Talanta 81 (2010) 327–333

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A greener and highly sensitive flow-based procedure for carbaryl determination exploiting long pathlength spectrophotometry and photochemical waste degradation

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ARTICLE INFO

Article history:
Received 29 October 2009
Received in revised form 4 December 2009
Accepted 7 December 2009
Available online 16 December 2009

Keywords:
Flow analysis
Multicommutation
Spectrophotometry
Green analytical chemistry
Liquid core waveguides
Carbaryl

ABSTRACT

An environmentally friendly analytical procedure with high sensitivity for determination of carbaryl pesticide in natural waters was developed. The flow system was designed with solenoid micro-pumps in order to improve mixing conditions and minimize reagent consumption as well as waste generation. A long pathlength (100 cm) flow cell based on a liquid core waveguide (LCW) was employed to increase the sensitivity in detection of the indophenol formed from the reaction between carbaryl and p-aminophenol (PAP). A clean-up step based on cloud-point extraction was explored to remove the interfering organic matter, avoiding the use of toxic organic solvents. A linear response was observed within the range 5–200 $\mu g\,L^{-1}$ and the detection limit, coefficient of variation and sampling rate were estimated as 1.7 $\mu g\,L^{-1}$ (99.7% confidence level), 0.7% ($n = 20$) and 55 determinations per hour, respectively. The reagents consumption was 1.9 μg of PAP and 5.7 μg of potassium metaperiodate, with volume of 2.6 mL of effluent per determination. The proposed procedure was selective for the determination of carbaryl, without interference from other carbamate pesticides. Recoveries within 84% and 104% were estimated for carbaryl spiked to water samples and the results obtained were also in agreement with those found by a batch spectrophotometric procedure at the 95% confidence level. The waste of the analytical procedure was treated with potassium persulphate and ultraviolet irradiation, yielding a colorless residue and a decrease of 94% of total organic carbon. In addition, the residue after treatment was not toxic for *Vibrio fischeri* bacteria.

1. Introduction

Carbamates are one of the most important classes of pesticides. Carbaryl (1-naphthyl-N-methyl carbamate) is a broad-spectrum insecticide, used to control more than 100 kinds of pests in crops such as beans, bananas, potatoes and domestic vegetables [1]. However, this substance inhibits the cholinesterase enzyme, impairing the functions of the central nervous system and can cause nausea, vomit, broncho-constriction, blurred vision, convulsions, coma and respiratory failure, in addition to its teratogenic characteristics [2].

Several procedures have been described in the literature for the analysis of carbaryl in different matrices [3–7], mainly based on chromatographic separation [8–11]. The 8318 reference method, recommended by the United States Environmental Protection Agency (EPA) for the determination of N-methylcarbamates in soil, water and wastes, for example, is based on high performance liquid chromatography (HPLC) with fluorimetric detection after post-column derivatization. Before analysis, the analyte is extracted with a mixture of ethylene glycol, methanol and acetonitrile [1].

The spectrophotometric determination of carbaryl can be performed using p-aminophenol (PAP), p-N,N-dimethylphenylenediamine dihydrochloride or 1-amino-2-naphthol-4-sulphonic acid as chromogenic reagents, forming a product with absorption maximum between 600 and 700 nm. For analyte extraction from water samples, up to 200 mL of chloroform are used per determination [3]. Other spectrophotometric procedures are based on diazotization with trimethylaniline (TMA) in a micellar medium containing sodium dodecyl sulfate [2]. The anionic surfactant was used to dissolve TMA and provides a suitable medium for the coupling reaction with 1-naphthol. Later, the same anionic surfactant was used in an acid-induced cloud-point extraction coupled to derivatization with 2-naphthylamine-1-sulfonic acid (ANSA) reagent for determination of carbaryl residues in waters and vegetables. The proposed method showed good analytical features, including low detection limit, but a large amount of concentrated HCl was used (5.0 mL per sample) [12]. Alternatively, a procedure based on double cloud-point extraction for determination of carbaryl in natural waters was proposed. The analyte was separated from the matrix using Triton X-114 in an alkaline medium in

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doi:10.1016/j.talanta.2009.12.005

order to avoid the use of toxic organic solvents. Cloud-point preconcentration of the indophenol product was explored to increase sensitivity and to improve the detection limit [13].

The spectrophotometric determination of carbaryl has also been implemented in flow systems [6,14–17]. A single-line manifold based on the reaction of carbaryl with diazotized sulfanilic acid in alkaline medium was used in the first work described in the literature [15]. A linear response was observed within the range 0.1–40 mg L^{-1} carbaryl, with a detection limit and coefficient of variation estimated as 0.08 mg L^{-1} and 3.8%, respectively. Other flow-based procedures have exploited the reaction of the hydrolyzed carbaryl with p-aminophenol in an alkaline medium, forming indophenol blue, with maximum absorption at 585 nm [14–17]. The carbaryl extraction step consumes large amounts of reagent—2.5 g of anhydrous sodium sulfate, 60 mL of methylene chloride and 10 mL of sodium hydroxide per determination [15] or 10 mL xylene, followed by back-extraction of the α-naftolate with 0.2 mol L^{-1} NaOH [16].

Recently, a flow procedure based on chemiluminescence [18] was developed, exploiting the oxidation of the pesticide by Ce(IV) in a nitric acid medium containing rhodamine 6G as a sensitizer. Solid-phase extraction was used to concentrate and separate the analyte from the matrix, followed by elution using 5 mL of a 1:1 ethyl acetate: n-hexane solution. The eluent was collected and evaporated to dryness under a nitrogen stream. Ethanol was directly added to the residue and the amount of analyte was then determined. A new solvent extraction and concentration procedure using microchip technology was based on thermal lens spectroscopy [19]. After pesticide hydrolysis in alkaline medium and coupling of 1-naphthol with diazotized trimethylaniline, the reaction product was extracted with toluene as a colored azo-dye.

Sensitivity in spectrophotometry can be enhanced by increasing the optical path, hence a larger amount of absorbing species is available to interact with the radiation beam. However, for cells fabricated with conventional materials, the increase in the optical path is limited by the excessive attenuation of the radiation beam, also demanding higher sample volumes [20]. Liquid core waveguides (LCWs) with capillary dimensions have been used to circumvent these drawbacks. The capillary tubes are constructed with a polymer with refractive index lower than that of water (e.g. Teflon AF family) or by fused silica covered with this material. In this way, radiation incident at a suitable angle in the liquid core is constrained inside the waveguide by total reflection that occurs between the liquid and the tube wall [21,22]. These devices have been used for the determination of species at nanomolar level in environmental samples [23–25], including gaseous analytes [26] and also for measurements based on turbidimetry [26,27] or luminescence [22].

In this work, an improved flow-based procedure for determination of carbaryl in natural waters is described. The procedure is based on the alkaline hydrolysis of carbaryl to 1-naphthol which is coupled to the oxidized form of PAP. The flow system was designed with solenoid micro-pumps to minimize reagent consumption and long pathlength spectrophotometry (LPS) was explored to increase sensitivity. The clean-up step, often carried out with organic solvents, was replaced by a simple, fast and environmentally friendly cloud-point extraction. The waste was photochemically decomposed and characterized before toxicity tests with the marine bacteria *Vibrio fischeri*.

2. Experimental

2.1. Apparatus

The flow system was constructed with four solenoid micro-pumps of 12 (P_1, P_2 and P_3) and 7 μL (P_4) nominal volume per pulse (Biochem Valve Inc., Boonton, NJ, USA; model 090SP), a pair of three-way solenoid valves (NResearch, West Caldwell, NJ, USA), 0.7 mm i.d. polyethylene tubes and a Perspex joint. A Pentium I microcomputer was used for system control and data acquisition. The solenoid micro-pumps and valves were controlled through a parallel port of the microcomputer by using a power drive based on a ULN2803 integrated circuit. Spectrophotometric measurements in dual-wavelength mode (585 and 750 nm) were carried out with a multi-channel CCD spectrophotometer (Ocean Optics, Dunedin, FL, USA; model USB2000) coupled to a tungsten-halogen light source (Ocean Optics; model LS-1). Optical fibers (100 or 600 μm) were used to transmit the radiation. A 100 cm optical path (250 μL internal volume, 0.55 mm i.d.) flow cell (Ocean Optics; model LPC-1) was also employed. The control software was developed in Visual Basic 6.0 (Microsoft, Redmond, WA, USA) and the software supplied by the manufacturer of the multi-channel spectrophotometer was used for data acquisition.

The photo-reactor used for waste degradation was similar to the previously described [28]. It consists of a 400 W low-pressure mercury lamp, obtained from a common street lightning fluorescent lamp (Philips) by removing the external glass bulb. The socket of the lamp was fixed at the ends of a PVC cylindrical tube (10.2 cm i.d., 60 cm length) commonly used in construction field to transport sewage. The lamp was powered by an appropriate power supply (Serwal RIM426B, 220 V) and shows a continuous emission spectrum, predominantly in the visible and emission lines typical of mercury vapor at 254, 313 and 366 nm. The waste generated was positioned in a beaker at 10 cm from the lamp and kept under constant agitation.

UV–vis absorption spectra were obtained with a spectrophotometer (HITACHI, Schaumburg, IL, USA; model U-3000), equipped with a quartz cuvette (1 cm). Total organic carbon was determined using a SHIMADZU TOC–5000A analyzer (Santa Clara, CA, USA). A SHIMADZU LC-20 AT liquid chromatograph with UV–vis detector was used in characterization studies of the generated waste. In toxicity tests, a luminometer MICROTOX 500-B-7120 (Carlsbad, CA, USA) with integrated control and measurement units was used.

2.2. Reagents and solutions

All solutions were prepared with analytical grade chemicals and distilled and deionized water. The reference solutions were prepared in the range 5.0–400.0 μg L^{-1} (2.5×10^{-8} to 2.0×10^{-6} mol L^{-1}) carbaryl (Sigma–Aldrich, St. Louis, MO, USA) by dilution of a 7.5×10^{-5} mol L^{-1} stock solution, prepared in 5% (v/v) ethanol (Merck, Darmstadt, Germany).

The reagents were 4.6×10^{-5} mol L^{-1} p-aminophenol (Sigma–Aldrich, R_1) and 2.0×10^{-4} mol L^{-1} potassium metaperiodate (Merck, R_2) solutions prepared by dissolving appropriate amounts of the chemicals in water. A 1.0×10^{-2} mol L^{-1} sodium hydroxide (Merck) solution was employed as carrier.

Carbamate pesticides (aminocarb, bendiocarb, carbofuran, pirimicarb and propoxur) were obtained from Sigma–Aldrich. Solutions at 1.2–9.9 $\times 10^{-7}$ mol L^{-1} concentrations were prepared in water.

River and lake water samples were collected in the state of São Paulo, Brazil. Samples were filtered through a 0.45 μm cellulose acetate membrane and preserved at −4 °C, being stabilized at ambient temperature immediately before analysis.

2.3. Flow diagram

The flow manifold, shown in Fig. 1, was operated according to the switching course of the active devices described in Table 1. The binary sampling approach [29] was adopted for solutions handling. Low volume aliquots of the solutions were inserted in tandem into the analytical path, generating a sampling profile that was repeated

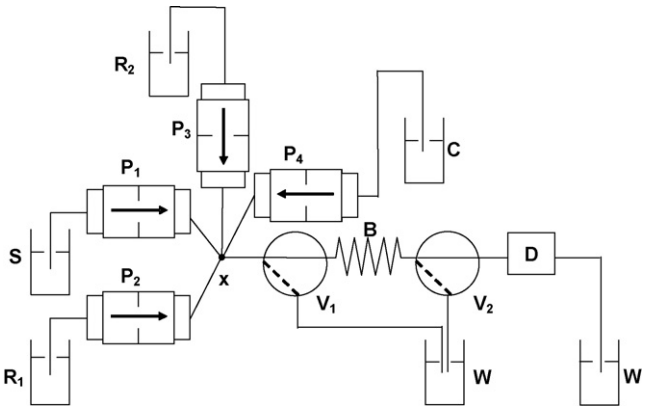


Fig. 1. Flow diagram of the system for carbaryl determination. P_1–P_4: solenoid micro-pumps; V_1, V_2: three-way solenoid valves; S: sample; C: carrier stream; R_1 and R_2: p-aminophenol and potassium metaperiodate reagents, respectively; B: 100 cm long polyethylene coil; D: spectrophotometer; x: Perspex joint point and W: waste vessel.

Table 1
Switching course of the solenoid micro-pumps and values for carbaryl determination with the flow system shown in Fig. 1. The status 1/0 indicates a pulse of current in the solenoid pump. 0 and 1 indicate that valves are switched off and on, respectively.

Step	Description	P_1	P_2	P_3	P_4	V_1	V_2	Pulses or time
1	Sample insertion	1/0	0	0	0	0	1	7[a]
2	Reagent 1 insertion	0	1/0	0	0	0	1	3[a]
3	Reagent 2 insertion	0	0	1/0	0	0	1	1[a]
4	Stopped flow	0	0	0	0	0	0	20 s
5	Sample transportation and signal measurement	0	0	0	1/0	0	0	220
6	Sample replacement	1/0	0	0	0	1	0	100
		0	0	0	1/0	1	0	50

[a] Eight sampling cycles

until the programmed number of sampling cycles was reached. The volume of each solution was defined by the programmed number of pulses of the corresponding micro-pump.

The analytical cycle was started by pumping sample and reagents through P_1, P_2 and P_3 pumps (steps 1, 2 and 3). These aliquots underwent fast mixing at the interfaces, establishing the first sampling cycle. The sequence was repeated eight times to form the sample zone and then the flow was stopped for 20 s (step 4). The sample zone was carried out towards detection at 585 nm by actuation of P_4 (step 5). The analytical signal was based on peak height and measurements were taken in triplicate.

Valve V_1 was used to replace solutions, avoiding passage through the analytical path and minimizing the risk of contamination. Before analyzing another sample, valve V_1 was switched on while pump P_1 was actuated (100 pulses) to fill the connection tube with a new sample. Pump P_4 was then actuated (50 pulses) to remove the sample aliquot by the carrier through valve V_1 (step 6). Valve V_2 was used to avoid drawbacks caused by the hydrodynamic impedance of the LCW cell in formation of the sampling zone.

High performance liquid chromatography [10] with UV detection at 220 nm was adopted as a reference for the analysis of carbaryl in water samples. The mobile phase was acetonitrile:water (40:60) at a flow-rate of 1.3 mL min^{-1}.

2.4. Sample clean-up

For carbaryl separation from interfering species, aliquots of 13 mL of the sample or reference solutions containing from 5.0 to 400.0 μg L^{-1} of the analyte, 400 μL of 1 mol L^{-1} NaOH and 1 mL Triton X-114 (7.5%, v/v) were transferred to 15 mL graduate flasks of polyethylene. The mixture was kept for 10 min in a thermostatic bath at 40 °C and phase separation was accomplished by centrifugation for 10 min. The supernatant aqueous phase containing carbaryl hydrolyzed to 1-naphthol was then completely separated from the organic matter in the sample and employed for the spectrophotometric determination.

2.5. Characterization of treated waste

The total organic carbon content was determined by the difference between measurements of total carbon and inorganic carbon. Measurements of total carbon were made by the introduction of the residue in a heated reaction chamber (670 °C) containing a platinum catalyst adsorbed on aluminum oxide. The water was vaporized and the residual carbon converted into CO_2, which was measured in an infrared analyzer. The inorganic carbon was measured by a similar approach, but with phosphoric acid in the chamber.

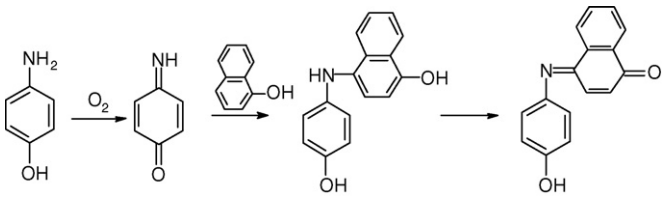
Characterization of the waste generated was also carried out by HPLC. The chromatographic conditions used were based on those described by Aceituno et al. [30]: C-18 column (25 mm × 4.6 mm), mobile phase (flow-rate 0.7 mL min^{-1})—95% of 0.68 mol L^{-1} formic acid/ammonium formate buffer and 5% methanol (isocratic mode, 2 min), 5–100% methanol (gradient, 13 min) and 100% methanol (isocratic mode, 15 min). The analytical separation of the residue with and without degradation in the presence of titanium dioxide or potassium persulphate was carried out.

Toxicity tests were based on the marine bacteria *Vibrio fischeri*. The parameter used for toxicity evaluation was the inhibition of the bacteria bioluminescence after contact with the toxic waste. The bacteria were grown in a liquid medium and then frozen at −30 °C. In order to reactivate the metabolism and emission of light, the bacteria were placed in a rebuilding medium, consisting mainly of NaCl and KCl [31,32]. After 20 min with temperature stabilized at 15 °C, the luminescence was measured before and after addition of the waste. The pH of all solutions was previously adjusted between 6 and 8.5 and the salinity between 20 and 50 g L^{-1} to avoid the effect of these parameters on the bioluminescence of bacteria.

3. Results and discussion

3.1. System optimization

Spectrophotometric carbaryl determination was based on the formation of indophenol blue from the product of carbaryl hydrolyzed and the oxidized PAP, which showed absorption maximum at 585 nm (Eq. (1)). The flow system (Fig. 1) was designed with solenoid micro-pumps in order to improve mixing conditions due to the inherent pulsed flow as well as to minimize reagent consumption and effluent generation. Optimization was carried out by the univaried method, using a 100 cm optical path flow cell. The effect of the sample/reagents ratio was evaluated with the flow system shown in Fig. 1. Best sensitivity was achieved with seven pulses of sample (84 μL), three pulses of p-aminophenol (36 μL) and one pulse of potassium metaperiodate (12 μL) in eight sampling cycles.



(1)

The magnitude of the blank signal is a critical parameter in LPS, affecting both the working range and the detection limit. Blank

Table 2
Ranges studied and optimized conditions for carbaryl determination.

Parameter	Range studied	Selected value
Pulses of sample	1–7	7
Pulses of R_1	1–6	3
Pulses of R_2	1–6	1
Sampling cycles	1–10	8
[PAP] (μmol L^{-1})	4.6–92	46
[KIO_4] (μmol L^{-1})	50–1000	200
[NaOH] (mol L^{-1})	0.01–1.0	0.01
Reactor (cm)	50–200	100
Stopped flow period (s)	0–200	20

signals can be originated from absorption by the reagent mixture or perturbations by Schlieren effect [33]. In some previous applications involving LPS, the concentration of reagents and thus the analytical performance were limited by the blank magnitude [25]. As the p-aminophenol reagent absorbs at the measurement wavelength, optimization was performed taking into account the magnitude of both analytical and blank signals. The analytical signal was ca. five times higher when the p-aminophenol concentration was varied from 4.6×10^{-6} to 9.2×10^{-5} mol L^{-1}. On the other hand, the blank signal increased 6.5 times under the same conditions and thus the p-aminophenol concentration was maintained at 4.6×10^{-5} mol L^{-1}.

The KIO_4 reagent was used to oxidize the p-aminophenol to yield benzoquinoneimine. The effect of this reagent was evaluated in the range 5.0×10^{-5} to 1.0×10^{-3} mol L^{-1}, resulting in significant increase in the analytical response up to 2.0×10^{-4} mol L^{-1} (analytical signal 50% higher than that obtained in the lowest concentration evaluated). The magnitude of the blank was not affected by the oxidant concentration.

Indophenol blue formation occurs in an alkaline medium that is also required for carbaryl hydrolysis. As NaOH concentrations up to 0.1 mol L^{-1} in the carrier did not significantly affect the analytical and blank signals, a concentration of 0.01 mol L^{-1} was selected. The reactor coil length was varied from 50 to 200 cm and the analytical signal increased gradually up to 150 cm, indicating that the effect of increased residence time on the formation of indophenol blue overcame the effect of the higher sample dispersion. A 100 cm long reactor coil was then selected, providing suitable mixing conditions and repeatability, with the sample residence time being increased by the stopped flow approach. The analytical signal was 14% higher with a 20 s stopping period, which is significant for the analysis of carbaryl at low concentrations. A trend to a signal decrease was observed after 20 s, because diffusion of the product was not compensated for the rate of indophenol formation. A summary of the ranges studied and the selected values is presented in Table 2.

3.2. Analytical features and application

A linear response was observed between 5.0 and 200.0 μg L^{-1} carbaryl, described by the equation: $A = -0.0163 + 0.0038C$ (μg L^{-1}), $r = 0.999$ (Fig. 2). Perturbations characteristic of the Schlieren effect were observed before the analytical signals, especially due to the use of NaOH solution as carrier. In order to compensate for this effect, measurements were based on dual-wavelength spectrophotometry (measurements at maximum absorption of the product and at the reference wavelength, in which the product does not absorb) [34]. The detection limit was estimated at 1.7 μg L^{-1} with a 99.7% confidence level, and the coefficient of variation was 0.7% ($n = 20$). The sampling rate was 55 determinations per hour with reagent consumption estimated at 1.9 μg of p-aminophenol and 5.7 μg of potassium metaperiodate per determination and waste volume of 2.6 mL per determination.

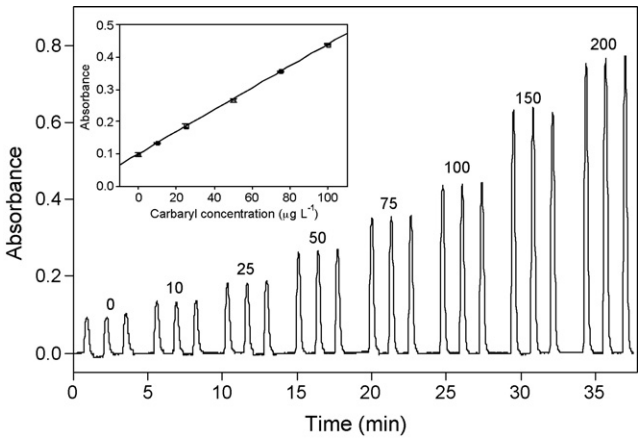


Fig. 2. Transient signals obtained for carbaryl reference solutions measured in triplicate. Numbers indicate carbaryl concentrations in μg L^{-1}. The inset shows the corresponding calibration graph.

When natural water samples were analyzed by the proposed procedure, interferences were observed due to absorption by organic matter in the samples. All samples evaluated absorbed significantly at the measurement wavelength, with absorbances between 0.095 and 0.399. Aiming to eliminate the spectral interference, a clean-up procedure based on cloud-point extraction was adopted. After sample clean-up, the absorption at 585 nm was reduced by at least 75%, while the surfactant rich-phase showed a dark color indicating extraction of the interfering species. The extraction method was highly efficient because carbaryl is highly soluble and easily hydrolyzed in the alkaline medium. This was demonstrated by a study carried out to evaluate the effect of the clean-up step on carbaryl determination. When a 5.0×10^{-7} mol L^{-1} carbaryl solution was analyzed in triplicate with and without the preliminary extraction, absorbance values were 0.208 ± 0.004 and 0.206 ± 0.007, respectively. The signals did not show significant differences, indicating that the analyte remains in the aqueous phase after cloud-point extraction.

The relative response for some carbamate pesticides (aminocarb, bendiocarb, carbofuran, propoxur and pirimicarb) was estimated from the slopes of calibration curves obtained in the same concentration range (2.6×10^{-7} to 2.6×10^{-6} mol L^{-1}) and the results are presented in Table 3. Carbamate pesticides did not cause interference (signal variation lower than 2%) showing that the procedure is selective for carbaryl. This occurred because the formation of indophenol blue from other carbamates is slower (ca. 8 min is required to reach the steady state condition for propoxur, for example), while product formation was instantaneous from carbaryl.

The proposed procedure was applied to carbaryl determination in natural waters after sample spiking with 50 or 100 μg L^{-1}. As shown in Table 4, recovery values within 84% and 104% were obtained by using the proposed procedure, indicating absence of matrix effects. The proposed procedure was then applied to car-

Table 3
Relative response for some carbamate pesticides with the proposed procedure.

Pesticide	Relative response (%)
Carbaryl	100
Aminocarb	2
Bendiocarb	0.8
Carbofuran	0.3
Pirimicarb	0
Propoxur	1.4

Table 4
Addition-recovery experiment of carbaryl in natural water samples.

Sample	Carbaryl ($\mu g\,L^{-1}$)		Recovery (%)
	Spiked	Found	
1	50.0	41.8 ± 2.1	83.6 ± 4.2
	100.0	93.4 ± 4.8	93.4 ± 4.8
2	50.0	44.8 ± 2.7	89.6 ± 5.4
	100.0	92.8 ± 2.6	92.8 ± 2.6
3	50.0	51.8 ± 0.4	104 ± 0.8
	100.0	103 ± 1	103 ± 1
4	50.0	42.5 ± 1.6	85.0 ± 3.2
	100.0	94.6 ± 2.1	94.6 ± 2.1

Table 5
Mean values and estimates ($n = 3$) for carbaryl determination in natural waters after addition of 50 or 100 $\mu g\,L^{-1}$ carbaryl.

Sample	Carbaryl ($\mu g\,L^{-1}$)	
	Proposed procedure	Reference procedure [10]
1	60.4 ± 1.0	57.9 ± 2.5
2	60.2 ± 1.5	59.2 ± 1.0
3	101 ± 4	101 ± 1
4	121 ± 1	121 ± 1

baryl determination in natural water after sample spiking and the results (Table 5) agreed with the reference procedure based on HPLC [10] at the 95% confidence level.

The analytical characteristics were better than those obtained by using other flow procedures based on the reaction with p-aminophenol and potassium metaperiodate in alkaline medium [14] (Table 6). The detection limit was 15 times lower than the observed in flow injection analysis with continuous addition of reagents and in the multicommutation-based system with solenoid valves, and 24 times lower than the observed in the sequential injection system. The sensitivity obtained with the LCW cell was 125, 270 and 80 times higher in comparison with the FIA, SIA and multicommutation-based procedures, respectively. The reagent consumption decreased considerably in comparison with the other evaluated procedures. The more significant reduction was in comparison to the flow injection system, in which reagent consumption 435- and 71-fold lower for KIO_4 and p-aminophenol were observed. For the multicommuted system with solenoid valves, the consumption was ca. 16 and three times lower for KIO_4 and p-aminophenol, respectively.

3.3. Waste photo-degradation

In aiming to develop cleaner analytical procedures, replacement of toxic reagents without affecting the analytical performance is rarely feasible. Alternatives to minimize the reagent consumption and generation of toxic wastes associated with waste treatment are then required. The complete oxidation (also called mineralization) of an organic compound generates CO_2, H_2O and mineral acids as final products. With this objective, the advanced oxidative processes are characterized by the generation of highly oxidizing species, especially hydroxyl radicals ($^{\bullet}OH$), capable of promoting the rapid degradation of various pollutant compounds [35]. Various alternatives can be explored for the production of these radicals, allowing adaptation to specific treatments. Waste degradation was carried out by heterogeneous photocatalysis ($TiO_2 + H_2O_2$) or by treatment with persulphate coupled to UV irradiation. The decomposition of the persulphate ion, with the formation of sulfate radical (Eq. (2)) can be induced by irradiation. In a second step, the sulfate radical reacts with water forming the hydroxyl radical (Eq. (3)). In subsequent stages, $S_2O_8^{2-}$ is decomposed as indicated by Eqs. (4) and (5) [36].

$$S_2O_8^{2-} \rightarrow 2\,SO_4^{\bullet -} \quad (2)$$

$$2\,SO_4^{\bullet -} + H_2O \rightarrow HSO_4^{-} + HO^{\bullet} \quad (3)$$

$$S_2O_8^{2-} + HO^{\bullet} \rightarrow HSO_4^{-} + SO_4^{\bullet -} + 1/2O_2 \quad (4)$$

$$SO_4^{\bullet -} + HO^{\bullet} \rightarrow HSO_4^{-} + 1/2O_2 \quad (5)$$

The effect of the mass of the reagents (TiO_2 and $K_2S_2O_8$) on the reduction of total organic carbon (TOC) was evaluated within the

Table 6
Analytical features of flow-based procedures for carbaryl determination based on formation of indophenol blue.

Procedure	Sensitivity ($L\,mol^{-1}$)	Detection limit[a] ($\mu g\,L^{-1}$)	Sampling rate (h^{-1})	Reagent consumption (μg/determination)	
				KIO_4	PAP
FIA	6.1×10^3	26	90	2480	135
SIA	2.8×10^3	40	20	193	11
Multicommutation with solenoid valves	9.5×10^3	26	70	92	5.0
Proposed procedure	7.6×10^5	1.7	54	5.7	1.9

[a] 99.7% confidence level.

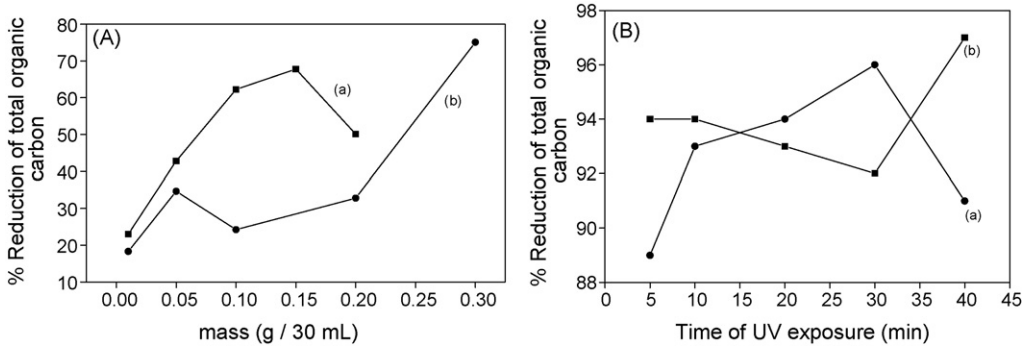


Fig. 3. Effect of mass (A) and time of exposure (B) of (a) TiO_2 (HCl 0.22 mol L^{-1} and H_2O_2 0.02 mol L^{-1}) and (b) $K_2S_2O_8$ on total organic carbon reduction of the waste generated in carbaryl determination.

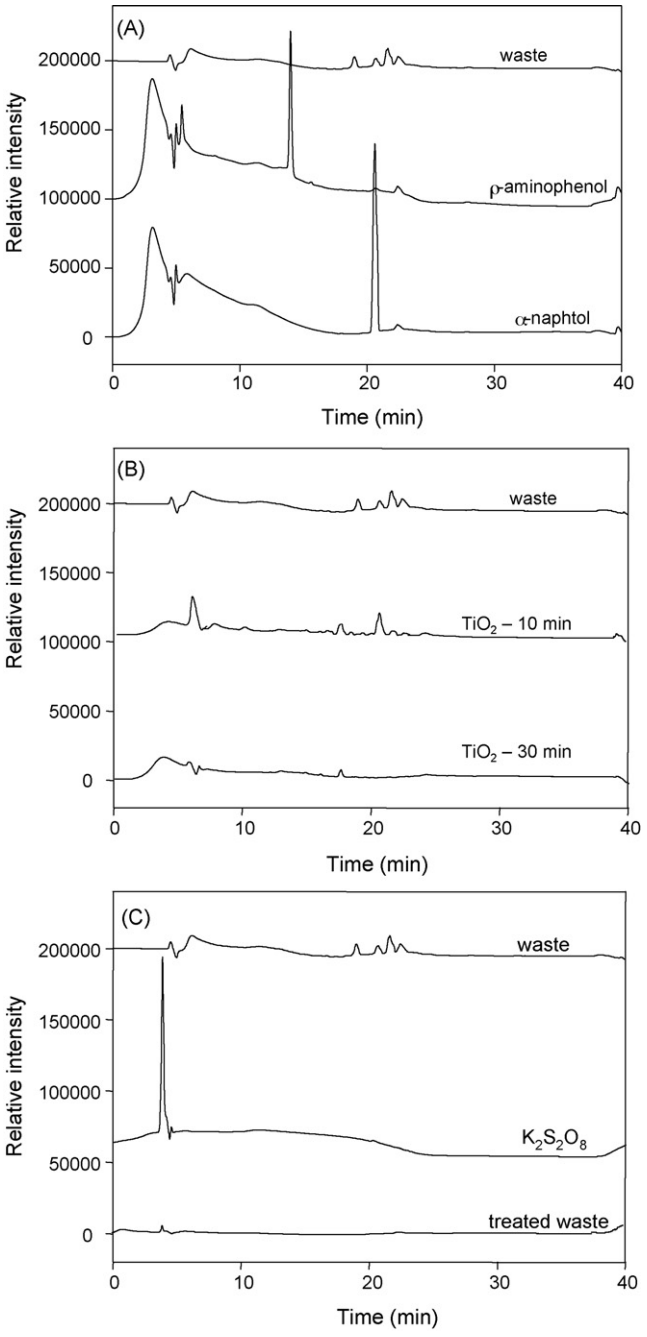


Fig. 4. Chromatograms of reagents and waste without degradation (A) and the residue before and after degradation with (B) titanium dioxide and (C) potassium persulphate. The chromatogram for a persulphate solution is also shown for comparison (C).

range 0.05–0.30 g in 30 mL of waste (Fig. 3A). The waste mineralization (generating CO_2 and H_2O) was favored when the mass of both reagents (TiO_2 and $K_2S_2O_8$) was increased. For 0.20 g of TiO_2, an increase on the residual organic carbon was observed. This effect may be due to scattering of incident radiation by solid particles in suspension. For lower reagent amounts, this effect must be offset by increased efficiency of the formation of hydroxyl radicals. The effect of the time of exposure to ultraviolet radiation was evaluated with 0.15 g of $K_2S_2O_8$ or 0.30 g of TiO_2 for 30 mL of waste, respectively. Reductions in total organic carbon above 94% were obtained with titanium dioxide with 20 min of exposure to UV radiation or by using potassium persulphate with 5 min of UV irradiation exposure (Fig. 3B).

Table 7
Degradation of the waste generated in carbaryl determination.

Parameter	TiO_2	$K_2S_2O_8$
Colorless residue	m = 0.15 g and t = 20 min	m = 0.30 g and t = 5 min
Reduction TOC > 94%	m = 0.15 g and t = 10 min	m = 0.30 g and t = 5 min
Toxicity[a]	Toxic, necessary 1:5 dilution	Non-toxic
Cost (US$ per liter of waste)	10.4	8.2

[a] Assay with *Vibrio fischeri* bacteria.

Characterization of the treated waste was performed by HPLC. The analytical separation was carried out for the waste before and after degradation in the presence of titanium dioxide or potassium persulphate (Fig. 4). It was possible to characterize the peaks of both reagents and the waste generated before treatment (Fig. 4A). After waste degradation using titanium dioxide with exposure to ultraviolet radiation for 10 min, a peak characteristic of the product of carbaryl hydrolysis was observed. However, by increasing the exposure time to 30 min, the total mineralization of the waste was observed (Fig. 4B). By using potassium persulphate, only the peak characteristic of the reagent was identified in the remaining solution after total mineralization of the residue (Fig. 4C).

A critical parameter is the toxicity of the waste before and after the degradation process. Toxicity tests were performed using the marine bacteria *Vibrio fischeri*, which are luminescent, gram-negative and facultative anaerobic. In favorable environmental conditions (pH and salinity of the medium), the bacteria show natural bioluminescence. The test is based on measuring the light emitted by the bacteria after exposure to a sample for 15 min [32]. The intensity of luminescence is compared to a control test, in which the sample has not been added. In the presence of toxic substances, the bioluminescence decreases proportionally to the waste toxicity. A residue is considered non-toxic if the inhibition of luminescence is lower than 20% [37]. The assays were carried out on the residue before and after degradation with titanium dioxide or potassium persulphate. Inhibition levels of 87% and 100% of bioluminescence were found for waste samples before and after treatment with titanium dioxide, respectively. Therefore, the residue treated with TiO_2 and H_2O_2 is considered more toxic to the *Vibrio fischeri* bacteria than the residue without degradation. This is probably due to the presence of H_2O_2 that cannot be completely eliminated during waste treatment and indicates that TOC measurements are not enough to conclude about the viability of waste treatment. According to these results, residues without degradation or treated with titanium dioxide should be three times or five times diluted before discarding. However, for the residue treated with potassium persulphate, inhibition of 19% characterizes the waste as non-toxic to the *Vibrio fischeri* bacteria.

Table 7 summarizes the results obtained with the procedures evaluated for waste degradation. The best results (fast treatment yielding a low toxicity waste) were achieved by using $K_2S_2O_8$ at a 22% lower cost compared to the process using TiO_2.

4. Conclusions

The analytical features of the proposed system with a 100 cm optical path flow cell were superior to those obtained by other procedures based on reaction with p-aminophenol and potassium metaperiodate in an alkaline medium. Furthermore, the procedure was selective for carbaryl with relative responses to the other carbamate pesticides lower than 2%. The determination of this pesticide in natural water samples was possible without interference from the matrix after cloud-point extraction, which avoids the use of organic solvents. The degradation of the generated waste yielded a colorless residue with 94% reduction in total organic carbon after 5 min of exposure to UV radiation. Toxicity tests with marine bac-

teria *Vibrio fischeri* classified the waste as non-toxic and the cost for degradation was estimated as US$ 8.00 per liter of waste.

Acknowledgements

The authors acknowledge fellowships and financial support from the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Companhia Ambiental do Estado de São Paulo (CETESB) is thanked for the toxicity measurements.

References

[1] U.S. Environmental Protection Agency, http://www.epa.gov/, accessed 16 July 2008.
[2] L. Alvarez-Rodríguez, L. Monferrer-Pons, J.S. Esteve-Romero, M.C. García-Alvarez-Coque, G. Ramis-Ramos, Analyst 122 (1997) 459.
[3] C.S.P. Sastry, D. Vijaya, D.S. Mangala, Analyst 112 (1987) 75.
[4] D.C. Portela, I.M.F. Pereira, P. Paíga, C. Delerue-Matos, M.C.V.F. Vaz, Anal. Bioanal. Chem. 377 (2003) 356.
[5] K.D. Khalaf, A. Morales-Rubio, M. de la Guardia, J.M. Garcia, F. Jimenez, J.J. Arias, Microchem. J. 53 (1996) 461.
[6] J. Ferré, R. Boqué, B. Fernández-Band, M.S. Larrechi, F.X. Rius, Anal. Chim. Acta 348 (1997) 167.
[7] N.L. Pacioni, A.V. Veglia, Anal. Chim. Acta 488 (2003) 193.
[8] A. Caballo-López, M.D. Luque de Castro, J. Chromatogr., A 998 (2003) 51.
[9] C. Sánchez-Brunete, A. Rodriguez, J.L. Tadeo, J. Chromatogr., A 1007 (2003) 85.
[10] L. Sun, H.K. Lee, J. Chromatogr. A 1014 (2003) 165.
[11] R.C. Prados-Rosales, M.C. Herrera, J.L. Luque-García, M.D. Luque de Castro, J. Chromatogr., A 953 (2002) 133.
[12] A. Santalad, S. Srijaranai, R. Burakham, T. Sakai, R.L. Deming, Microchem. J. 90 (2008) 50.
[13] W.R. Melchert, F.R.P. Rocha, Int. J. Environ. Anal. Chem. 89 (2009) 969.
[14] B.F. Reis, A. Morales-Rubio, M. de la Guardia, Anal. Chim. Acta 392 (1999) 265.
[15] P. Yánez-Sedeno, C.N. Nova, L.M.P. Díez, Microchem. J. l38 (1988) 370.
[16] K.D. Khalaf, A. Morales-Rubio, M. de la Guardia, Anal. Chim. Acta 280 (1993) 231.
[17] E. Ródenas-Torralba, F.R.P. Rocha, B.F. Reis, A. Morales-Rubio, M. de la Guardia, J. Autom. Methods Manage. Chem. 2006 (2006) 1.
[18] J.A.M. Pulgarín, A.A. Molina, P.F. López, Talanta 68 (2006) 586.
[19] A. Smirnova, K. Mawatari, A. Hibara, M.A. Proskurnin, T. Kitamori, Anal. Chim. Acta 558 (2006) 69.
[20] W. Lei, K. Fujiwara, K. Fuwa, Anal. Chem. 55 (1983) 951.
[21] K. Tsunoda, A. Nomura, J. Yamada, S. Nishi, Appl. Spectrosc. 43 (1989) 49.
[22] T. Dallas, P.K. Dasgupta, Trends Anal. Chem. 23 (2004) 385.
[23] J. Zhang, J. Chi, Environ. Sci. Technol. 36 (2002) 1048.
[24] J. Zhang, Anal. Sci. 22 (2006) 57.
[25] K.O. Lupetti, F.R.P. Rocha, O. Fatibello Filho, Talanta 62 (2004) 463.
[26] P.K. Dasgupta, Z. Genfa, S.K. Poruthoor, S. Caldwell, S. Dong, S. Liu, Anal. Chem. 70 (1998) 4661.
[27] W.R. Melchert, F.R.P. Rocha, Anal. Chim. Acta 616 (2008) 56.
[28] A. Cavicchioli, I.G.R. Gutz, Anal. Chim. Acta 445 (2001) 127.
[29] F.R.P. Rocha, B.F. Reis, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, J.L.M. Santos, Anal. Chim. Acta 468 (2002) 119.
[30] M. Aceituno, C.D. Stalikas, L. Lunar, S. Rubio, D. Pérez-Bendito, Water Res. 36 (2006) 3582.
[31] L. Sepúlveda, F. MacRitchie, J. Colloid Interface Sci. 28 (1968) 19.
[32] Companhia de Saneamento do Estado de São Paulo, http://www.cetesb.sp.gov.br, accessed 27 February 2009.
[33] C.M.C. Infante, F.R.P. Rocha, Microchem. J. 90 (2008) 19.
[34] F.R.P. Rocha, P.B. Martelli, B.F. Reis, Talanta 55 (2001) 861.
[35] O. Legrini, E. Oliveros, A.M. Braun, Chem. Rev. 93 (1993) 671.
[36] J. Golimowski, K. Golimowska, Anal. Chim. Acta 325 (1996) 111.
[37] A.R. Fernández-Alba, D. Hernando, A. Agüera, J. Cáceres, S. Malato, Water Res. 36 (2002) 4255.