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Article

A Chemiluminescence Flow-based Procedure for Determination of Carbaryl in Natural Waters Exploiting Multicommutation and Enzymatic Reaction

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Um procedimento empregando detecção quiluminescente para determinação de carbaril em águas naturais utilizando acetilcolinesterase e colina oxidase é descrito. O módulo de análise projetado para implementar o processo de multicomutação controlado por microcomputador foi constituído por cinco válvulas solenóides, duas colunas com enzimas imobilizadas em esferas de vidro e cela de fluxo para detecção de quimiluminescência. Nas melhores condições experimentais foi obtida faixa linear entre 25 a 700 μg L^{-1} de carbaril. Amostras de água foram fortificadas com carbaril e as recuperações variaram entre 95 e 101%. Limite de detecção e coeficiente de variação foram estimados em 11 μg L^{-1} (99,7% nível de confiança) e 1,3% (n = 20), respectivamente. Outras características analíticas tais como freqüência de amostragem de 72 determinações por hora, consumo de reagente de 2,1 mg de hexacianoferrato de potássio(III) e 0,069 mg de luminol por determinação, também, foram obtidas.

A chemiluminescence procedure for the determination of carbaryl in natural waters using acetylcholinesterase and choline oxidase is described. The flow system designed to implement multicommutation approach controlled by microcomputer comprised five solenoid valves, two columns with immobilized enzymes on controlled pore glass beads and chemiluminometric flow cell. In the best experimental conditions a linear response ranging from 25 to 700 μg L^{-1} carbaryl was obtained. Water samples were spiked with carbaryl in order to access the accuracy and recoveries between 95 and 101% were obtained for a concentration level ranging from 25 to 100 μg L^{-1} carbaryl. Detection limit and variation coefficient were estimated as 11 μg L^{-1} (99.7% confidence level) and 1.3% (n = 20), respectively. Other profitable features such as a sample throughput of 72 determinations *per* hour, a reagent consumption of 2.1 mg potassium hexacyano ferrate(III) and 0.069 mg luminol *per* determination were also achieved.

Keywords: multicommutation, flow analysis, pesticide carbaryl, chemiluminescence, enzymatic reaction

Introduction

The use of pesticides in agriculture has increased dramatically in the two past decades, thus contributing to the environmental contamination mainly soil and surface waters. The carbamate family of pesticides are among those widely used due to the high activity, low bioaccumulation and moderate degradation in the environment, nevertheless some formulations present high toxicity. Carbaryl (1-naphthyl methylcarbamate) commonly known as Sevin, is a carbamate pesticide widely used on agriculture practices as a contact insecticide because it is very effective to prevent numerous insects that infest fruits, vegetables, cotton and many other crops.[1] The potential biological activities of carbaryl and its main hydrolysis metabolite 1-naphthol have been

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investigated[2] and the authors pointed out that toxic effects are caused by the inhibition of the cholinesterase activity. For humans the symptomatic effects caused by carbaryl poisoning are nausea, diarrhoea, bronchoconstriction, blurred vision, excessive salivation, muscle twitching, cyanosis, convulsion, coma and respiratory failure.[3]

The use of the carbaryl represents a potential risk for the environmental contamination and because of that its presence in surface waters should be controlled. To satisfy this requirement, analytical procedures employing as detection techniques amperometry,[4,5] potentiometry,[6,7] conductimetry,[8] spectrophometry[3,9] have been reported. Nowadays, the methods widely used for pesticide determination are based on gas chromatography,[10] high-performance liquid chromatography[11] and gas chromatography coupled with mass spectrometry.[12,13] These detection techniques require expensive equipments, and furthermore, laborious sample preparation step prior to analysis is generally required. The long time consumed to carry out the sample preparation could become a drawback when a lot of samples must be analyzed. In this sense, attention should be focused to search for analytical procedures with high sampling throughput. Nevertheless, to attain this objective the sample-processing steps prior to analysis should be simplified. Analytical procedures based on flow injection approach implemented employing enzymatic reaction and spectrophotometric detection can attain this requirement.[9]

The pesticide concentration in water must be very low and in the Environmental National Agency (CONAMA) administrative rules carbaryl received individual specification, where it was established that the maximum concentration acceptable is 20 μg L^{-1}.[17] In this sense, high sensitivity is a feature that must be provided by the analytical procedure. This requirement could be afforded by analytical procedures based on chemiluminescence.[14,15]

In this context, an enzymatic method for the determination of pesticides using chemiluminescence detection could be proposed based on the reaction of acetylcholine with acetylcholinesterase (AchE) producing choline and acetic acid. Afterwards choline reacts with choline oxidize delivering hydrogen peroxide.[16] The pesticide inhibits the activity of the acetylcholinesterase, thus causing a decrease of the choline production and, as a consequence, reduction of the hydrogen peroxide generation. The reduction effect presents a directly relationship with pesticide concentration in the sample, thus analytical procedures employing chemiluminescence detection based on this feature have been developed.[14,15]

Low reagent consumption is another parameter that should be considered because it affects favorably both the cost of analysis and decreases the waste generation. This requisite could be easily achieved by implementing the analytical procedure in a flow system based on multicommutation process, which afforded ability to handle small volumes of sample and reagent solutions.[18]

In this paper we intend to develop an automatic flow procedure based on multicommutation[19-21] for the determination of carbaryl in water. The procedure is based on the enzymatic reaction and detecting by chemiluminescence using an inexpensive instrument based on photodiode.

Experimental

Apparatus

The equipment set-up consisted of a home-made chemiluminescence detection unit based in the silicon photodiode (71608, Oriel instruments) coupled to a flow cell (78 μL inner volume) machined in acrylic;[22] a 586 microcomputer equipped with a PCL-711S electronic interface card (Advantech Corp); an IPC8 Ismatec peristaltic pump equipped with Tygon pumping tubes; a 12 V regulated power supply for the solenoid valves; a home-made electronic interface to match the voltage and current intensity required to drive the solenoid valves;[18] two mini-columns (10 mm length, 5 mm inner diameter) to pack the glass beads with immobilized enzyme; joint devices machined in acrylic; mixing coils and flow lines of polyethylene tubing (0.8 mm i.d.); and accessories. To allow the synchronization of the pumping pulsation with the solenoid valves switching pattern, the roller count output of the peristaltic pump was coupled to the microcomputer through the analog input (A_1) of the PCL711s interface card.

Reagents

All chemicals were of analytical reagent grade. Freshly purified water presenting conductivity less than 0.1 μS cm^{-1} was used throughout.

Carbaryl (purity 99.5%) was obtained from Bayer CropScience. A fresh stock solution of 1.0 mg L^{-1} of carbaryl was prepared by dissolving 1.0 mg of the pesticide in 70 mL of acetone and made up to 1000 mL with water. Reference solutions within the range 25 to 700 μg L^{-1} carbaryl were daily prepared by appropriated dilutions from the stock solution with water.

Acetylcholinesterase (AchE) (EC 3.1.1.7, from electric eel type V-S, 1430 U mg^{-1}) and choline oxidase (EC 1.1.3.17, alcaligenes species, 13.1 U mg^{-1}) were purchased from Sigma Chemical. Acetylcholine chloride (Sigma) solution of 5.0 mmol L^{-1} was used as substrate. It was

daily prepared by dissolving the solid in a 1.0 mmol L^{-1} KH_2PO_4 buffer solution (pH 7.0).

A 2.0 mmol L^{-1} luminol solution was prepared by dissolving 35.4 mg of 5-amino-2,3-dihydro-1,4-phthalazinedione (Sigma) in 100 mL of a 0.2 mol L^{-1} K_2CO_3 solution with pH adjusted to 10.5 using a 0.1 mol L^{-1} HCl solution. This solution was prepared 24 hours before use and maintained in a freezer at 4 °C.

A 0.1 mol L^{-1} potassium hexacyanoferrate(III) solution was daily prepared by dissolving 3.29 g of salt (Merck) in 100 mL of water. Phosphate buffer solutions 0.1 mol L^{-1} (pH 6.0 and 7.5) and 1.0 mmol L^{-1} (pH 7.0) were prepared by dissolving 13.6 g and 0.136 g of KH_2PO_4 (Merck) in water, respectively. The pH was adjusted with a 0.1 mol L^{-1} NaOH solution and volumes were made up to 1000 mL with water.

Water samples (volume 1000 mL) were collected from Corumbataí River (São Paulo, Brazil) and stored at 4 °C. Prior to analysis the samples were left to reach the laboratory temperature (22 °C) and filtered using membrane filters with pore size of 0.45 μm.

Enzyme immobilization

The AchE and choline oxidase enzymes were immobilized on aminopropyl glass beads (200-400 mesh, 170 Å pore size) as previously described.[23] About 0.2 g of glass beads were activated by maintaining in a 2.5% (v/v) glutaraldehyde solution for 1 hour under stirring with brief nitrogen deoxygenation every 10 min for the first 30 min. Afterwards, the glass beads were washed with water and with phosphate buffer solution (pH 7.0).

AchE (1 mg, 1430 U) was dissolved in 3.0 mL of 0.1 mol L^{-1} phosphate buffer solution (pH 6.0). This solution was added to the activated glass beads and was maintained at 4 °C for 20 hours to complete the immobilization step. The glass beads were washed first with cold phosphate buffer solution and then with cold water to remove the unlinked enzyme. The choline oxidase was immobilized following a similar procedure using a mass of 2.31 mg, 30 U.

Water slurry of the beads with immobilized enzymes was inserted into the column by using a needle-less syringe. When the columns were not in use, they were rinsed with phosphate buffer solution (pH 7.5) to fill dead volume and stored at 4 °C.

Procedure

The flow set-up was designed to implement the multicommutation approach and the diagram is depicted in Figure 1. In this configuration, the system is in the stand by condition assigned as Sb in the valves timing course. Under this condition, all valves are switched off and carrier solution (Cs) flows through valve V_1, enzymatic columns (C_1, C_2) and chemiluminescence detector (Det) towards waste (W). Sample (S), substrate (R_1) and reagents (R_2, R_3) solutions are pumped towards their storing vessels (VS, VR_1, VR_2, VR_3).

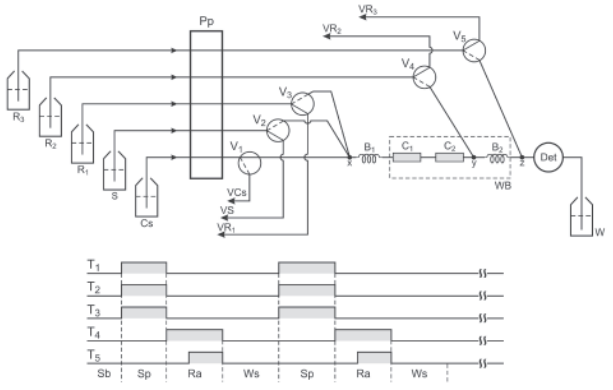

Figure 1. Flow diagram of the system. V_1, V_2, …,V_5 = three-way solenoid valves; *S* = sample solution, flow rate 17 μL s^{-1}; C_s = carrier phosphate buffer solution, flow rate 27 μL s^{-1}; R_1 = acetylcholine chloride solution, flow rate 5 μL s^{-1}; R_2 = luminol solution, flow rate 13 μL s^{-1}; R_3 = hexacyano ferrate (III) solution, flow rate 13 μL s^{-1}; *x, y, z* = joint devices; C_1 and C_2 = acetylcholinesterase and choline oxidase columns, respectively; B_1 and B_2 = coiled reactors, 50 cm long, 0.8 mm i.d.; WB = water bath; Det = chemiluminescence detector; *W* = waste; VS, VCs, VR_1, VR_2 and VR_3 = solutions storing vessels. T_1, T_2,…,T_5 = timing course of valves V_1, V_2, …,V_5, respectively; Sb = stand by condition; Sp = sampling step; Ra = reagent additions step; Ws = washing step. The shadow surfaces beneath the timing course lines indicate that the corresponding valves are switched on. Solid lines into the valve symbols indicate the actual flow pathway when valves were maintained off. Dashed line indicates the flow pathway when valve is switched on.

When the microcomputer runs the software, it was necessary to wait for the synchronization signal generated by peristaltic pump roller count, which was read through analog input (A_1) of the PCL711S interface card. Afterwards, the microcomputer sent a sequence of electric pulses through the digital output of the PCL711S interface to switch on/off the valves V_1, V_2, V_3, V_4 and V_5 as depicted in the valves timing course (Figure 1). In the first step (Sp), valves V_1, V_2 and V_3 are switched on at the same time, thus carrier solution was directed towards its storing vessel (VCs). Under this condition, solutions of sample (S) and acetylcholine (R_1) merged into column C_1 that was packed with AchE, thus allowing the development of the reaction of the substrate (R_1) with the immobilized enzyme producing choline and acetate. This operational sequence was settled considering the reaction conditions pointed out.[15] The formed choline reacted with choline oxidase into the column C_2 generating hydrogen peroxide. Carbaryl inhibited the reaction between acetylcholine and acetylcholinesterase, thus causing a decrease in the

generation of choline and hydrogen peroxide. Afterwards, valves V_1, V_2 and V_3 were switched off, solutions S and R_1 were pumped towards the storing vessels (VS, VR_1) and carrier solution flowed again through the columns. The step (Ra) settled for reagents addition was carried out by switching valves V_4 and V_5 on as indicated in the valves timing course (Figure 1). Under this condition, luminol and potassium hexacyanoferrate(III) solutions merged with the sample zone at the joint devices *y* and *z*, respectively. As indicated by the shadow surface in the valves timing course a delay time (5 s) between the switching valves V_4 and V_5 was settled in order to save the hexacyanoferrate(III) solution (R_3).

The peroxide generated by the enzymatic reaction in the sampling step (Sp) reacted with luminol catalysed by hexacyanoferrate(III) generating electromagnetic radiation with a λ_{max} around 420 nm. In order to assure that light emission occurred into the flow cell the catalyzing solution was added to the sample zone (joint z) 10 mm far from of the flow cell input. While the *Ra* step was carried out the signal generated by the photodiode was read through the analog input of the PCL711S interface. The digital values were stored as ASCII file to permit further treatment. At the same time, a plot of the signal as a time function was displayed in the microcomputer screen to allow its visualization in real time. As showed in the valves timing course, the next analytical run should begin after the washing step (Ws).

Flow rates of the carrier (Cs), sample (S), acetylcholine chloride solution (R_1), luminol (R_2) and potassium hexacyanoferrate(III) (R_3) solutions were maintained at 27, 17, 5, 13 and 13 μL s^{-1}, respectively. Thus, to find the best condition experiments were carried out using concentrations ranging from 0.05 to 0.4 mol L^{-1} hexacyanoferrate(III) solution, 1.5 to 4.5 mmol L^{-1} luminol solution and 0.5 to 10 mmol L^{-1} acetylcholine chloride solution. The experimental variables studied were the volume of the solution aliquots, which were done by changing the time interval to maintain switched the corresponding valve on, the temperature of the water bath (Figure 1) in the 15 to 45 °C range and the pH of the carrier solution that was changed from 6.0 to 8.0. These experiments were carried out using a set of carbaryl standard solutions with concentration ranging from 25 to 700 μg L^{-1}.

Measurements were based on the chemiluminescence emission caused by the reaction between hydrogen peroxide and luminol. Because pesticide caused a decrease of the hydrogen peroxide generation, the maximum signal was recorded without the insertion of the carbaryl solution. In this sense, the luminescence detector was adjusted prior to begin the analytical run and it was done by simultaneously switching valves V_1, V_3 V_4 and V_5 on. After a time interval of 60 s, enough to obtain a constant measurement, the readout signal was adjusted to 200 mV. This step was carried out everyday prior to begin the work.

After definition of the experimental variables, a set of water samples collected at several points of the Corumbataí River was analyzed. The water samples were spiked with 25, 50, 75 and 100 μg L^{-1} carbaryl solutions to allow the accuracy assessment. To verify the system robustness it was ran during four hours every day for one week using a set of pesticide standard solutions maintaining the laboratory temperature at 22 °C.

Results and Discussion

In the next section will be commented the assays carried out involving parameters, such as, sample and reagent volumes, reagent concentration, pH of the carrier solution, reaction coil length and temperature of the water bath, which were selected in order to find the best conditions for maximum light emission and better signal reproducibility.

Effect of sample and reagent volumes

The effects of sample (S) and substrate solution (R_1) volumes on the analytical signal were investigated by varying the time intervals to switch on valves V_2 and V_3 (Sp step, Figure 1) from 2.0 to 30 s. Under this condition, the volume of sample solution that flowed through the enzymatic columns was varied from 34 to 510 μL, while the volume of substrate solution was changed from 10 to 150 μL. From data of Table 1 one can deduce that the ratio between solutions aliquot was maintained at 3.4. The measurements displayed in this Table show that the maximum signal was recorded when the volumes of the aliquots were 255 and 75 μL for

Table 1. Effect of the sample and substrate volumes on the luminescence signal. Standard solution concentration = 200 μg L^{-1} carbaryl, flow rate 17 μL s^{-1}; luminol solution concentration = 2.0 mmol L^{-1}, flow rate 13 μL s^{-1}; potassium hexacyano ferrate(III) solution concentration = 0.1 mol L^{-1}, flow rate 13 μL s^{-1}; acetylcholine chloride solution, concentration = 8 mmol L^{-1}; flow rate =5 μL s^{-1}; temperature = 22 °C. Results average of 3 consecutive reference solution processing

Volume of sample/μL	Volume of substrate/μL	Signal/ mV
34	10	115.5 ± 1.2
85	25	129.7 ± 1.5
170	50	135.1 ± 0.8
255	75	148.3 ± 1.5
340	100	129.8 ± 1.7
425	125	117.3 ± 2.1
510	150	110.6 ± 2.1

sample and substrate solutions, respectively. These volumes were selected for the further experiments.

The addition of the luminol (R_2) and hexacyanoferrate(III) (R_3) solution to sample zone was controlled by varying the time intervals to switch on valves V_4 and V_5 from 5 to 25 s and from 3 to 20 s, respectively. Considering that both flow rates were maintained at 13 μL s^{-1}, the volumes of luminol and hexacyano ferrate(III) solutions varied from 65 to 325 μL and from 39 to 260 μL, respectively. Considering the results showed in Table 2 we could observe that the maximum signal was achieved when the solutions volumes were 195 μL and 65 μL for R_2 and R_3, respectively.

Table 2. Effect of the reagent volumes on the luminescence signal. Standard solution concentration = 200 μg L^{-1} carbaryl, flow rate = 17 mL s^{-1}; luminol solution concentration = 2.0 mmol L^{-1}; 13 μL s^{-1}; potassium hexacyanoferrate(III) solution concentration = 0.1 mol L^{-1}, flow rate = 13 μL s^{-1}; acetylcholine chloride solution concentration = 8 mmol L^{-1} ; flow rate = 5 μL s^{-1}; temperature = 22 °C. Results average of 3 consecutive reference solution processing

luminol (R_2)/μL	hexacyanoferrate III (R_3)/μL	Signal/mV
65	39	113.2 ± 2.1
130	39	125.7 ± 1.3
195	39	142.3 ± 2.1
260	39	120.1 ± 1.3
325	39	113.8 ± 1.3
195	65	159.7 ± 2.5
195	130	137.2 ± 2.2
195	195	126.4 ± 1.7
195	260	118.8 ± 1.1

Effect of reagent concentration

The luminol concentration was changed from 1.5 up to 4.5 mmol L^{-1} maintaining the concentration of potassium hexacyanoferrate(III) solution at 0.2 mol L^{-1}. When luminol concentration was changed between 1.5 and 2.0 mmol L^{-1} the electrical signal related to light emission delivered inserting blank solution increased from 92 to 110 mV. No significant increase was observed using solutions with concentration higher than 2.0 mmol L^{-1}, thus indicating that a 2.0 mmol L^{-1} luminol concentration was enough to satisfy the stoichiometry of the reaction.

The measurements obtained by varying the potassium hexacyanoferrate(III) concentration from 0.05 to 0.1 mol L^{-1} increased from 65 to 140 mV. Nevertheless, using concentration higher than 0.1 mol L^{-1} a remarkable decrease was observed. In this sense, the solution 0.1 mol L^{-1} potassium hexacyanoferrate(III) was selected. This set of assays was performed using the blank solution and a 2.0 mmol L^{-1} luminol solution.

To verify the effect of the substrate (acetylcholine) on the signal magnitude, assays were carried out varying its concentration from 1 to 10 mmol L^{-1} yielding the results showed in Figure 2. As we can see the maximum measurement was recorded when substrate concentration was about 5 mmol L^{-1}, therefore this value was chosen to carring out additional assays.

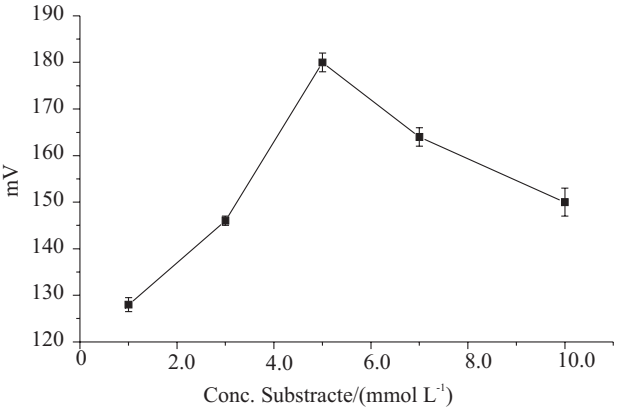

Figure 2. Effects of acetylcholine concentration on the analytical signal. Standard solution, concentration = 200 mg L^{-1} carbaryl, flow rate 17 μL s^{-1}; luminol solution, concentration = 2.0 mmol L^{-1} (13 μL s^{-1}); potassium hexacyano ferrate(III) solution, concentration = 0.1 mol L^{-1} (13 μL s^{-1}); carrier phosphate buffer solution, concentration = 0.1 mol L^{-1} (27 μL s^{-1}); temperature = 22 °C. Results average of 3 consecutive reference solution processing.

Effects of pH and temperature

The experiments commented in previous section were done by using a phosphate buffer solution at pH 7.0. Because enzymatic reaction could be affected by the pH and temperature of the reaction medium, experiments were carried out varying these parameters yielding the results showed in Table 3. Considering these data, the carrier solution was maintained at pH 7.0. Better results were obtained when temperature was around 22 °C, which was the temperature usually settled to the laboratory, thus

Table 3. Effect of pH and temperature on the enzymatic activity. Standard solution concentration = 200 μg L^{-1} carbaryl, flow rate = 17 μL s^{-1}, luminol solution concentration = 2.0 mmol L^{-1}, flow rate = 13 μL s^{-1}; potassium hexacyano ferrate(III) solution concentration = 0.1 mol L^{-1}, flow rate = 13 μL s^{-1}; acetylcholine chloride solution concentration = 5 mmol L^{-1}, flow rate = 5 μL s^{-1}. Results average of 3 consecutive reference solution processing

pH	Temperature/(°C)	Signal/mV
6.0	22	112.3 ± 1.8
6.5	22	121.5 ± 1.0
7.0	22	143.3 ± 1.6
7.5	22	136.0 ± 1.3
8.0	22	121.2 ± 0.8
7.0	15	119.8 ± 2.0
7.0	22	143.7 ± 1.3
7.0	30	130.4 ± 1.7
7.0	35	118.1 ± 1.2
7.0	45	97.6 ± 1.2

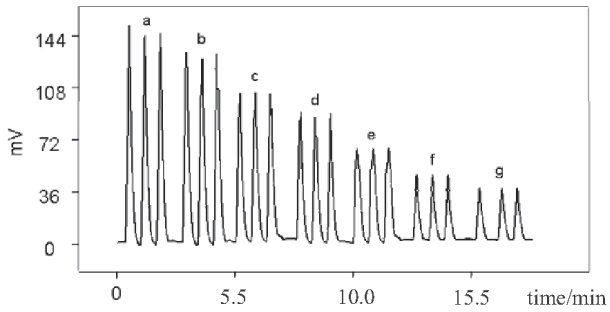

Figure 3. Signal records of the reference solutions. From *a* to *g* references solutions with 0, 25, 50, 100, 200, 400 and 700 mg L^{-1} carbaryl. Luminol solution, concentration = 2.0 mmol L^{-1}, flow rate 13 μL s^{-1}; potassium hexacyanoferrate(III) solution, concentration = 0.1 mol L^{-1} , flow rate 13 μL s^{-1}; acetylcholine chloride solution, concentration = 5 mmol L^{-1}, flow rate 5 μL s^{-1}; carrier phosphate buffer solution concentration = 0.1 mol L^{-1} (27 μL s^{-1}), temperature = 22 °C, replicates = 3.

permitting to work without the use of water bath to control temperature of the flow system.

Analytical parameters

The overall system performance was ascertained by processing a set of carbaryl standard solutions and water samples fortified with four concentration levels of carbaryl. The records of Figure 3 show that the precision of the measurements was very good. No significant baseline drift occurred, thus indicating that the system was very stable. This figure shows that the time elapsed to carry out 18 determinations was 15 min, therefore a sampling throughput of 72 determinations *per* hour could be easily attained. For analyte concentrations ranging from 25 up to 700 μg L^{-1} carbaryl a linear response (R = 0.995, n = 7) characterized by the equation, Signal (mV) = (0.879 ± 0.011) - (24.242 ± 1.21) × (× = μg L^{-1} carbaryl) was achieved.

The applicability of the proposed procedure was ascertained by processing a set of natural water samples, which were fortified with different concentrations of carbaryl yielding the results presented in Table 4. Recoveries ranging from 95 to 102% were achieved, thus indicating that the proposed system can be employed to monitor this pesticide in surface waters. Other profitable features, such as, a relative standard deviation of results of 1.3% (n = 20), reagent consumption 0.069 mg luminol and 2.1 mg potassium hexacyanoferrate(III) *per* determination and a detection limit of 11 μg L^{-1} at the

Table 4. Carbaryl determination in spiked water samples. Standard solution, concentration = 200 μg L^{-1} carbaryl, flow rate = 17 μL s^{-1}; luminol solution concentration = 2.0 mmol L^{-1}, flow rate = 13 μL s^{-1}; potassium hexacyanoferrate(III) solution concentration = 0.1 mol L^{-1}, flow rate = 13 μL s^{-1}; acetylcholine chloride solution, concentration = 5 mmol L^{-1}, flow rate = 5 μL s^{-1}; temperature = 22 °C. Results average of 3 consecutive reference solution processing

Sample	Carbaryl/(μg L^{-1})	SpikedCarbaryl/(μg L^{-1})	Carbaryl Found/(μg L^{-1})	Recovery/(%)	Average/(%)
1	ND	25	25.5	102	
1	ND	25	24.8	99.2	
1	ND	25	25.7	102.4	101.2 ± 1.7
2	ND	50	49.6	98.6	
2	ND	50	48.2	96.4	
2	ND	50	49.4	98.4	97.8 ± 1.2
3	ND	75	73.8	98.4	
3	ND	75	73.4	97.9	
3	ND	75	75.4	100.5	98.9 ±1.4
4	ND	100	99.2	99.2	
4	ND	100	98.4	98.4	
4	ND	100	95.1	95.1	97.6 ± 2.2

ND: not detected.

Table 5. Figures of merit of the proposed method and chemiluminescence based procedures[14,15]

Parameters	Proposed method	Reference 14	Reference 15
Sample consumption *per* determination/μL	255	70	60
Solutions consumption *per* hour/mL			
Carrier solution	97	300	6
Luminol solution	47	300	12
Catalyst solution	47	180	Not indicated
Waste generated *per* determination *per* hour/mL	270	780	18
Linear range/(μg L^{-1})	25-700	5-100	0-75
Correlation coefficient (r)	0.995	0.998	0.995
Limit of detection/(μg L^{-1})	11	4.9	4
Throughput/h^{-1}	72	Not indicated	15
RSD/(%)	1.3	2	3.7

99.7% confidence level were also achieved. Furthermore, maintaining the established operational conditions, the immobilized enzymes could be used up to 60 days allowing 1200 determination.

Aiming to demonstrate the effectiveness of the proposed procedure, the analytical parameters presented in Table 5 were compared with those of reference chemiluminescence methods.[14,15] An overall comparison would be favorable to proposed procedure, presenting as advantage, a wider linear response range and higher throughput.

Conclusions

The flow system is low cost, fast and simple to operate and does not require any sample pre-treatment (except filtration), thus minimizing errors that could be caused by sample manipulation.

Chemiluminescence detection unit presented a good working performance, which could be attained using inexpensive instrumentation. The low reagent consumption and, consequently, waste generation are other additional advantages, which were easily attained exploiting the facilities provided by the multicommutation approach.

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References

1. Mathew, L. ; Reddy, M.L.P. ; Rao, T.P. ; Iyer, C.S.P. ; Damodaran, A. D. ; *Analyst* **1995**, *120*, 1799.
2. Gosselin, R.E.; Powell, S.R.; Hodge, H.C.; Smith, R.P.; Gleaon, M.V.; *Clinical Toxicology of Commercial Products*, 4th ed., Williams and Wilkins: Baltimore, Sect. III, 1976.
3. Khalaf, K.D.; Morales-Rubio, A.; de la Guardia, M.; *Anal. Chim. Acta* **1993**, *280*, 231.
4. Budnikov, H.C.; Evtugyn, G.A.; *Electroanalysis* **1996**, *8*, 817.
5. Martorell, D.; Céspedes, F.; Martinez-Fabregas, E.; Alegret, S.; *Anal. Chim. Acta* **1994**, *290*, 343.
6. Ivanov, A.N.; Evtugyn, G.A.; Gyurcsány, R.E.; Tóth, K.; Budnikov, H.C.; *Anal. Chim. Acta* **2000**, *404*, 55.
7. Jaffrezic-Renault, N.; Semillou, A.; Martelet, C.; Wan, K.; Chovelon, J.M.; *Sens. Actuators B* **1999**, *59*, 154.
8. Dzydevich, S.V.; Shul'ga, A.A.; Soldatkin, A.P.; Nyami Hendji, A.M.; Jaffrezic-Renault, N.; Martelet, C.; *Electroanalalysis* **1994**, *6*, 752.
9. Danet, A.F.; Bucur, B.; Cheregi, M.C.; Badea, M.; Serban, S.; *Anal. Lett.* **2003**, *36*, 59.
10. Hernandez-Romero, A.H.; Tovilla-Hernandez, C.; Malo, E.A.; Bello-Mendoza, R.; *Mar. Pollut. Bull.* **2004**, *48*, 1130.
11. Kot-Wasik, A.; Dabrowska, D.; Kartanowicz, R.; Namiesnik, J.; *Anal. Lett.* **2004**, *37*, 545.
12. Bao, M.L.; Pantani, F.; Barbieri, K.; Burrini, D.; Griffini O.; *Int. J. Environ. Anal. Chem.* **1996**, *64*, 233.
13. Parilla, P.; Martinez Vidal, J.L.; *Chromatographia* **1996**, *43*, 265.
14. Huertas-Pérez, J.F.; García-Campaña, A.M.; Gámiz-Gracia, L.; González-Casado, A.; Iruela, M.O.; *Anal. Chim. Acta* **2004**, *524*, 161.
15. Roda, A.; Rauch, P.; Ferri, E.; Girotti, S.; Ghini, S.; Carrea, G.; Bovara, R.; *Anal. Chim. Acta* **1994**, *294*, 35.
16. Karousos, N. G.; Aouabdi, S.; Way, A. S.; Reddy, S. M.; *Anal. Chim. Acta* **2002**, *469*,198.
17. Ministério do Meio Ambiente, Conselho Nacional do Meio Ambiente-CONAMA, Resolução No. 357 de 17 de março de 2005 (http://www.mma.gov.br/port/conama/res/res05/res35705.pdf, in portuguese).
18. Reis, B.F.; Giné, M.F.; Zagatto, E.A.G.; Lima, J.L.F.C.; Lapa, R.A.S.; *Anal. Chim. Acta* **1994**, *293*, 129.
19. Kronka, E.A.M.; Paim, A.P.S.; Tumang, C.A.; Latanze, R.; Reis, B.F.; *J. Braz. Chem. Soc.* **2005**, *16*, 46.
20. Pires, C.K.; Martelli, P.B.; Reis, B.F.; Lima, J.L.F.C.; Saraiva, M.L.M.F.S.; *J. Agric. Food Chem.* **2003**, *51*, 2457.
21. Rocha, F.R.P.; Martelli, P.B.; Reis, B.F.; *J. Braz. Chem. Soc.* **2004**, *15*, 38.
22. Stieg, S.; Nieman, T.A.; *Anal. Chem.* **1978**, *50*, 401.
22. Leon Gonzalez, M.E.; Townshend, A.; *Anal. Chim. Acta* **1990**, *236*, 267.

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