DETERMINATION OF CARBARYL AND ITS METABOLITE
1-NAPHTHOL IN COMMERCIAL FORMULATIONS
AND BIOLOGICAL FLUIDS

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Summary—A simple and sensitive method for the determination of carbaryl in whole blood and commercial formulations, based on normal, and synchronous first- and second-derivative fluorescence spectra, is presented. Solvent effects on the spectral characteristics of carbaryl solutions and the influences of instrumental parameters are described in detail. Two methods have been developed, with neutral (for carbaryl) and basic (for 1-naphthol) media. Detection limits of 0.9 and 0.7 ng/ml were achieved for carbaryl and 1-naphthol, respectively, with the first-derivative approach.

Carbaryl (1-naphthyl methylcarbamate) is one of the major pesticides used today, owing to its effectiveness against numerous insect pests, along with its relatively short half-life and low plant and mammalian toxicity. Nevertheless, various studies have indicated that carbaryl may cause toxic effects by inhibition of cholinesterase enzyme and by its teratogenic character.1–3

Fluorimetry has attracted much interest as a method for the determination of carbaryl and 1-naphthol, owing to their native fluorescence.4 Most procedures involve a separatory technique such as TLC,5,6 GC7,8 or HPLC9–15 to avoid matrix interferences. In some cases, a prior derivatization is used to obtain greater sensitivity.16–18 Spectrophotometric detection has also been widely used as an alternative to spectrofluorimetry,19–23 with which it competes in speed and sensitivity.

Determination of carbaryl residues in crops and of the compound in formulations is clearly important.24–26 The method of McDermott26 has been recommended for the analysis of formulations, and that of Lawrence and Leduc16 for crops, water and soil. Both are HPLC methods with photometric detection.

Fluorimetry offers excellent detection limits in the determination of trace amounts of many organic molecules. However, synchronous spectrofluorimetry27 and derivative spectrometry28 are two very valuable techniques for improving sensitivity and reducing interferences. The application of derivative techniques in luminescence spectroscopy was first reported by Green and O’Haver29 and has been widely extended since.30–36 This paper describes a quick and efficient method for the determination of both carbaryl and 1-naphthol and is a useful alternative to the spectrophotometric method proposed earlier.37

EXPERIMENTAL

Apparatus
All spectra were obtained at room temperature (25°) over the range 250–600 nm, with a Perkin–Elmer MPF-43A spectrofluorimeter equipped with an Osram XBO 150-W xenon lamp, 1 x 1 cm fused-silica cells, an R-777 Hamamatsu photomultiplier, and a Perkin–Elmer 023 recorder. The derivative spectra were obtained with a Perkin–Elmer model DCSU-2 unit connected to the spectrofluorimeter.

Reagents
Carbaryl and 1-naphthol (purity ≥ 99%) of Pestanal quality were purchased from Riedel–de Haen AG. Stock solutions in ethanol were prepared weekly (1 x 10⁻³M carbaryl and 1 x 10⁻²M 1-naphthol). The solvents used were analytical-grade ethanol, methanol, butan-1-ol, propan-1-ol, acetone, dimethylformamide and acetonitrile. Demineralized water was used throughout the work.

Analytical procedure
Take appropriate volumes of the carbaryl sample solution (in ethanol) to obtain final
concentrations between 1 and 100 ng/ml. Add 0.5 ml of 0.2M sodium hydroxide (to provide a basic medium) and enough ethanol to give a final concentration of 5% v/v and dilute to volume with demineralized water. Measure the fluorescence intensity at 333 nm with excitation at 285 nm (neutral medium) and at 460 nm with excitation at 330 nm (basic medium) against a solvent blank. Record the synchronous first- and second-derivative spectra with Δλ = 50 nm (neutral medium) or 130 nm (basic medium) with a response-time of 0.3 sec, wavelength increment Δλ' = 10 nm, and a scan speed of 60 nm/min (neutral medium) or 120 nm/min (basic medium). Convert the relative fluorescence intensity (RFI) and the derivative values (expressed in cm) into concentration units by applying the corresponding regression equations or calibration curves.

RESULTS AND DISCUSSION

Solvent effects on the spectral data

The effects of solvents on molecular spectra are of interest in analytical spectroscopy because the information they give can be used to increase sensitivity and selectivity. To evaluate these effects, the fluorescence and absorption spectra of carbaryl solutions (2.5 × 10⁻⁵ M) in solvents of different polarities and hydrogen-bonding capacities were recorded.

The spectral characteristics found are summarized in Table 1. There is a slight bathochromic shift of the fluorescence and absorption maxima when the dielectric constant decreases. As a result, the Stokes shift, Δλ, increases from 52 nm in water to 57 nm in butan-1-ol, but this effect is too small to be significant.

As a general rule, both the relative fluorescence intensity (RFI) and the relative efficiency (RE = RFI/ε) increase with solvent polarity except for propan-1-ol and butan-1-ol. The hydrogen-bonding capacity of these two solvents also probably favours the fluorescence emission. This hypothesis agree well with the behaviour in acetonitrile, which is more polar than dimethylformamide (DMF) but exclusively a hydrogen-bonding acceptor, and gives only half the RFI observed for the DMF solution.

From Table 1, water seems to be the best solvent for the spectrofluorimetric determination of carbaryl, but carbaryl is only slightly soluble in water and is hydrolysed in basic media to its main metabolite 1-naphthol. However, examination of use of an aqueous ethanol medium showed that the RFI decreased only slightly with the increase in ethanol content, owing to the decrease in the dielectric constant of the medium (Table 1). A fixed ethanol percentage of 5% v/v was chosen for use.

Fluorescence spectra and effect of experimental variables

Earlier, we used the influence of the acidity of the medium on the chemical behaviour of carbaryl and its hydrolysis product 1-naphthol to determine the hydrolysis rate and the ground and excited state pKᵢ values by spectrophotometry and spectrofluorimetry to establish the best pH values for the determination of carbaryl and 1-naphthol by the two techniques. That work shows that it is possible to determine carbaryl spectrofluorimetrically

Extraction procedures

Commercial formulations. An accurately weighed sample containing 0.04 ± 0.01 g of active carbaryl was placed in a 30-ml medium-porosity fritted-glass Buchner funnel, fitted in a 250-ml Buchner flask. Ten ml of methanol were added to the funnel, and after 5 min suction was applied until all the liquid was in the flask. This extraction step was repeated twice more in the same way. The contents of the flask were then transferred quantitatively to a 50-ml standard flask and diluted to volume with methanol. Aliquots of this solution were used for the analytical determination.

Blood samples. A white Wistar rat of approximate weight 260 g was anaesthetized with sodium pentobarbital (50 mg), and blood was extracted from the left ventricle with a heparinized syringe and kept in the refrigerator (at 4°C) until used.

To 0.25-ml portions of blood sample (in centrifuge tubes) different amounts of a standard ethanolic solution of carbaryl and/or 1-naphthol (200 μg/ml) were added, and the tubes were placed in an ultrasonic bath for 20 sec for haemolysis to take place. Then 2.5 ml of ethyl acetate were added to each tube. The tubes were shaken for 10 min, then centrifuged at 3500 r.p.m. Two ml of the supernatant liquid were transferred to a round-bottomed flask and evaporated to dryness at 40°C under reduced pressure. The residue was dissolved with 10 ml of demineralized water, and this solution was analysed as above.
Determination of carbaryl

Table 1. Spectral characteristics of carbaryl in different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>DIElectric constant</th>
<th>$\lambda_{em}$, nm</th>
<th>$\lambda_{ex}$, nm</th>
<th>$\Delta\lambda^*$, nm</th>
<th>RFI</th>
<th>$10^3 \times RE$</th>
<th>log $\epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>76.5</td>
<td>278</td>
<td>330</td>
<td>52</td>
<td>138</td>
<td>27.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Water/ethanol (50% v/v)</td>
<td>51.5</td>
<td>279</td>
<td>334</td>
<td>55</td>
<td>120</td>
<td>19.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>37.5</td>
<td>279</td>
<td>333</td>
<td>54</td>
<td>60</td>
<td>12.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>36.7</td>
<td>281</td>
<td>337</td>
<td>56</td>
<td>117</td>
<td>18.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Methanol/acetone (50% v/v)</td>
<td>35.1</td>
<td>279</td>
<td>336</td>
<td>57</td>
<td>64</td>
<td>12.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.7</td>
<td>278</td>
<td>336</td>
<td>57</td>
<td>56</td>
<td>9.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Butanol/DMF (50% v/v)</td>
<td>27.1</td>
<td>281</td>
<td>337</td>
<td>56</td>
<td>101</td>
<td>19.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.5</td>
<td>279</td>
<td>336</td>
<td>57</td>
<td>67</td>
<td>10.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>20.3</td>
<td>279</td>
<td>336</td>
<td>57</td>
<td>78</td>
<td>10.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Butan-1-ol</td>
<td>17.5</td>
<td>280</td>
<td>337</td>
<td>57</td>
<td>85</td>
<td>15.1</td>
<td>3.7</td>
</tr>
</tbody>
</table>

$* $Stokes shift, $\Delta\lambda = \lambda_{ex} - \lambda_{em}$.  
$\tau$ expressed in 1.mole$^{-1}.cm^{-1}$.  

either as such in neutral medium or as 1-naphthololate in basic medium. The fluorescence spectra of both species are presented in Fig. 1.

The emission spectrum taken at pH 6 has two maxima, at 320 and 333 nm, with excitation at 285 nm. When the 5% v/v ethanol/water solution of carbaryl is treated with 0.2M sodium hydroxide, there is a strong bathochromic shift of the excitation to maximum to 330 nm and of both emission maxima to 460 nm.

Variation of the pH used showed that the hydrolysis takes place instantaneously at room temperature when the pH is higher than the ground state pK$_a$ value for carbaryl (9.5), and that a small change in pH at this level does not affect the RFI. A $4 \times 10^{-3}M$ sodium hydroxide concentration in the final solution was selected.

A study of the influence of sunlight on the fluorescence intensity of both neutral and basic carbaryl solutions showed that carbaryl is stable for 2 hr when exposed to sunlight but there is a slight decrease in the RFI of basic solutions after 1 hr, in agreement with previous findings.

The onset of self-reversal of fluorescence was examined, and it was found that the response curves were usable over the range $10^{-5}$--$10^{-4}M$.

No temperature effects were observed over the range between 10 and 55°, so all measurements were made at 25 ± 0.5°.

Optimization of instrumental variables

The use of normal fluorescence spectra for determination of carbaryl in real samples without prior clean-up is generally very difficult, because most co-extracted species would interfere. The suitability of synchronous spectrofluorimetry for the determination was therefore examined. The selectivity of the method can be improved by using different wavelength scanning intervals ($\Delta\lambda$), and derivative synchronous spectra.

![Fig. 1. Excitation and emission spectra of carbaryl in (a) neutral and (b) basic media. Carbaryl concentration 14 µg/ml.](image-url)
To optimize the spectral bandwidth of the synchronous signal, the effect of the wavelength difference between the two monochromators (Δλ) was examined. Various synchronous spectra were recorded with Δλ varied from 20 to 90 nm (neutral medium) and from 20 to 150 nm (basic medium). As demonstrated earlier, the fluorescence intensity depends strongly on Δλ, and is maximal when Δλ is equal to the Stokes shift. Hence Δλ values (cf. Fig. 1) of 50 nm (neutral medium) and 130 nm (basic medium) were employed.

The main parameters affecting the shape of the derivative spectra are the scan speed, response time and wavelength increment (Δλ'). The apparatus used gave a choice of three response times and four scan speeds. A combination of 0.3 sec response time, 10 nm Δλ' and 60 nm/min (neutral medium) or 120 nm/min (basic medium) scan speed was found to be best.

**Calibration graphs and statistical analysis**

The relationships between the fluorescence intensity, or the peak heights of first and second-derivative synchronous spectra and the carbaryl concentration in neutral and basic media, were found to be linear over the range 1–100 ng/ml. The main characteristics of the calibration graphs are summarized in Table 2. When corrected for the blank, the calibration lines all pass through the origin. The peak-to-trough method of measurement was used for the determination of carbaryl in the derivative mode.

The statistical data for analysis of 11 samples at both the 50 and 10 ng/ml levels in both media by the various methods proposed are also summarized in Table 2. The precision is indicated by the correlation coefficients and the relative standard deviation, and the analytical sensitivity (sA) is derived from the slopes and is defined as $s_A = s_x/m$, where $s_x$ is the standard deviation of the analytical signal and $m$ is the slope of the calibration graph.

The linear dynamic range (LDR) varied from 1.9–100 to 5.0–100 ng/ml, and the minimum detectable quantity (cL = 3s_b/m, where $s_b$ is the standard deviation of the blank signal) of 0.6 ng/ml, obtained with the synchronous second-derivative at neutral pH, is particularly low in comparison with the values obtained by the previous spectrophotometric method and other methods. The correlation coefficients are larger than 0.99 in all cases, which shows reasonable precision.

It can be deduced from Table 2 that despite the better detection limits obtained in neutral medium, the sensitivity, precision and relative error are much better in basic medium. It is noteworthy that the detection limit, which is a function of the standard deviation of the blank signal, is lower in neutral than in basic medium, whereas the analytical sensitivity, a function of the standard deviation of the analytical signal, is higher.

**Analysis of commercial formulations and whole blood samples**

The N-methyl and N,N-dimethylcarbamate insecticides are widely used owing to their effectiveness as pesticides and low mammalian toxicity. Carbaryl in particular has wide use in Spain and numerous commercial formulations are available.

The extraction system used in the present method is based on the one proposed by

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
<th>Sensitivity, $s_A$, ng/ml</th>
<th>cL, ng/ml</th>
<th>LDR, ng/ml</th>
<th>Error, %</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>neutral A</td>
<td>0.99*</td>
<td>-4.0*</td>
<td>0.9970</td>
<td>0.90</td>
<td>1.5</td>
<td>5.0–100</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>A</td>
<td>0.63</td>
<td>-0.1</td>
<td>0.9994</td>
<td>0.98</td>
<td>0.9</td>
<td>3.0–100</td>
<td>6.9</td>
<td>10.2</td>
</tr>
<tr>
<td>C</td>
<td>0.58</td>
<td>+0.2</td>
<td>0.9983</td>
<td>0.91</td>
<td>0.6</td>
<td>1.9–100</td>
<td>6.2</td>
<td>9.3</td>
</tr>
<tr>
<td>basic A</td>
<td>0.96*</td>
<td>-0.5*</td>
<td>0.9998</td>
<td>0.64</td>
<td>1.1</td>
<td>3.9–100</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>A</td>
<td>0.79</td>
<td>-1.1</td>
<td>0.9997</td>
<td>0.26</td>
<td>0.8</td>
<td>2.3–100</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>C</td>
<td>0.66</td>
<td>+0.2</td>
<td>0.9970</td>
<td>0.23</td>
<td>1.1</td>
<td>3.6–100</td>
<td>1.6</td>
<td>2.3</td>
</tr>
</tbody>
</table>

A, B, C: Normal, synchronous first and synchronous second derivatives.

*Values expressed in (relative fluorescence intensity units).ml ng⁻¹.

†Limit of detection.
§Linear dynamic range.
‡Relative standard deviation.
Determination of carbaryl

Table 3. Application of the normal spectrofluorimetric method to determination of carbaryl in commercial formulations

<table>
<thead>
<tr>
<th>Formulation*</th>
<th>Neutral pH</th>
<th>Basic pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found, †%</td>
<td>Recovery, %</td>
</tr>
<tr>
<td>1</td>
<td>4.8 ± 0.4</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>7.0 ± 0.9</td>
<td>93 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>87 ± 1.8</td>
<td>102 ± 2</td>
</tr>
</tbody>
</table>

†Mean ± standard deviation of 2 extractions and 2 determinations for each.

*Nominal content: 1—carbaryl 5% (Patatol AC); 2—carbaryl 7.5% (Agres S-7.5); 3—carbaryl 85% (Sevin 85).

McDermott, 26 which employed 5% methanol in methylene chloride, instead of 10% methanol in chloroform (to avoid the health hazard associated with chloroform).

As indicated by the WHO, 42 the usual methods employed for carbaryl are based on the colorimetric determination of 1-naphthol. 42 In 1982 this organization recommended a special investigation to find an easy extraction method applicable with simple equipment in any laboratory.

The results obtained for analysis of some commercial formulations by the proposed direct spectrofluorimetric method are shown in Table 3. The accuracy and precision in terms of the relative standard deviation for duplicate determinations on the solutions from two separate samples compare favourably with those for established methods. 7, 25, 26

Although carbaryl is not very toxic to human beings, there are studies that have indicated that it may be a viral enhancer and a teratogen. 3 For this reason accurate and sensitive methods for its determination are needed.

The proposed method has been applied to the determination of carbaryl in samples of whole rat blood spiked with different volumes of standard carbaryl solution and treated as indicated above. The results obtained are given in Table 4 together with the relative standard deviations for duplicate analyses of each of three extracts from the same sample.

These values demonstrate the applicability of the proposed analytical method to determination of the insecticide in two types of residue analysis, at concentrations relevant to this type of analysis.

Acknowledgement—We thank the Dirección General de Investigación Científica y Técnica (Project PB86-0247) for supporting this study.

REFERENCES


Table 4. Application of normal spectrofluorimetric method for determination of carbaryl in whole blood samples

<table>
<thead>
<tr>
<th>Added*, ng/ml</th>
<th>Extraction No.</th>
<th>Found, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>75</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>

*Concentration in the final solution.