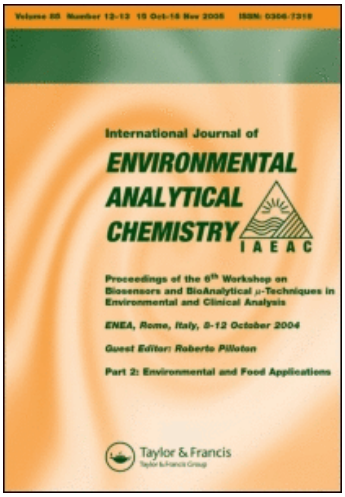

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Enzymatic Stopped-Flow Determination of Carbofuran Residues at the Nanomolar Level in Environmental Waters

M. C. Quintero[a]; M. Silva[a]; D. Pérez-Bendito[a]
[a] Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Cordoba, Spain

To cite this Article Quintero, M. C. , Silva, M. and Pérez-Bendito, D.(1990) 'Enzymatic Stopped-Flow Determination of Carbofuran Residues at the Nanomolar Level in Environmental Waters', International Journal of Environmental Analytical Chemistry, 39: 3, 239 — 243
To link to this Article: DOI: 10.1080/03067319008032067
URL: http://dx.doi.org/10.1080/03067319008032067

Intern. J. Environ. Anal. Chem., Vol. 39, pp. 239–243
Reprints available directly from the publisher
Photocopying permitted by license only

ENZYMATIC STOPPED-FLOW DETERMINATION OF CARBOFURAN RESIDUES AT THE NANOMOLAR LEVEL IN ENVIRONMENTAL WATERS

M. C. QUINTERO, M. SILVA and D. PÉREZ-BENDITO

Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, 14004 Cordoba, Spain

(*Received 7 December 1989*)

A method for the direct determination of carbofuran residues in environmental (river and swamp) and tap water is reported. It is based on the enzymatic inhibition of acetylcholinesterase by the pesticide, using DTNB (5,5'-dithiobis(2-nitrobenzoic) acid) as chromogenic reagent and using a stopped-flow technique. Carbofuran was determined in sample waters containing between 1.0 and 6.0 ng ml^{-1} of the pesticide with an average recovery of 99.7% and a relative standard deviation of 1.9%. Organophosphorous and chlorinated pesticides in 1000-fold excess do no interfere, while carbaryl, propoxur and paraoxon can be tolerated in 8-, 4-, and 1-fold excess, respectively.

KEY WORDS: Carbofuran, enzymatic, stopped-flow, environmental.

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzo(b)furanyl methylcarbamate) is a broad-spectrum insecticide currently used widely on a variety of crops; consequently, it can be found in environmental waters. This N-methylcarbamate pesticide not only acts as an insecticide, but is also highly toxic to species living in aquatic systems and, despite the ease with which it is hydrolysed (mainly to carbofuran phenol) it has been detected in dead mollusks.[1] Moreover, it is highly toxic to mammals (acute oral LD_{50} about 11 mg kg^{-1} for rats) which calls for its occasional analysis in tap water. To preserve aquatic life, some international organizations[2] which have established concentration limits of pesticides in environmental waters (that for carbofuran is 6 μg ml^{-1}), recommend that tap water should be free from this pesticide.

Many analytical methods have been proposed for the determination of carbofuran residues. Among these, enzymatic inhibition methods are particularly popular on account of their high sensitivity and reasonable capability of discerning between different pesticides. The method based on the inhibitory effect of the pesticide on acetylcholinesterase, which enzymatically hydrolytes acetylcholine, is one of the most frequently used.

In this work we used the above-mentioned enzymatic reaction for the determination of carbofuran in water samples, by using the stopped-flow technique,

which allows the photometric monitoring of the fast reaction between the choline released in the enzymatic reaction and the DTNB (5,5'-dithiobis(2-nitrobenzoic) acid) used as chromogenic reagent. The enzymatic stopped-flow method is quite selective and sensitive and allows the determination of carbofuran at the nanomolar level[3]; this, in turn, allows it to be directly applied to the determination of this pesticide in environmental and tap waters without further sample pretreatment.

In earlier works we successfully applied the stopped-flow technique to the determination of carbofuran in soil[4] and of other N-methylcarbamate pesticides such as carbaryl and its hydrolysis product in water and vegetable samples,[5] and maneb in formulations and residues on grains[6] by using various nonenzymatic kinetic methods.

EXPERIMENTAL

Reagents

A carbofuran (Chem. Service Inc.) solution (6.63×10^{-6} M) was made by dilution of a more concentrated stock solution prepared by dissolving 100.0 mg of the compound in 100 ml of 1,4-dioxane. This solution was stored in a refrigerator in PTFE bottles. An electric eel acetylcholinesterase (Sigma Chemical) solution of 4.0 units ml^{-1} was prepared from the lyophylized powdered enzyme containing 5% ammonium sulphate and featuring a protein activity of 200–400 units mg^{-1}. An acetylcholine iodide (3.77×10^{-2} M) solution was prepared by dissolving 109 mg of this compound in 10 ml of distilled water. A 1×10^{-3} M DTNB solution was prepared by dissolving 100 mg in 250 ml of Tris buffer of pH 7.4; 0.05 M Tris buffers of pH 7.4, 8.0 and 9.0 were also made.

Apparatus

A Perkin–Elmer Lambda 5 spectrophotometer coupled on-line with a stopped-flow module[7] was used. The acquisition and treatment of kinetic data was performed with a Hewlett-Packard 98561AE computer and a 16-bit Hewlett-Packard 98640A analogue-to-digital converter. The software required for application of the kinetic method was written by the authors themselves.

Procedure

Fifty ml of the water sample were spiked with various volumes of a 2.0 $\mu g\,ml^{-1}$ carbofuran solution containing up to 2.0 μl of 1,4-dioxane. 2-ml aliquots of this solution were added to a 5-ml standard flask, in which 0.5 ml of electric eel acetylcholinesterase solution of 4.0 units ml^{-1} had been previously placed (Solution A). Then the mixture was diluted to the mark with 0.5 M Tris buffer of pH 8.0. This solution was incubated at 37 °C for 30 min. Another solution (Solution B) was prepared by mixing 0.8 ml of a 9.4×10^{-3} M acetylcholine iodide

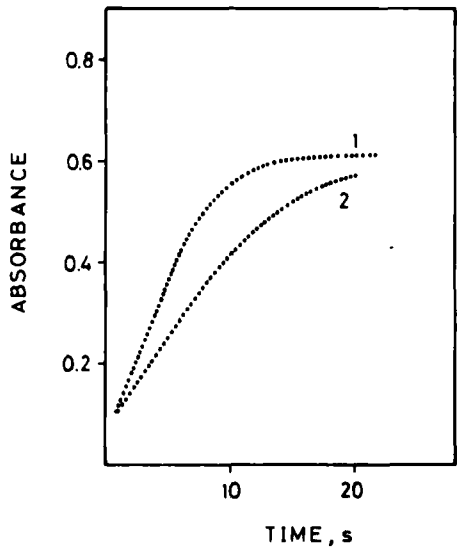

Figure 1 Absorbance-time curves obtained (1) in the absence of carbofuran and (2) in the presence of 3 ng ml^{-1} of carbofuran by stopped-flow mixing of DTNB/acetylcholine and the solution from the incubation process. Conditions as described under Experimental.

solution and 2.5 ml of 10^{-3} M DTNB in a 10-ml standard flask, diluting to the mark with 0.05 M Tris buffer of pH 9.0. Equal volumes of both solutions were mixed in the stopped-flow system and the reaction was monitored at 400 nm. The temperature was kept constant throughout at 35 ± 0.1 °C. The kinetic curve, the initial rate (both in the presence of the pesticide and in its absence) and the analyte concentration were automatically acquired by the computer.

RESULTS AND DISCUSSION

The method for the determination of carbofuran in environmental waters reported in this paper is based on an enzymatic stopped-flow approach used earlier for the determination of N-methylcarbamate pesticides.[3] It relies on the inhibitory effect of these pesticides on electric eel acetylcholinesterase and on the use of cholinesterase iodide as substrate and DTNB as chromogenic reagent for the choline released in the enzymatic hydrolysis of the substrate. The reaction is monitored photometrically by the stopped-flow technique. As the chromogenic reaction develops in a few seconds, the stopped-flow technique is mandatory to obtain the kinetic data required to calculate the pesticide concentration.

The method is especially useful for the analysis of carbofuran residues. In Figure 1 the absorbance-time kinetic curves recorded at 400 nm in the absence and presence of 1 ng ml^{-1} of carbofuran are shown. The determination of carbofuran is based on the plot of the per cent inhibition vs. the carbofuran concentration. The per cent inhibition is calculated from the absorbance-time curves by applying the

Table 1. Selectivity achieved in the enzymatic stopped-flow determination of carbofuran.[a]

Compound assayed	*Tolerance limit*
Maneb, zineb, ziram, 1-naphthol, 2-isopropoxyphenol, carbofuran phenol, parathion, aldrin, dieldrin, α-HCH, lindane, methylparathion, malathion, fenitrothion, dimethoate	1000
Dicrotophos	100
Carbaryl	8
Propoxur	4
Paraoxon	1

[a]Initial concentration in the syringe, 1 ng ml^{-1}.

kinetic initial-rate method to both curves. This method allows the determination of carbofuran[3] in the range of 0.1–5.0 ng ml^{-1}, with a relative standard deviation of 0.55%. The sensitivity is superior to that achieved so far with other enzymatic inhibition reactions.[8]

In order to apply the proposed method to the analysis of carbofuran residues in real samples, we made a study of its selectivity.

Selectivity of the Method

The study was conducted under the optimal conditions reported under Experimental. The influence of other pesticides on the determination of carbofuran was studied by adding variable amounts of up to 1 μg ml^{-1} to samples containing 1 ng ml^{-1} of carbofuran (these concentrations correspond to the initial concentration of the compounds in the syringe) and applying the procedure described under Experimental.

For each pesticide the tolerance level was taken as the concentration resulting in an error of less than ±5% in the per cent inhibition of carbofuran in the absence of the foreign species. The results of these experiments are listed in Table 1. A number of pesticides including organophosphorous and organochlorine compounds do not interfere at concentrations 1000-fold higher than that of carbofuran. Carbaryl and propoxur are tolerated in 8-fold and 4-fold excess, respectively. The most serious interference is posed by paraoxon; this organophosphorous pesticide can only be tolerated at concentrations equal to or lower than those of carbofuran.

The high selectivity achieved is a distinct advantage, since both organophosphorous and chlorinated pesticides are stated also to inhibit the enzymatic reaction.[9,10] The selectivity can be attributed to the use of electric eel acetylcholinesterase as the enzyme source.

Carbofuran in Water Samples

Water samples from the river Guadiato, the Breña swamp and tap water from the

Table 2 Recovery of carbofuran from water samples

Type of water	*Added* ($ng\,ml^{-1}$)	*Found* ($ng\,ml^{-1}$)	*Average recovery* (%)
River	1.25	1.23, 1.24, 1.25	99.2
	2.50	2.51, 2.43, 2.53	99.6
	5.00	4.90, 4.66, 4.98	96.9
Swamp	1.50	1.50, 1.49, 1.52	100.2
	3.00	2.98, 3.07, 3.02	100.7
	6.00	5.78, 6.17, 5.68	97.9
Tap	1.00	1.05, 0.99, 1.07	103.6
	2.00	1.99, 1.98, 1.96	98.8
	4.00	3.95, 4.04, 4.10	100.8
		Average	99.7
		Average standard deviation	1.9

city of Córdoba were analyzed by adding appropriate volumes of the $2\,\mu g\,ml^{-1}$ carbofuran standard solution to obtain the carbofuran concentrations listed in Table 2. The proposed method was directly applied to each sample in triplicate without further pretreatment, following the procedure described under Experimental. The results are given in Table 2. Recoveries ranged from 97% to 103% with an average of 99.7% and an average relative standard deviation of $\pm 1.9\%$. There were no significant differences in the recoveries for the different types of water or between the different levels of spiking.

The results show the suitability of the proposed method for the analysis of carbofuran residues at low concentrations in environmental and tap waters.

Acknowledgement

The authors gratefully acknowledge financial support from the CICYT (Project No. PB87-0821).

References

1. C. C. Yu, G. M. Booth, D. J. Hansen and J. R. Larsen, *J. Agric. Food Chem.* **3,** 975 (1974).
2. *New and Revised Great Lakes Water Quality Objectives*, Vol. 1, International Joint Commission, Windsor, Ontario (Canada), 1977.
3. M. C. Quintero, M. Silva and D. Pérez-Bendito, *Z. Anal. Chem.*, in press.
4. M. C. Quintero, M. Silva and D. Pérez-Bendito, *Analyst* **114,** 497 (1989).
5. M. C. Quintero, M. Silva and D. Pérez-Bendito, *Talanta* **35,** 943 (1988).
6. M. C. Quintero, M. Silva and D. Pérez-Bendito, *Anal. Chim. Acta* **222,** 269 (1989).
7. A. Loriguillo, M. Silva and D. Pérez-Bendito, *Anal. Chim. Acta* **199,** 29 (1987).
8. W. S. G. Morgan, P. C. Kuehn and P. P. Van Niekerk, *Water Sci. Technol.* **17,** 855 (1985).
9. M. H. Sädar, S. S. Kuan and G. G. Guilbault, *Anal. Chem.* **42,** 1770 (1970).
10. J. R. Cobertt, K. Wright and A. C. Baillie, *The Biochemical Mode of Action of Pesticides*, Academic Press, New York (U.S.A.), 1984.