Flow-injection determination of carbaryl and carbofuran based on KMnO₄–Na₂SO₃ chemiluminescence detection

Amir Waseem, Mohammad Yaqoob* and Abdul Nabi

Department of Chemistry, University of Balochistan, Quetta, Pakistan

Received 7 December 2006; revised 27 January 2007; accepted 28 January 2007

ABSTRACT: A flow-injection method is described for the determination of carbaryl and carbofuran. It was found that a strong chemiluminescence (CL) signal was generated when these pesticides were mixed with Na₂SO₃ and KMnO₄ in acidic medium. Under the optimum experimental conditions, the enhanced CL intensity was linear, with the concentrations in the range 0.1–2.0 µg/mL ($r^2=0.9996$ and 0.9993, $n=6$) with relative standard deviation ($n=4$) in the range 1.0–2.3%. The limits of detection (3σ blank) were 10 and 50 ng/mL, respectively, with a sample throughput of 180/h. The proposed method was applied to determine carbaryl and carbofuran in freshwaters with satisfactory results. Most metal and non-metal ions and some pesticides, such as carbophenothion and aldicarb, do not interfere with the determination. Dinoseb, diazinon and malathion calibration graphs (in the range 0.2–2.0 µg/mL, $r^2=0.9966–0.9988$, $n=6$) were also established with relative standard deviations ($n=4$) in the range 1.2–2.0% with limits of detection (3σ blank) in the range 100–300 ng/mL. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: flow injection analysis; acidic potassium permanganate; sodium sulphite; chemiluminescence; carbaryl; carbofuran

INTRODUCTION

Carbamate pesticides have been widely used because some are more biodegradable than the formerly popular organochlorine insecticides and have lower dermal toxicities than most common organophosphates pesticides. Carbaryl (1-naphthyl-N-methylcarbamate) (Figure 1) has been widely used as an insecticide on lawns and gardens and generally has low toxicity to mammals. Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate) (Figure 1) has high water solubility and acts as a systematic plant insecticide taken up by the roots and leaves of plants, so that insects are poisoned by the plant material on which they feed (1).

These compounds are inhibitors of acetylcholinesterase and considered toxic to human beings. This inhibition activity may result from hydrolysis of the carbamate in vivo (2).

Various methods have been reported for the determination of carbofuran and carbaryl in diverse samples. These include; spectrophotometry (3–6), flow injection analysis (FIA) (7–9), fluorescence (10–12), HPLC-UV (13–15), GC–MS (16, 17) and immunoassay (18). These methods are accurate and selective, but involve expensive instrumentation and are time consuming and have poor sample throughput.

Flow injection with chemiluminescence detection (FI–CL) has been extensively used for the determination of numerous analytes in environmental, pharmaceutical, clinical, biochemical, food and beverage samples at low concentrations (19–21). Advantages of FI–CL methods include high sensitivity, a wide linear dynamic range and simple instrumentation. There are many inorganic and organic chemical reactions that produce CL in the liquid phase. However, only a few systems have been used for analytical purposes, including luminol, lucigenin, lophine, gallic acid, morphine, codeine, pyrogallol, acridinium esters, acidic potassium permanganate and tris(2,2-bipyridyl)ruthenium(II) (22–24).

Numerous FI–CL methods have been reported for the determination of carbaryl and carbofuran in environmental, biological, food and pharmaceutical samples. A FI–CL method has been reported, based on the enhancing effect of carbaryl on the CL emission generated by the oxidation of luminol with potassium permanganate.
in an alkaline medium. CL intensity is linear for a carbaryl concentration over the range 5–100 ng/mL, with a limit of detection (3σ) of 4.9 ng/mL. Another direct FI–CL method has been reported for the determination of carbaryl based on the oxidation of carbaryl with potassium permanganate in acidic medium, using 4 mol/L H₂SO₄ (26). The limit of detection (S:N = 3) is 14.8 ng/mL with a high reproducibility (RSD = 2.29%) for 0.1 µg/mL carbaryl (n = 10), using a sample volume of 135 µL and PMT voltage of 1400 V. Another FI method using photolytic decomposition and photogenerated tris(2,2'-bipyridyl)-ruthenium(III) processes has been reported for the determination of carbaryl (27). The method is based on the on-line photoconversion of carbaryl into methylamine, which subsequently reacts with Ru(bpy)_3^{2+} generated through the on-line photo-oxidation of Ru(bpy)_3^{2+} with peroxydisulfate. The limit of detection is 0.012 µg/mL, using a sample volume of 135 µL and a sample throughput of 200 samples/h. Carbofuran is determined using an FI–CL method based on photolysis decomposition and photogenerated tris(2,2'-bipyridyl)-ruthenium(III) processes (28), based on the fact that carbofuran can enhance CL intensity in the CL reaction between Ce(IV) and Na₂SO₃ in acidic medium. The limit of detection (3 s/K) is 2.84 × 10⁻⁸ mol/L. Carbofuran is also determined by using a FI–CL method based on the reaction of luminol with KMnO₄ in alkaline medium by its enhancement effect (29). The CL intensity is linear for carbofuran concentration over the range 0.06–0.5 µg/mL, with a limit of detection of 0.02 µg/mL, using a flow rate of 4.0 mL/channel. Carbamate determination has also been reported in natural water, based on the enhancement of electrochemiluminescent of Ru(bpy)_3^{2+} at the multi-wall carbon nanotube-modified electrode (30), in which the limits of detection are 2.0 × 10⁻⁸, 1.8 × 10⁻⁸, 1.2 × 10⁻⁷ and 1.7 × 10⁻⁷ mol/L, for pirimicarb, methomyl, aldicarb and carbofuran, respectively. Inhibition methods using immobilized enzymes have also been reported for the determination of organophosphorus and carbamate pesticides (31–34).

In this work, a FI–CL method is proposed for the determination of carbaryl and carbofuran. The method is based on the enhancement of CL intensity in the reaction between KMnO₄ and Na₂SO₃ in acidic medium. The proposed method is simple and sensitive, and has been applied to the determination of carbamates in freshwater. A possible reaction mechanism for the enhancement of the CL system is also described.

**EXPERIMENTAL**

**Chemicals and reagents**

All solutions were prepared using ultra-high purity (UHP) water (18.2 mol/L·Ω·cm; Elgastat, Maxima, UK) and all reagents were of analytical grade, supplied by Merck BDH, unless stated otherwise. Carbaryl, carbofuran, dinoseb, diazinon and malathion (Dr. Ehrenstorfer Laboratories GmbH) stock solutions (100 mg/L) were prepared by dissolving the required amount in 100 mL acetonitrile (50% v/v), stored in the dark at 4°C. Working solutions were freshly prepared in UHP water by serial dilution whenever required. Potassium permanganate stock solution (0.01 mol/L) was prepared by dissolving the required amount of compound in UHP water, filtered and stored in the dark. Diluted solutions were prepared by mixing portions of the stock solution with the required amount of H₂SO₄ (0.01 mol/L). Fresh solutions were prepared weekly. Sodium sulphite solution (0.01 mol/L) was prepared daily by dissolving the required amount of anhydrous sodium sulphite in UHP water and diluted solutions were prepared in UHP water whenever required. Stock solutions (100 mg/L) of carbofenthion; aldicarb prepared in acetonitrile 50% v/v; ascorbic acid and pyrocatechol in UHP water; 1000 mg/L of cations (K⁺, NH₄⁺, Ca²⁺, Mg²⁺, Fe²⁺, Fe³⁺ and Cu²⁺ prepared in 0.01 mol/L HCl) and anions (Cl⁻, SO₄²⁻, PO₄³⁻, NO₃⁻, NO₂⁻, and HCO₃⁻ in UHP water), for interference studies from their respective salts and various working solutions were prepared from these stock solutions in UHP water.

**Instrumentation and procedures**

The FI–CL manifold used for this work is shown in Fig. 2. A peristaltic pump (four channels; Ismatec Reglo, Switzerland) was used to deliver the sample carrier and reagent solutions at a flow rate of 1.9 mL/min. A rotary injection valve (Rheodyne 5020; Anachem, Luton, UK) was used to inject the sample carrier and reagent solutions at a flow rate of 4.0 mL/channel. Carbamate determination has also been reported in natural water, based on the enhancement of electrochemiluminescent of Ru(bpy)_3^{2+} at the multi-wall carbon nanotube-modified electrode (30), in which the limits of detection are 2.0 × 10⁻⁸, 1.8 × 10⁻⁸, 1.2 × 10⁻⁷ and 1.7 × 10⁻⁷ mol/L, for pirimicarb, methomyl, aldicarb and carbofuran, respectively. Inhibition methods using immobilized enzymes have also been reported for the determination of organophosphorus and carbamate pesticides (31–34).

In this work, a FI–CL method is proposed for the determination of carbaryl and carbofuran. The method is based on the enhancement of CL intensity in the reaction between KMnO₄ and Na₂SO₃ in acidic medium. The proposed method is simple and sensitive, and has been applied to the determination of carbamates in freshwater. A possible reaction mechanism for the enhancement of the CL system is also described.

**Figure 2.** Flow injection–chemiluminescence (FI–CL) manifold for the determination of carbamate pesticides.
2 kV power supply (PF1053, Burle, USA). The detector output was recorded using a chart recorder (Kipp & Zonen BD 40, The Netherlands).

RESULTS AND DISCUSSION

Optimization of the FI manifold

In order to establish the optimum conditions for the determination of carbamate pesticides, various parameters were investigated using a univariate approach. The key parameters optimized were sulphuric acid, potassium permanganate and sodium sulphite concentrations, sample volume, and flow rate of sample carrier and reagent streams (see Table 1). All of these studies were performed with a 1.0 mg/L carbofuran standard solution and a PMT voltage of 900 V.

The efficiency of potassium permanganate CL is highly dependent on acid conditions and typically gives a higher response at 0.01 mol/L sulphuric acid (range studied, 0.005–0.2 mol/L). The effect of potassium permanganate concentration was studied over the range 5.0 × 10⁻⁶–5.0 × 10⁻⁴ mol/L, using the optimized sulphuric acid conditions. The CL response increased up to 1.0 × 10⁻⁴ mol/L potassium permanganate (used in all subsequent experiments), above which the response decreased. Na₂SO₃ played an important role in the CL reaction and no signal was detected in the absence of Na₂SO₃. The influence of Na₂SO₃ concentration on the CL reaction was studied over the range 1.0 × 10⁻⁵–1.0 × 10⁻³ mol/L. The CL response increased with increase in Na₂SO₃ concentration up to 0.01 mol/L with high background, and therefore a solution of 1.0 × 10⁻⁵ mol/L was used for subsequent studies.

The effect of flow rate and sample volume on the CL response was investigated in terms of sensitivity; speed and reagent consumption and the results are shown in Table 1. A flow rate of 1.9 mL/min gave the maximum CL response and no signal was detected in the absence of potassium permanganate and sodium sulphite concentrations, sample volume, and reagent streams (see Table 1). All of these studies were performed with a 1.0 mg/L carbofuran standard solution and a PMT voltage of 900 V.

The efficiency of potassium permanganate CL is highly dependent on acid conditions and typically gives a higher response at 0.01 mol/L sulphuric acid (range studied, 0.005–0.2 mol/L). The effect of potassium permanganate concentration was studied over the range 5.0 × 10⁻⁶–5.0 × 10⁻⁴ mol/L, using the optimized sulphuric acid conditions. The CL response increased up to 1.0 × 10⁻⁴ mol/L potassium permanganate (used in all subsequent experiments), above which the response decreased. Na₂SO₃ played an important role in the CL reaction and no signal was detected in the absence of Na₂SO₃. The influence of Na₂SO₃ concentration on the CL reaction was studied over the range 1.0 × 10⁻⁵–1.0 × 10⁻³ mol/L. The CL response increased with increase in Na₂SO₃ concentration up to 0.01 mol/L with high background, and therefore a solution of 1.0 × 10⁻⁵ mol/L was used for subsequent studies.

The effect of flow rate and sample volume on the CL response was investigated in terms of sensitivity; speed and reagent consumption and the results are shown in Table 1. A flow rate of 1.9 mL/min gave the maximum CL response (range studied, 0.5–3.0 mL/min) with a steady baseline and reproducible (<2.0% RSD) peak height and was used subsequently for all three channels. Similarly, a sample injection volume of 90 µL gave almost the highest CL response and was used for economy of sample consumption and speed of response, respectively.

Table 1. Ranges investigated and optimized for FI-CL manifold parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Optimized</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂SO₄/mol/L</td>
<td>0.005–0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>KMnO₄/mol/L</td>
<td>5.0 × 10⁻⁶–5.0 × 10⁻⁴</td>
<td>1.0 × 10⁻⁴</td>
</tr>
<tr>
<td>Na₂SO₃/mol/L</td>
<td>1.0 × 10⁻⁵–1.0 × 10⁻³</td>
<td>1.0 × 10⁻³</td>
</tr>
<tr>
<td>Flow rate, mL/min</td>
<td>0.5–3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Injection volume, µL</td>
<td>30–120</td>
<td>90</td>
</tr>
</tbody>
</table>

Analytical figures of merit

Calibration graphs of CL intensity vs. [carbaryl and carbofuran] over the range 0.1–2.0 µg/mL under the optimum conditions described above are shown in Fig. 3, with the correlation coefficients (r²) 0.9996 and 0.9993 (n = 6) respectively. The relative standard deviation (RSD) was 1.0–2.3% (n = 4) over the range studied. The limits of detection (3σ blank) were 10 and 50 ng/mL, respectively, with a sample throughput of 180/h.

The calibration data of CL intensity vs. [Dinoseb, Diazinon and Malathion] over the range 0.2–2.0 µg/mL were then obtained, using optimum conditions as given in Table 2.

Interference studies

The influence of metal and non-metal ions and related organic compounds was studied by preparing solutions (Ca²⁺, 100 mg/L; Mg²⁺, 20 mg/L; Fe²⁺, 0.5 mg/L; Fe³⁺ and Cu²⁺, 1.0 mg/L; K⁺ and NH₄⁺, 25 mg/L; Cl⁻, 200 mg/L; SO₄²⁻, 250 mg/L; HCO₃⁻, 100 mg/L; NO₃⁻, 25 mg/L; NO₂⁻, 0.1 mg/L; PO₄³⁻, 1.0 mg/L; thiourea and urea, 5.0 mg/L; carbofuran and aldicarb, 1.0 mg/L; ascorbic acid, 0.3 mg/L; and pyrocatechol, 0.1 mg/L containing 0.3 µg/mL carbofuran. No interference was found from these metal and non-metal ions and related organic compounds on carbofuran signals except Cl⁻ (200 mg/L), which suppressed the CL signal 60%. However, ascorbic acid (0.3 mg/L) and pyrocatechol (0.1 mg/L) in the absence of carbofuran give signals of 2.0 and 3.0 mV, respectively.

Applications

The proposed method was applied to the recovery of carbofuran and carbaryl from natural water samples by spiking experiments. Lake water samples (from Hanna valley, Quetta) and tap water samples (from the university) were collected into acid-washed (10%
Table 2. Analytical characteristics of the proposed method

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Linear range (µg/mL)</th>
<th>Correlation coefficient ($r^2$)</th>
<th>Regression equation*</th>
<th>Detection limit S:N = 3/ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>0.1–1.0</td>
<td>0.9996 (n = 6)</td>
<td>$y = 150.58x + 1.2027$</td>
<td>10</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.1–1.0</td>
<td>0.9993 (n = 6)</td>
<td>$y = 49.603x + 1.2219$</td>
<td>50</td>
</tr>
<tr>
<td>Dinoseb</td>
<td>0.2–2.0</td>
<td>0.9977 (n = 6)</td>
<td>$y = 21.659x + 1.8690$</td>
<td>100</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.3–1.5</td>
<td>0.9988 (n = 6)</td>
<td>$y = 19.333x + 1.8$</td>
<td>100</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.6–1.8</td>
<td>0.9966 (n = 6)</td>
<td>$y = 15x + 0.4$</td>
<td>300</td>
</tr>
</tbody>
</table>

* $y = CL$ intensity (mV); $x =$ concentration (µg/mL).

Table 3. Recoveries of carbaryl and carbofuran residue from fortified water samples

<table>
<thead>
<tr>
<th>Spiked (µg/mL)</th>
<th>Found (µg/mL)*</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbaryl</td>
<td>Carbofuran</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UHP water</td>
<td>0.1</td>
<td>0.102 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.248 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.1</td>
<td>0.097 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.246 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.486 ± 0.07</td>
</tr>
<tr>
<td>Lake water</td>
<td>0.1</td>
<td>0.085 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.215 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.435 ± 0.08</td>
</tr>
</tbody>
</table>

* Mean of five injections.

After collection, the samples were filtered through a cellulose membrane filter (cellulose acetate, pore size 0.45 µm, 47 mm diameter; Whatman, Maidstone, UK) to remove the suspended solids, and kept refrigerated in the dark at 4°C. The data are shown in Table 3. The recoveries were satisfactory except for lake water, possibly due to the high organic matter content of the samples (mostly humic acids), which may reduce the analytical signal, due to oxidant consumption or retardation of the kinetically controlled luminescent oxidation reaction of carbaryl (26).

**Possible CL mechanism**

The mechanism of the CL reaction between KMnO$_4$ with Na$_2$SO$_3$ has been investigated and it is proposed that the excited SO$_2^*$ is the emitter during the reaction (emission spectrum in the range 450–600 nm) (36, 37). This weak CL reaction can be sensitized by several fluorescent and non-fluorescent compounds (38, 39).

The fluorescence studies of KMnO$_4$–Na$_2$SO$_3$–H$_2$SO$_4$ in the presence and absence of carbaryl/carbofuran were performed in the range 450–600 nm, using a fluorescence spectrophotometer (RF-1501; Shimadzu, Japan). Both spectra had the same maximum emission wavelength at 464 nm as reported previously (40). The CL spectra of the KMnO$_4$–Na$_2$SO$_3$–H$_2$SO$_4$ reaction with and without carbaryl were also examined using the FI–CL manifold shown in Fig. 1. In the first two carrier streams UHP water was used, while in the third channel KMnO$_4$ solution ($1.0 \times 10^{-4}$ mol/L) was used. In the sample carrier stream Na$_2$SO$_3$ solution ($1.0 \times 10^{-3}$ mol/L, 90 µL) was injected, which produced a weak CL signal. The CL signal was markedly increased when injecting Na$_2$SO$_3$ solution containing carbaryl (100 µg/L; 75% increase in CL signal) shown in Fig. 4. Therefore, it may be concluded that carbaryl played a role of an enhancer in the reaction.

![Figure 4. CL intensity vs. time profile, Na$_2$SO$_3$ (1.0 $\times 10^{-3}$ mol/L, 90 µL) in the absence (a) and presence (b) of carbaryl (100 µg/L).](image-url)
CONCLUSION

The proposed FI–CL method for the determination of carbofuran and carbaryl is simple and rapid (180 samples/h) and the recoveries are within the acceptable range for pesticide residue analysis. The method has superior sensitivity and sample throughput compared with FI–photometric methods (7–9). The method has detection limits of 10 and 50 ng/mL for carbaryl and carbofuran, respectively.

Acknowledgments

The authors are grateful to the Higher Education Commission, Pakistan, for financial support in the form of an Indigenous PhD Scholarship (IQC-03600101) and a research project (639).

REFERENCES


