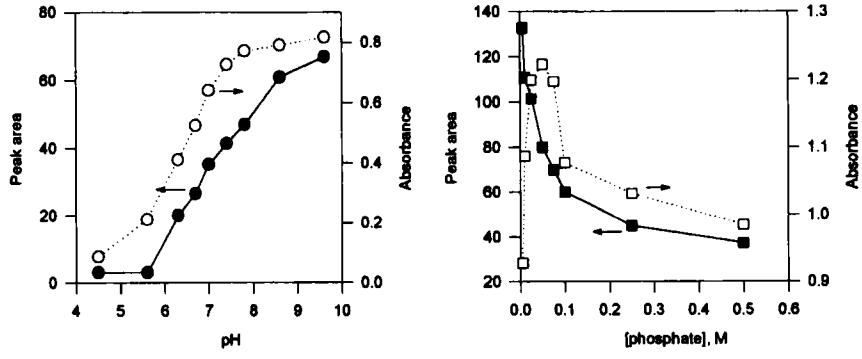

FIGURE 2 Influence of the pH and the phosphate buffer concentration on both the reaction inhibited by injecting 1 μg/ml of paraquat (solid line) and the enzymatic reaction (dotted line)

The influence of other chemical variables is shown in Figure 3 . The ATC concentration was varied between 1.0×10^{-4} and 5.0×10^{-3} M in the presence of a

0.25 U/ml AChE concentration. All solutions were prepared in 0.05 M phosphate buffer (pH 7.5). The signals obtained from both catalyzed and inhibited reactions rapidly increased and an ATC concentration of 10^{-3} M was chosen as optimal. The signals obtained with different DTNB concentrations were studied in the 1×10^{-4}- 4×10^{-3} M range and it was observed that the inhibition by paraquat levelled off at above 10^{-3} M. An increase in temperature had a favourable effect on the signal up to approximately 30–37°C, above which the inhibition decreased. The reactor coil was, therefore, thermostated at 37 ± 0.1 °C. The effect of varying the enzyme concentration was studied and the signals from both the enzymatic and the inhibited reactions continuously increased with higher concentrations.

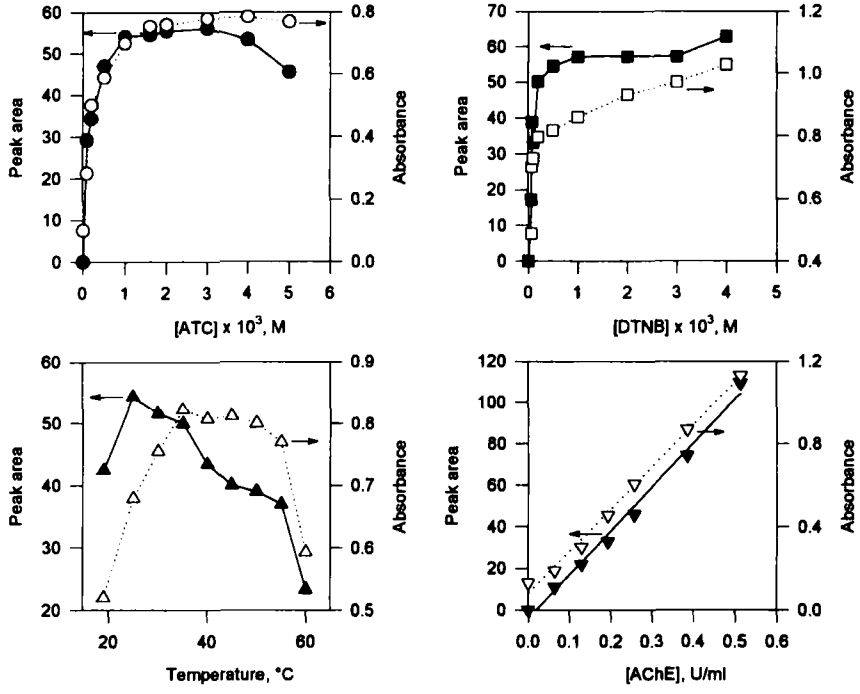

FIGURE 3 Influence of the ATC, DTNB and AChE concentrations and temperature on both the reaction inhibited by injecting 1 μg/ml of paraquat (solid line) and the enzymatic reaction (dotted line)

Influence of the FI variables

Several FI manifolds were tried and the best results were obtained when the inhibitor paraquat and the enzyme AChE were simultaneously mixed with the

ATC and DTNB by means of a four-way connector. The influence of the reactor coil length was studied in the 0.5–6.5 m range. Figure 4 shows that the inhibition by paraquat increased with increasing the reactor lengths up to about 3 m, which was selected; greater reactor lengths resulted in smaller peak areas probably due to sample dispersion. The total flow rate was then varied between 1 and 3.5 ml/min to obtain a compromise optimal value which would lead to maximum peak area and sensitivity as well as a reasonable sample throughput rate. Figure 4 shows that the peak area decreased continuously as the flow rate increased because it considerably affected the reaction time. A 1 ml/min value was selected as a compromise between a good signal, sharp Fl peaks and an adequate sampling frequency. The effect of the sample loop size was examined between 30 and 350 µl and linearity was observed up to approximately 200 µl, which was the size chosen (Figure 4). Deviation from linearity with greater injection volumes may be attributed to increased dispersion of the sample plug[8].

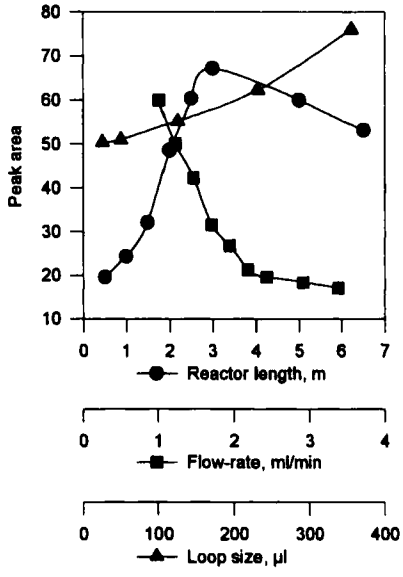

FIGURE 4 Effect of FIA variables (reactor coil length, flow-rate and sample loop size) on the reaction inhibited by injecting 1 µg/ml of paraquat

Calibration, interferences and applications

Calibration of paraquat was performed by plotting concentration against the area of the negative peaks and linearity was obtained between 0.2 and 1.5 µg/ml. The precision and accuracy of the method was demonstrated by repeated analyses

and the average relative standard deviation (RSD) calculated for ten replicate determinations of 0.26 μg/ml of paraquat was ±4.1%. In separate experiments, several other pesticides were tested for their ability to inhibit the enzyme. An inhibition effect on the enzyme was observed for diquat and diazinon at concentrations of 20 μg/ml and for atrazine and malathion at concentrations above 10 μg/ml. For other type of organic compounds such as chlorpromazine and perphenazine, no inhibition effect could be measured below a concentration of 20 μg/ml. Since some inhibitors were dissolved in organic solvents, their effect was investigated and it was found that up to 5% v/v methanol, ethanol and acetonitrile had no influence. Interferences caused by the common ions present in water samples were studied by injecting solutions containing paraquat (0.5 μg/ml) and different amounts of the ions. No interferences were found for calcium, magnesium, chloride or sulphate at [interferent]/[paraquat] ratios up to 100/1. Higher concentrations were not assayed. The tolerance limit was taken as the concentration causing an error of no more than ±4% in paraquat recovery.

The reliability of the proposed method was tested by analyzing a commercial product containing paraquat (Gramoxone, available in the form of the dichloride salt) and several samples of water of different origin. The results are included in Table I. Since none of the water samples contained any paraquat, they were spiked with the analyte at two concentrations. The concentrations found in water samples spiked with known amounts of the herbicide (0.5 and 1.0 μg/ml) were compared with the concentrations obtained by direct injection of standard solutions at each concentration level. As can be appreciated, the recovery was essentially quantitative.

TABLE I Determination of paraquat in different samples

Water sample	*Added (μg/ml)*	*Found**
Tap water	0.5	0.51 ± 0.02
	1.0	0.96 ± 0.05
Bottled water	0.5	0.49 ± 0.01
	1.0	0.98 ± 0.04
Spring water	0.5	0.52 ± 0.03
	1.0	1.06 ± 0.06
Commercial product	Labeled (g/l)	Found*
Gramoxone	200	195 ± 6

* Mean ± standard deviation (SD) (n =6)

Acknowledgements

The authors are grateful to the Spanish DGICYT (Project PB96–1100) and Comunidad Autónoma de la Región de Murcia (Project PB/7/FS/97) for financial support.

References

[1] D.J. Holme and H. Peck, *Analytical Biochemistry* (2nd edn., Longman, Essex, 1993).
[2] M. Valcárcel and M.D. Luque de Castro, *Flow-Injection Analysis. Principles and applications* (Ellis Horwood, Chichester, 1987).
[3] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone, *Biochem. Pharmacol.*, **7**, 88–95 (1961).
[4] T. Yao, *Anal. Chim. Acta*, **153**, 169–174 (1983).
[5] P. Lainé-Cessac, A. Turcant and P. Allain, *Clin. Chem.*, **35**, 77–80 (1989).
[6] I. López-García, F. Ruiz-Abellán and M. Hernández-Córdoba, *Anal. Biochem.*, **200**, 176–179 (1992).
[7] R. Kindervater, W. Künnecke and R.D. Schmid, *Anal. Chim. Acta*, **234**, 113–117 (1990).
[8] D. Narinesingh, R. Mungal and T.T. Ngo, *Anal. Chim. Acta*, **292**, 185–190 (1994).
[9] J. Marcos, A. Ríos and M. Valcárcel, *Anal. Chim. Acta*, **308**, 152–158 (1995).
[10] G. Zwig, *Pesticides, Plant Growth Regulators and Food Additives*(Academic Press, New York, 1967).
[11] M.T. Corasaniti and G. Nisticò, *J. Chromatogr. A*, **643**, 419–425 (1993).
[12] Y. Kambhampati, K.S. Roinestad, T.G. Hartman, J.D. Rosen, E.K. Fukuda, R.L. Lippincott and R.T. Rosen, *J. Chromatogr. A*, **688**, 67–73 (1994).
[13] M. Ibañez, Y. Pico and J. Manes, *J. Chromatogr. A*, **728**, 325–331 (1996).
[14] P. Shivhare and V.K. Gupta, *Analyst*, **116**, 391–393 (1991).
[15] E. Alvárez, M.T. Sevilla, J.M. Pinilla and L. Hernández, *Anal. Chim. Acta*, **260**, 19–23 (1992).
[16] T. Pérez-Ruíz, C. Martínez- Lozano and V. Tomás, *Intern. J. Environ. Anal. Chem.*, **44**, 243–252 (1991).
[17] M. Agudo, A. Ríos and M. Valcárcel, *Anal. Chim. Acta*, **281**, 103–109 (1993).