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Chemiluminescence determination of carbofuran at trace levels in lettuce and waters by flow-injection analysis

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Abstract

A simple, fast chemiluminescence (CL) flow-injection (FI) method based on the reaction of luminol with $KMnO_4$ in alkaline medium has been described for the direct determination of carbofuran. The method is based on the enhancing effect in the emission light from the oxidation of luminol produced in presence of carbofuran. The optimisation of instrumental and chemical variables influencing the CL response of the method has been carried out by applying experimental design, using the proposed flow-injection manifold. Under the optimal conditions, the CL intensity was linear for a carbofuran concentration over the range of 0.06–0.5 μg ml^{-1}, with a detection limit of 0.02 μg ml^{-1}. The method has been successfully applied to the determination of carbofuran residues in spiked water and lettuce samples.

Keywords: Carbofuran; Chemiluminescence; Luminol; Flow-injection analysis

1. Introduction

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a broad-spectrum pesticide belonging to the *N*-methylcarbamate group. These pesticides are biodegradable and have low soil persistence. They are degraded in water by hydrolysis, biodegradation, oxidation, photolysis, biotransformation and metabolic reactions in living organisms [1,2]. However, some carbamate pesticides are suspected to be endocrine disruptors [3,4]. Because these chemicals in minute quantities affect to human body, very low concentrations of these pesticides in the environment need to be measured. Carbofuran products are used worldwide for control of insects on a wide variety of fruit, vegetables, forage, cotton and other crops including bananas, coffee beans, grapes, potatoes, corn, rice, sugarcane, and wheat [5,6].

Currently, methods to monitor *N*-methylcarbamates in several types of matrices usually require sample enrichment, followed by high-performance liquid chromatography (HPLC). UV–vis detection and post column reaction-derivatization followed by fluorescence detection are widely used as detection tool in HPLC [7,8]. Gas chromatography (GC) has been less used, since these compounds are somewhat polar and thermally unstable. Different authors propose methods based on GC with derivatization reactions [9,10], but it seems that the direct determination of carbamate pesticides is possible at very low concentrations [11,12]. Recently, mass spectrometry (MS) is becoming the detection system of choice for liquid chromatography, and has been applied for the monitoring of traces of this pesticide in fruits, vegetables [13] and water samples [14].

Chemiluminescence (CL) is a luminescence technique showing as main advantages its high sensitivity, easy of use and simple instrumentation, being actively applied for detection of small amounts of chemical species at ultra-trace levels [15]. Considering the kinetic characteristics of this technique,

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it can be easily coupled to a flow-injection (FI) manifold as detection mode [16]. However, in pesticide residue control only few contributions have appeared using this detection mode, and particularly for the determination of carbamates, two different CL systems have been proposed, based on the tris (2,2′-bipyridyl)ruthenium(III) reaction [17,18] and on the peroxyoxalate CL oxidation in presence of a fluorophore [19]. The CL reaction based on the oxidation of luminol in alkaline medium by different oxidants, such as, hydrogen peroxide, molecular oxygen, hypochlorite, iodide or permanganate, mainly in the presence of some type of initiator or catalyst such as peroxidase, ferricyanide, heme compounds or metal ions (Co(II), Cu(II), Cr(III), Ni(II), etc.) has been only used to the determination of dithiocarbamate fungicides due to the CL emission produced by some of these fungicides with luminol in presence of hexacyanoferrate(III) as a catalyst/cooxidant and hexacyanoferrate(II) as an emission depressor in an alkaline solution without hydrogen peroxide [20]. This CL reaction has been used for the enzyme-linked inhibitory CL detection of carbofuran and other organophosphorous pesticides, using two consecutive enzymatic reactions of choline oxidase and peroxidase which produce photons when luminol is used as a substrate [21]. In this case, the pesticides inhibit the acetylcholinesterase activity, so the acetylcholine is not hydrolised in choline and subsequently it is not oxidized into hydrogen peroxide, blocking the oxidation of luminol and decreasing the CL emission. This method has been reported only for the analysis of tap water at $\mu g\,l^{-1}$ level. The method is highly sensitive and selective but the enzymes employed are very expensive and unstable.

In this study, we have found that the pesticide carbofuran produces a great enhancement on the CL emission from the luminol oxidation by potassium permanganate in alkaline medium without catalyst. This enhancement in the CL emission is proportional to the concentration of the studied compound, which can be determined by measuring the increase in the CL intensity. Based on these findings, a simple and fast new direct FIA-CL method has been developed for the determination of carbofuran, which has been satisfactorily applied in different natural waters and in vegetal food.

2. Experimental

2.1. Apparatus

The manifold used to deliver all solutions is shown in Fig. 1. A peristaltic pump (Gilson Minipulse-3) was used to drive the carrier and CL reagent streams through the flow system. Each stream was pumped at a constant flow rate of $4\,ml\,min^{-1}$. The sample solution was injected into the carrier stream of sodium hydroxide using a Rheodyne 5020 injection valve. The intensity of CL emission was measured with a Camspec CL-1 detector (Camspec), equipped with a quartz flow-cell (120 μl, volume), data control and acquisition software. A rotavapor (Büchi RE 121, Büchi Laboratoriums-Technik AG), a vacuum manifold system (Supelco) and a domestic blender (Taurus) were used for sample preparation.

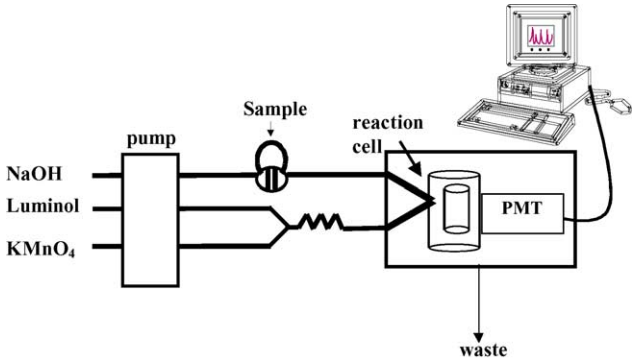

Fig. 1. Schematic FIA manifold. PMT: photomultiplier tube.

2.2. Chemicals and reagents

All the reagents used were of analytical grade and solvent of HPLC grade. The water used to prepare the solutions was purified with a Milli-Q Plus water system (Millipore Iberica).

A 1 mM stock solution of luminol (3-aminophthalhydrazide), purchased from Sigma, was prepared in 0.1 M sodium hydroxide (98% Panreac) and stored at least three days in refrigerator to attain stability before use [22,23]. This solution is stable at least for one week.

A 1 mM stock solution of potassium permanganate (98%, Panreac) was prepared by dissolving the product in water and the corresponding working solutions were prepared daily by appropriate dilutions. A stock standard solution of carbofuran ($100\,mg\,l^{-1}$) was prepared by dissolving 2.5 mg of the product (99%, ChemService) in 25 ml of methanol (Panreac). Working standard solutions were prepared by dilution in water.

Filter paper (0.45 μm pore size, Millipore), ethyl acetate (Panreac), anhydrous sodium sulphate (Panreac) and alumina (Merck) homemade cartridge (1.5 g) were used for sample preparation.

2.3. General procedure and possible mechanism

The FIA configuration consisted on a three-channel manifold where either the standard or sample solutions were incorporated to a NaOH solution carrier with the aid of a manual injection valve (Fig. 1). Prior to the CL measurement acquisition corresponding to the solutions containing carbofuran, the alkaline luminol solution stream was mixed with the permanganate solution stream in a three-way “T” connector. The resulting stream was then mixed with the NaOH carrier solution into the detection cell and the CL emission resulting from the oxidation of luminol was recorded as the background blank signal (baseline). Solutions containing carbofuran were injected into the NaOH stream and allowed to mix with the luminol–$KMnO_4$ mixture in the CL detector.

The determination of carbofuran was based on the increase in the CL intensity, calculated as $\Delta I = I - I_0$, where I is the net CL signal of the system in presence of carbofuran and I_0 is the CL intensity corresponding to the baseline.

The CL mechanism of luminol system has been extensively investigated and many research works have confirmed that 3-aminophthalate anion was the emitter in luminol system, regardless of the medium and oxidant being used [24]. There are some papers about the role of other pesticides (as organophosphorous pesticides) in the luminol reaction [25,26], producing a similar effect that this one observed in our study, but as far as we know, the role of the carbamates in this reaction has not been studied in deep. Nevertheless, based on the discussions proposed in the literature for these CL direct systems using luminol, and considering our previous studies of the reaction using different oxidants, a possible mechanism of present reaction could be as follows: carbofuran is firstly oxidized by permanganate, to produce an intermediate which could subsequently oxidized luminol to form the excited state 3-aminophthalate anion; finally this excited anion decayed to the ground state and produce CL.

2.4. Sample preparation

For the application of the method, water samples of different origins were filtered through a filter paper and collected in a glass bottle previously cleaned with nitric acid. The samples were stored in the dark at 4 °C until analysis.

For the determination of carbofuran in vegetable, portions of lettuce sample (20 g) were spiked with different amounts of carbofuran (2–4 mg l^{-1}). After 5 min, samples were chopped and homogenized for 5 min with 35 ml of ethyl acetate and 30 g of anhydrous sodium sulphate. The mixture was filtered through a filter paper with vacuum and collected in a flask. A 15 ml volume of ethyl acetate was used to clean both the filter and flask. The obtained solution was concentrated in a rotavapor to a low volume (2 ml), and then 3 ml of ethyl acetate were added. The obtained extract was passed through an alumina cartridge, containing 1.5 g of alumina, which was previously conditioned with 3 ml of methanol and 3 ml of water. The sample was eluted and collected in a glass vial. The ethyl acetate extract was evaporated under nitrogen stream until dryness. The residue was re-dissolved with 2 ml of methanol and analysed in the FIA–CL system.

3. Results and discussion

3.1. Optimisation of the method

The optimisation of the method was carried out by application of the experimental design and surface response methodologies for those factors, chemical and instrumental, affecting the analytical response. This multivariate optimisation implies two steps: (a) a preliminary evaluation, using a screening factorial design, in order to select the significant factors in the analytical procedure and (b) an appropriate estimation of the real functional relationship (response function) between the analytical response (CL intensity) and the significant factors, so as to obtain their optimum values. The analysis of the data was carried out using the Statgraphics software [27].

3.1.1. Screening of the selected variables

In order to check the effect of varying the concentration of the each solution, the flow rate of the three channels and the length from the mixing "T" to the detector on the CL intensity, a 2^{7-2} screening design plus three central points was carried out, selecting the levels showed in Table 1 to define the experimental domain. The measurements were carried out using a 1 μg ml^{-1} carbofuran concentration solution, and an injection loop of 70 μl. Considering the enhancement on CL intensity as the analytical response of the model, the total effects of the different variables as well as their second order interactions were evaluated. The study showed that sodium hydroxide concentration, luminol concentration and potassium permanganate flow rate as well as their second order interactions were significant factors affecting the response when their values change into the selected region. The second order interaction between sodium hydroxide flow rate and reactor length was also significant. However, this interaction was not considered in further studies as, under a chemical point of view, it has no sense because the sodium hydroxide channel is independent from the distance between the mixing "T" and the detector, that is, the reactor length. For non-significant variables, the nominal values of the cero level (central value of the experimental region) were selected for further studies, corresponding to 4.0 ml min^{-1} for sodium hydroxide and luminol flow rates, 1×10^{-5} M for potassium permanganate concentration and 50 cm (0.5 mm i.d.) for the reaction coil length.

Table 1
Studied factors and selected levels used in the screening of variables in the experimental region

Variable	Low (−1)	High (+1)
log[Sodium hydroxyde concentration (mol l^{-1})]	log 0.03 = −1.5	log 0.3 = −0.5
log[Luminol concentration (mol l^{-1})]	log 5×10^{-6} = −5.3	log 5×10^{-5} = −4.3
log[Potassium permanganate concentration (mol l^{-1})]	log 3.2×10^{-6} = −5.5	log 3.2×10^{-5} = −4.5
Length of the reactor (cm)	0	100
Sodium hydroxide flow rate (ml min^{-1})	2.5	5.5
Luminol flow rate (ml min^{-1})	2.5	5.5
Potassium permanganate flow rate (ml min^{-1})	2.5	5.5

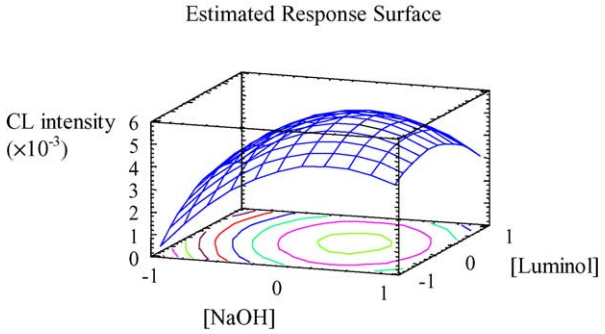

Fig. 2. Response surface for luminol and sodium hydroxide solution concentrations in the optimisation of the factors affecting the FIA system by using a 2^3 central composite plus centred face star design (with three central points). The concentrations are expressed in coded values, which correspond to an interval of 1×10^{-5} to 2×10^{-5} M and 0.1–0.5 M for luminol and sodium hydroxide concentrations (levels −1 and +1), respectively.

3.1.2. Optimisation of the significant variables

In order to optimise simultaneously the significant factors, a 2^3 central composite plus centred face star design (with three central points) was applied. With the aim of obtaining more accurate optimum values and taking into account the results obtained in the previous screening, the experimental domain for the studied variables was reduced. Thus, the concentration of luminol was studied in the range of 1×10^{-5} to 2×10^{-5} M and the sodium hydroxide concentration in the range of 0.1–0.5 M. The total flow rate for the potassium permanganate stream was varied over the range of 2–4 ml min^{-1}. The optimum obtained conditions were 0.39 and 1.46×10^{-5} M for sodium hydroxide and luminol concentrations, respectively, and a 4 ml min^{-1} flow rate for the potassium permanganate stream. One of the estimated response surfaces is shown in Fig. 2. According to these results, further experiences with increasing potassium permanganate flow rates were required, as the optimum value was just in the upper limit of the selected experimental domain. Thus, a univariate study was developed testing flow rates in the range of 4–5.5 ml min^{-1}. Finally a flow rate of 4 ml min^{-1} was selected, as higher flow rates did not enhance the CL intensity.

Under these optimum conditions the effect of the injection volume was examined over the range of 20–150 μl, using a 0.25 μg ml^{-1} carbofuran solution, which assured a non-saturated signal in all cases. An injection volume of 50 μl was selected as optimum as it provided the best signals in terms of both repeatability and CL peak height. The final selected values for the studied variables are summarised in Table 2.

3.2. Analytical performance characteristics

Under the optimal conditions above described and using the proposed FIA manifold, the calibration curve for the determination of carbofuran was established by triplicate injections of analyte concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 μg ml^{-1}. Using least square regression, CL intensity (I_{CL}) was proportional to the carbofuran concentration (C) over the studied range ($I_{CL} = -92.00 + 6101.76C$) with a determination coefficient, $R^2 = 99.84\%$. The performance characteristics, calculated from the calibration data set [28] provided a linear dynamic range from 0.06 to 0.5 μg ml^{-1}, an analytical resolution of 0.01 μg ml^{-1}, a linearity (expressed as relative standard deviation of slope) of 99.07%, a precision of 1.09% (expressed as relative standard deviation for 0.3 μg ml^{-1}) and detection and determination limits of 0.02 and 0.06 μg ml^{-1}, respectively.

Table 2
Final selected values for the studied variables

Variable	Selected value
Sodium hydroxyde concentration (mol l^{-1})	0.39
Luminol concentration (mol l^{-1})	1.5×10^{-5}
Potassium permanganate concentration (mol l^{-1})	1.0×10^{-5}
Length of the reactor (cm)	50
Sodium hydroxide flow rate (ml min^{-1})	4.0
Luminol flow rate (ml min^{-1})	4.0
Potassium permanganate flow rate (ml min^{-1})	4.0

3.3. Interference study

An extensive interference study was carried out on a selected concentration of carbofuran (0.2 μg ml^{-1}), to test the influence of foreign species that could be present in real samples. Several ions, carbamates (aldicarb and promecarb), organophosphorous (dichlorvos and chlorpyriphos-methyl) and pirethroid (fempropatrhin and acrinatrhin) pesticides were studied. Firstly, a confidence interval on the analytical response was established by repetitive injections ($n = 4$) of standard solutions of 0.2 μg ml^{-1} of carbofuran. This interval was defined as [29]:

Mean signal ± Student′s t (one-tailed; α = 0.05; $(n - 1)$ degrees of freedom) × standard deviation

Then, standard solutions containing a concentration of 0.2 μg ml^{-1} of carbofuran and increasing concentrations of each potential interferent were injected into the flow system and their signals were compared with the defined interval, with the purpose to estimate the maximum rate of interferent tolerated by the method. A substance was considered as an interferent at a certain level if the obtained signal was out of the confidence interval, being a *positive interference* if the signal was above the upper limit of the interval, and a *negative interference* if the signal was below the lower limit of the interval. Thus, each species was studied until the obtained signal corresponding to a specific analyte:interferent ratio was into the confidence interval, considering this concentration as the *maximum tolerance level*.

It can be concluded that the tolerated interferent concentrations were in most cases higher than those normally present in real samples. No interference effect was found for sulphate, phosphate, potassium(I), sodium(I), calcium(II), zinc(II) and

chloride in the studied range (1000-fold was the maximum tested), while the maximum tolerate rate was 500-fold excess for fempropathrin, acrinathrin, chlorpyriphos-methyl, aldicarb, 100-fold for magnesium(II), dichlorvos and 10-fold for promecarb.

Some ions, such as iron(III), cobalt(II) and copper(II) interfered in low concentrations (0.1-fold was the less tested), because they could act as a catalyst in the oxidation of luminol.

3.4. Applications

The applicability of the proposed FIA-CL method to real samples was demonstrated with recovery studies of carbofuran in different water samples (river, ground and tap water) and lettuce samples obtained from ecological agriculture, spiked at different concentrations. The water samples were filtered and stored in a refrigerator.

For the determination of carbofuran in vegetable, portions of lettuce sample (20 g) were spiked with different amounts of carbofuran (2, 3 and 4 mg l^{-1}, which corresponds to a final injected concentration of 0.1, 0.15 and 0.2 mg l^{-1}) then analysed, after the appropriate treatment above described. Two different recoveries can be considered according to the moment in which the spiking procedure was carried out, namely: apparent recovery (R^*), calculated from samples spiked at the beginning of the extraction process and related with the overall systematic error of the whole analytical process; and calibration recovery (R^c), calculated from sample extracts spiked just before the measurement process, and related with the type of systematic error due to the matrix effect [30].

In the case of water samples, as no extraction procedure was required, only calibration recovery (R^c) was estimated. The obtained results are shown in Table 3 and Table 4 for water and lettuce analysis, respectively. Taking into account that in pesticide residue analysis the acceptable range for recovery is usually between 60 and 140% for routine analysis [31,32], the results obtained with the proposed method can be considered in agreement with the current demands.

Table 3
Determination of carbofuran in different kinds of waters (ground, river and tap water)

Type of water	Added (μg ml^{-1})	Found (μg ml^{-1})[a]	Recovery (%)
Ground	0.10	0.12 ± 0.01	117 ± 6
	0.15	0.15 ± 0.01	98 ± 4
	0.20	0.23 ± 0.01	113 ± 3
River	0.10	0.14 ± 0.02	137 ± 15
	0.15	0.15 ± 0.01	102 ± 4
	0.20	0.24 ± 0.01	118 ± 3
Tap	0.10	0.09 ± 0.00	98 ± 1
	0.15	0.15 ± 0.01	100 ± 7
	0.20	0.19 ± 0.01	93 ± 4

[a] Mean of four determinations ± standard deviation.

Table 4
Determination of carbofuran in lettuce ($n = 4$)

Sample	Added (mg l^{-1})	Final injected concentration (mg l^{-1})	R^{*a}	R^{cb}
Lettuce	2	0.10	69 ± 2	72 ± 3
	3	0.15	73 ± 1	70 ± 1
	4	0.20	64 ± 2	76.2 ± 0.4

Mean of four determinations ± standard deviation.
[a] R^*: apparent recovery.
[b] R^c: calibration recovery.

4. Conclusions

The results presented in this work demonstrate that the coupling of luminol–$KMnO_4$ CL reaction and FIA is a very suitable approach to determine carbofuran residues at trace levels in environmental and vegetable samples, being a fast and cheap alternative.

The proposed method offers several advantages which are associated with the use of both FIA technique (low reagent consumption, high throughput and ease automation) and CL detection (high sensitivity, wide dynamic range and simple instrumentation).

The method implies a previous extraction and sample clean-up in vegetal samples, while in waters, no pre-treatment is required. Nevertheless, in the case of tap water for human consumption, the maximum limit of residue for carbofuran is 0.1 μg l^{-1}, and a previous pre-concentration step would be required. The method shows good recoveries in compliance with the current guidelines. Furthermore, this method could be easily coupled with liquid chromatography for the jointly determination of several carbamate residues. Further research will be developed in this sense.

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