Determination of the Pesticide Carbaryl by Microemulsion Room-Temperature Phosphorescence in Real Samples

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A room-temperature phosphorescence method for the determination of the pesticide carbaryl is described. The proposed method is based on the formation of a microemulsion obtained by mixing an apolar solvent with the surfactant sodium dodecyl sulfate as the micellar medium in the presence of thallium nitrate as the external heavy atom salt. To avoid the oxygen quenching, a sodium sulfite solution was used. A complete and exhaustive statistical analysis of experimental data by a multivariate optimization approach was performed to establish the optimum experimental variables. Good results have been obtained when the insecticide have been analyzed in synthetic and real samples.

Keywords Room-temperature phosphorescence, microemulsion, experimental design, carbaryl

The group of commercial insecticides known as carbamates have increased in popularity during the years as a consequence of their selective insecticidal properties, low mammalian toxicity and lack of undue persistence.

A great number of analytical methods have been proposed in the literature for the determination of this insecticide in numerous matrices by different instrumental methods[1–9], mainly spectrophotometric and spectrofluorometric (Table 1).

Micelle-stabilized room temperature phosphorescence (MS-RTP) is a type of RTP in solution first reported by Kalyanasundaram *et al.*[10] which has been developed by several authors during the last decade.[11–13] In all these works, a dramatic enhancement of RTP signals has been observed when the phosphors are incorporated into the micellar assembly. However, deoxygenation is needed in all cases and the traditional MS-RTP method of using N_2 purging to remove oxygen from solution, which is limited in application because of foam generation and other concomitant problems, has been recently replaced by a method proposed by Díaz-García and Sanz-Medel[14] based on a chemical deoxygenation with sodium sulfite, which represents a great technical advance in the use of MS-RTP.

MS-RTP suffers from a limitation due to the slow dissolution of apolar analytes in the aqueous micellar solutions. Many polycyclic aromatic hydrocarbons (PAHs) have been determined by this technique;[15–17] not only is the procedure for preparing samples relatively time consuming, but also the solubilizing capability of micelle solutions for some PAHs with very low polarities and vapor pressures, is limited, even after long sonication times.

This problem has been recently overcome by the methodology proposed by Ramos *et al.*[18] using a microemulsion aqueous solution of the analyte in an apolar solvent. The advantage of microemulsions with respect to normal micelles is that the highly hydrophobic core is adequate for dissolving relatively high concentrations of hydrophobic molecules in the aggregate and also large organic molecules with dimensions approaching those of many micelles.[19]

In many microemulsions, the mass fraction of a solvent can be varied over a fairly wide range (*e.g.*, 0 – 80%) without destruction of the monophase system. Although microemulsions offer more possibilities than normal micelles for the development of new analytical procedures, to date only a few applications have been developed.[20,21]

Although the determination of carbaryl by chemical deoxygenation MS-RTP[22] has been described recently, microemulsion room temperature phosphorimetry (ME-RTP) has been never used before for this purpose.

In order to obtain the optimum spectrophosphorimetric response, a statistical model was applied: type of central composite design.[23] The project has been to evaluate the significance of each variable in addition to the interaction between variables that affects the phosphorescence of carbaryl.

In the present work, the applicability of ME-RTP has

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Table 1 A review of phosphorimetric methods for the quantitative determination of carbaryl

Analytical method	Detection limit/ng ml^{-1}	Reference
Colorimetric	120	24
GC-fluorometric	50	25
Fluorometric	100	26
HPLC-fluorometric	30	27
Fluorometric	200	28
HPLC-photometric	100	29
HPLC-fluorometric	40	6
Photometric	300	30
LC-fluorométric	50	31
Phosphorimetric	40	20
Phosphorimetric	25	this work

been demonstrated by the succesful results obtained when the proposed method have been applied to different synthetic mixtures and soil samples.

Experimental

Reagents and chemicals

The solvents dichloromethane and 1-pentanol, the surfactant sodium dodecyl sulfate (SDS) (Sigma Chemical Co.), reagent grade thallium(I) nitrate and anhydrous sodium sulfite (Sigma Chemical Co.) and sulfuric acid (Sigma Chemical Co.) were used as received. Aqueous solutions were made with doubly distilled water. The sodium sulfite solutions were daily prepared and kept in tightly stopped containers.

Carbaryl (Fluka Chemie AG) was used without further purification. Microemulsion stock solutions were prepared by dissolving 5 mg of carbaryl in 1 ml of dichloromethane, adding 1 ml of 1-pentanol and making up to 50 ml with 0.5 M SDS.

Apparatus

All recordings of uncorrected luminescence spectra and measurements of ME-RTP intensities were carried out with an Aminco Bowman series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp. Measurements required a personal computer with 40 MB hard disk, 4 MB RAM memory, 3.5-inch 1.44 MB floppy disk drive, VGA color monitor with VGA graphics adapter card, serial 2-button mouse, DOS 6.0, OS/2 version 2.0, and a GPIB (IEEE-488) interface card for computer instrument communication. The spectrometer was equipped with a thermostated cell holder.

Software

STATGRAPHICS Statistical Institution Edition Version 6.0. Inc. and Statistical Graphics Corporation 1992.

Sample preparations and procedure

Basic procedure. A 100 µl aliquot of the carbaryl stock microemulsion, 0.64 ml of 0.5 M SDS, 1 ml of 0.25 M thallium nitrate and 0.49 ml of 0.1 M sodium sulfite and 0.79 ml of 0.02 M sulfuric acid were introduced into a 10-ml standard flask and made up to volume with water. If a precipitate appears after the addition of the heavy atom salt, the flask was warmed until the precipitate disappeared, before the other reagents were added. After thorough mixing, the flask was placed in a water bath at 25±1°C for 10 min. Standard 10-mm fused silica cells are filled with this solution.

Phosphorescence intensities were measured at 490 nm with excitation at 292 nm. Reagents blanks lacking carbaryl were prepared and measured following the same procedure.

Procedure for soil samples. The present method have been applied to soil samples from Gójar village (Granada, Spain). A certain quantity of carbaryl is added to 10 g of sample in such a way that the final concentration was included in the calibration graph. The samples were extracted twice with 30 ml dichloromethane, filtering on vacuum, and washing the dry residue with 10 ml dichloromethane. Both extracts are mixed and taken to dryness in a rotary evaporator at 40°C.

The residue is diluted to 10 ml with dichloromethane, 1 ml is taken and 1 ml 1-pentanol is added, diluting the mixture to 50 ml with SDS 0.5 M. The basic procedure is applied to this solution (Note: thallium(I) salts and dichloromethane are very toxic).

Results and Discussion

Mixing dichloromethane, 1-pentanol and SDS aqueous solution results in the formation of a oil-in-water (o/w) microemulsion. In this microenvironment, phosphors such as carbaryl are protected and escape partially or entirely from quenching caused by collisional deactivation. For forming this special aggregate containing a hydrocarbon core, critical concentrations for the surfactant SDS as well as the proper proportions of the apolar solvent and the alcohol, should be carefully selected. However, an external heavy atom addition, together with the deoxygenation carried out by sodium sulfite, seems to be decisive in obtaining RTP.

Optimization of experimental variables and spectral characteristics

In order to achieve maximum sensitivity, the optimum conditions should be selected. The effect of each experimental variable and their interactions were investigated using four-variable, composite hypercube-star design.[23]

The variables used in this multivariate optimization were SDS, $TlNO_3$, Na_2SO_3 and H_2SO_4 concentrations. In order to have a forecast of high quality, we applied the

Table 2 Experimental field for a design matrix: variables and carbaryl phosphorescence measurement

No.	[SDS]/ 10^{-2} M	[$TlNO_3$]/ 10^{-2} M	[Na_2SO_3]/ 10^{-3} M	[H_2SO_4]/ 10^{-3} M	I_{Phos}
1	2.00	2.00	4.50	1.80	0.80
2	2.00	3.00	4.50	1.80	0.74
3	2.00	2.00	4.50	1.40	0.89
4	4.00	3.00	4.50	1.40	0.91
5	2.00	2.00	5.50	1.40	0.75
6	4.00	2.00	5.50	1.40	0.68
7	4.00	3.00	4.50	1.80	0.73
8	4.00	2.00	4.50	1.40	0.88
9	3.00	2.50	5.00	1.60	0.92
10	4.00	2.00	5.50	1.80	0.89
11	2.00	3.00	5.50	1.40	0.65
12	4.00	3.00	5.50	1.40	0.73
13	4.00	2.00	4.50	1.80	0.70
14	2.00	3.00	4.50	1.40	0.90
15	2.00	3.00	5.50	1.80	0.90
16	4.00	3.00	5.50	1.80	0.89
17	2.00	2.00	5.50	1.80	0.88
18	9.42	2.50	5.00	1.60	0.87
19	3.00	3.52	5.00	1.60	0.90
20	5.06	2.50	5.00	1.60	0.90
21	3.00	1.47	5.00	1.60	0.76
22	3.00	2.50	5.00	1.60	0.94
23	3.00	2.50	5.00	1.19	0.69
24	3.00	2.50	5.00	2.01	0.78
25	3.00	2.50	3.97	1.60	0.70
26	3.00	2.50	6.03	1.60	0.68

I_{Phos}: phosphorescence intensity.

experimental matrix of the type composite blocked hypercube-star design. The 26 experiments dictated by the matrix are presented in detail in Table 2.

The results of this study (Table 2) show that the experimental response, as a function of the variables levels, can be approximated by a quadratic equation. One can conclude from the magntitude of the coefficients in this equation that the interaction between the variables is small. Therefore, terms that contain the interaction between variables would be important only at high values of the correspondent variables.

The maximum response is obtained with [SDS] of 3.20×10^{-2} M, [$TlNO_3$] of 2.67×10^{-2} M, [Na_2SO_3] of 4.94×10^{-3} M and [H_2SO_4] of 1.59×10^{-3} M. These concentrations were used as the working conditions, and selected for the rest of the experimental work.

The response surfaces of SDS and $TlNO_3$, SDS and Na_2SO_3, SDS and H_2SO_4, $TlNO_3$ and Na_2SO_3, $TlNO_3$ and H_2SO_4 and also Na_2SO_3 and H_2SO_4 at the optimum values obtained are represented in Fig. 1.

As can be deduced from these figures, there is a great stability in the phosphorescence response for a wide range of the micellar solution concentration, when varying the concentration of the deoxygenation compound and/or the external heavy atom (see Figs. 1a, 1b, 1c). But, as can be seen in Fig. 1f, there is a marked effect on carbaryl phosphorescence response when the sulfuric acid concentrations are varied in the solution in presence of different concentrations of the oxygen scavenger. As

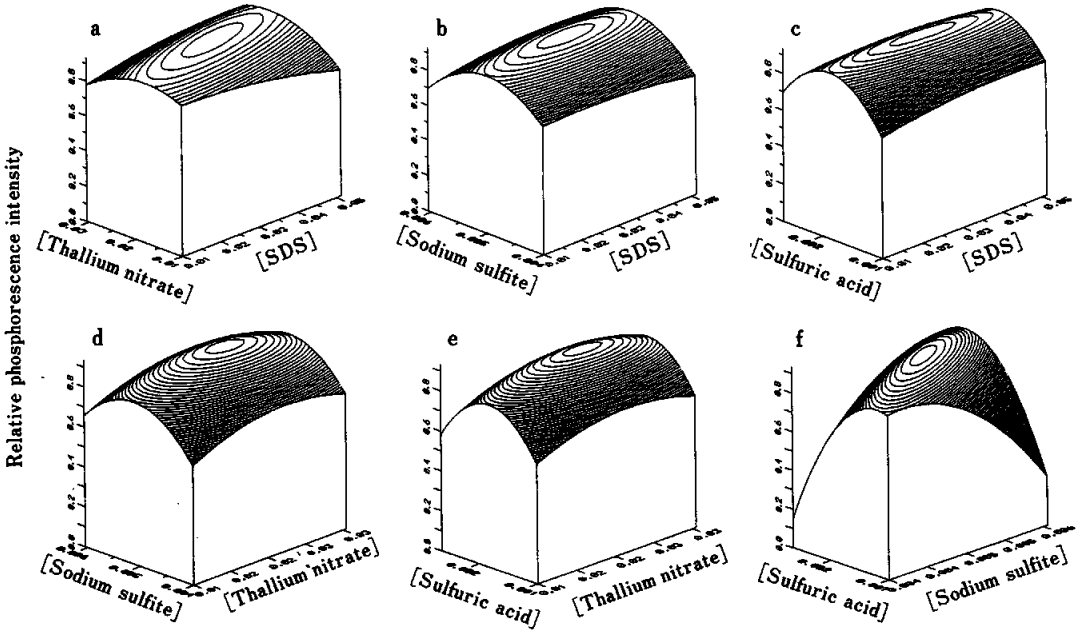

Fig. 1 Surface response of carbaryl for (a) SDS and $TlNO_3$ concentrations at Na_2SO_3 and H_2SO_4 concentrations 4.94×10^{-3} M and 1.49×10^{-3} M, respectively; (b) SDS and Na_2SO_3 concentrations at $TlNO_3$ and H_2SO_4 concentrations of 2.57×10^{-2} M and 1.59×10^{-3} M, respectively; (c) SDS and H_2SO_4 concentrations at $TlNO_3$ and Na_2SO_3 concentrations of 2.57×10^{-2} M and 4.94×10^{-3} M, respectively; (d) $TlNO_3$ and Na_2SO_3 concentrations at SDS and H_2SO_4 concentrations of 3.20×10^{-2} M and 1.59×10^{-3} M, respectively; (e) $TlNO_3$ and H_2SO_4 concentrations at SDS and Na_2SO_3 concentrations of 3.20×10^{-2} M and 4.94×10^{-3} M, respectively and (f) Na_2SO_3 and H_2SO_4 concentrations at SDS and $TlNO_3$ concentrations of 3.20×10^{-2} M and 2.57×10^{-2} M, respectively.

Table 3 Optimum instrumental parameters

Fluorescence wavelengths (ex/em)	292/337 nm
Phosphorescence wavelengths (ex/em)	292/490 nm
Slits (ex/em)	16/16 nm
Minimum period pulse	5 ms
Scanning speed	2 nm/s
Decay time	200 μs
Gate time	1000 μs
Detector sensitivity	1100 V

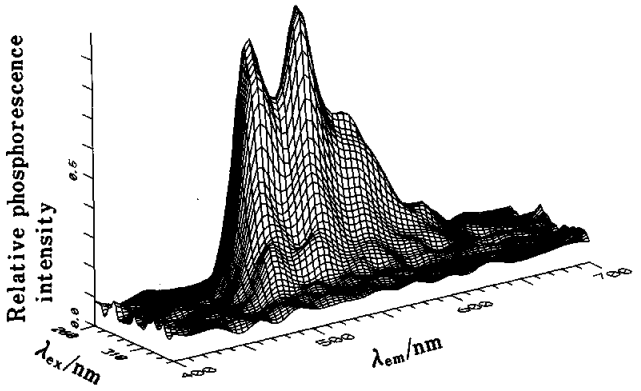

Fig. 2 Projected three-dimensional spectrum of carbaryl. ppb carbaryl= 400 ng ml^{-1}, [SDS]=3.20×10^{-2} M, [$TlNO_3$]= 2.57×10^{-2} M, [Na_2SO_3]=4.94×10^{-3} M, [H_2SO_4]=1.59×10^{-3} M; emission 400 - 700 nm, excitation 250 - 350 nm; scanning speed 2 nm/s; decay time 200 μs; gate time 1000 μs; detector sensitivity, 1100 V.

a result of these experiments, the optimum working conditions to obtain the highest phosphorescence response were selected.

As for the optimum instrumental parameters for RTP carbaryl determination, the data selected are summarized in Table 3.

The total luminescence spectra of carbaryl in a dichloromethane/1-pentanol emulsion agent in SDS micelles, with $TlNO_3$ as heavy atom and Na_2SO_3 as oxygen scavenger, is shown in Fig. 2.

The results indicate that stable and homogeneous microemulsions can be obtained in the range 0.5 - 3% (v/v) dichloromethane or 1-pentanol. Hence 2% dichloromethane and 1-pentanol were used throughout this work. After dilution, the dichloromethane and 1-pentanol concentrations in the final solutions were 0.02%. Under these conditions, stable and very intense RTP can be obtained.

The RTP intensities decrease almost linearly with increase in temperature. The decrease is greater in a microemulsion system than in a micelle system when the temperature increases by 5°C. The residual fluorescence intensities decrease slightly with increase in temperature. These effects are mainly related to molecular thermal motion and intermolecular energy conversion.

Table 4 Effect of foreign species on the determination of 400 ng ml^{-1} carbaryl

Foreign species	Tolerance level/ng ml^{-1}
p-Chlorophenoxyacetic acid	>40000
2,4-Dichlorophenoxyacetic acid	>40000
Carbendazime	>30000
2-(4-Thiazolyl)benzimidazole	>400
2-Aminophenol	>400
3-Amino-1,2,4-triazole	>400
α-Naphthol	>40

The molecular thermal motion causes collisional deactivation of the phosphors. A temperature of 25±1°C was selected for the rest of the experimental work.

Validation of the method

The method was tested for linearity, precision, reproducibility and specificity. Phosphorescence response was linear in relation to the concentration of carbaryl over the range 80 - 600 ng ml^{-1} calculated in the final solution.

The regression equation was

$$P = 0.104 + 2.1 \times 10^{-3} C$$

where P is the phosphorescence intensity and C the concentration of carbaryl in ng ml^{-1}. The correlation coefficient r=0.999 (n=7), indicating excellent linearity. A detection limit of 23.8 ng ml^{-1} was determined.

The precision of the method was determined at two different concentrations. The relative standard deviation (RSD) (n=7) was 3.44 and 1.48% for concentrations of carbaryl of 200 and 500 ng ml^{-1}, respectively.

The microemulsion room-temperature phosphorescence method proposed was applied to the analysis of carbaryl in the presence of different plant growth regulators and insecticides. Table 4 summarizes the results calculated from the calibration graph.

The phosphorescence spectra were always found to be identical to the corresponding carbaryl spectra.

Application of the method to soil samples

A soil sample from Gójar village (Granada, Spain) was spiked with carbaryl by adding appropriate volumes of a standard solution. Different recovery experiments in the spiked soil samples were performed. Samples of carbaryl containing 400 ng ml^{-1} were analyzed. A mean value of 95.8% (n=3), with an RSD of 2.55%, has been obtained.

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