Determination of the Carbamate Herbicide Propham by Synchronous Derivative Spectrofluorometry following Fluorescamine Fluorogenic Labeling

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Three methods for the determination of propham by fluorogenic labeling with fluorescamine and synchronous derivative spectrofluorometry are described and discussed. The herbicide is hydrolyzed in 1 M NaOH at 100 °C for 40 min, and the liberated aniline reacts instantaneously with fluorescamine in an aqueous buffer solution (pH 4.0). Reaction rates, fluorescence phenomena, and synchronous derivative parameters are investigated. To enhance sensitivity, the analysis of the fluorescamine derivative is used following extraction in ethyl acetate. Linear analytical curves are obtained between 24 and 2000 ng/mL. The minimum detectable quantity is estimated to be 7.2 ng/mL. Wellwater samples containing low concentrations of carbamate are also analyzed.

The esters of carbamic acid are physiologically quite active; propham (isopropyl phenylcarbamate, IPC) is very representative. Discovered in 1945, it is used primarily as a selective, preemergence herbicide that kills plants by interfering with cell division and plant growth.

Various analytical methods have been proposed for IPC determination (*1–5*). Thin-layer chromatography using fluorogenic labeling with dansyl chloride has been used to analyze lake water samples for the herbicide (*1*). This method was later modified for food crops (*2*); its detection limits were 50 ng/mL.

Fluorescence techniques are seldom used for analysis of pesticide residues because few pesticides possess inherent fluorescence. However, these methods are very useful if a highly fluorescent moiety is introduced into the pesticide molecule. This fluorogenic labeling approach has proved its usefulness in numerous analytical applications for some 30 years.

Udenfriend (*6*) introduced a labeling reagent, fluorescamine, to determine primary amines; this is superior to dansyl chloride because both the reagent and its hydrolysis products are nonfluorescent. On this basis, it was decided to develop a fluorescamine quantitative labeling method and to modify its application to a secondary amine as this would be less affected by interferences than the dansyl chloride methods.

Despite their extensive use, both gas–liquid chromatography and thin-layer chromatography require time-consuming and rigorous preparatory purifying steps. Recently, the synchronous derivative technique has been shown to be most useful in many types of trace analysis (*7–11*) and this is both simple and rapid. Its band narrowing effect plus the greater discrimination of derivative spectroscopy enhances both sensitivity and selectivity of the analytical methods.

This paper describes the use of the synchronous derivative technique to determine the fluorescent derivative of propham herbicide, which was obtained by converting the secondary amine into primary amine and then subsequently reacting it with the fluorescamine labeling reagent. This method may be used for the quantitative analysis of water samples at levels of 24 ng/mL without prior preparation.

EXPERIMENTAL SECTION

Apparatus. Fluorescence measurements were made with a Perkin-Elmer MPF-43A spectrofluorometer equipped with a 150-W Osram XBO lamp, excitation and emission monochromators, 1 × 1 cm quartz cells, R-777 Hamamatsu photomultiplier, and a Perkin-Elmer 023 recorder. Instrument sensitivity was adjusted daily, using a rhodamine B bar as a reference standard. A differentially corrected spectra unit, Perkin-Elmer Model DCSU-2, connected to the MPF-43A spectrofluorometer was used to obtain the derivative spectra.

Reagents and Solutions. Stock solution of propham (>99% pure, Serva, Feinbiochemica, Heidelberg) was prepared in ethanol (1 mg/mL). Standard working solutions were prepared from this by dilution with ethanol.

Analytical reagent grade fluorescamine (4-phenylspiro[furan-2(7*H*),1′-phtlan]-3,3′-dione) obtained from Aldrich Chemical Co., Inc., was dissolved in acetone (1 mg/mL).

The herbicide was hydrolyzed by a 1 M solution of sodium hydroxide. A 0.1 M pH 4.0 potassium phthalate buffer was also prepared. The ethyl acetate used for extraction and the other solvents used were of analytical reagent grade.

Reaction Procedure. A stock solution of the herbicide (0.2 mL) was placed in a 15-mL test tube. A 1 M sodium hydroxyde solution (0.5 mL) was added and the mixture was taken to a final volume of 5 mL with deionized water, heated in a water bath at 100 °C for 40 min, then left to cool at room temperature. Aliquots of the aniline were taken to give a final concentration between 24 and 2000 ng/mL. These were transferred to 10-mL standard flasks. The fluorescamine solution (0.5 mL) was added to each flask, and the contents were mixed and taken to the final volume of 10 mL. They were mixed again and made up to the final volume with the potassium phthalate buffer and agitated. The fluorescence intensity was measured at λ_{ex} = 400 nm and λ_{em} = 510 nm, against a reagent blank.

For the extraction with ethyl acetate, 5-mL portions of the prepared solutions were placed in 15-mL test tubes; the same volume of ethyl acetate was added and the mixture was shaken vigorously for a few seconds and allowed to stand for 10 min. The fluorescence intensity of the organic phase was measured at λ_{ex} = 410 nm and λ_{em} = 493 nm.

Analysis of Water Samples. Three 1-mL and three 2-mL water samples taken from a well near cultivated ground (Benagalbón, Málaga, Spain) were spiked with a standard solution of IPC in ethanol to give final concentrations of 200, 300, and 400 ng/mL. The samples and a blank were transferred to 15-mL test tubes with 0.5 mL of 1 M NaOH and made up to 5 mL with deionized water. They were heated in a water bath at 100 °C for 40 min and allowed to cool to room temperature. Aliquots of 100 μL were taken and treated as described under reaction procedure.

RESULTS AND DISCUSSION

Reaction Conditions. Fluorescamine, itself nonfluorescent, reacts rapidly with primary aliphatic or aromatic amines to give highly fluorescent pyrrolidine derivatives and nonfluorescent hydrolysis products. Since IPC is a secondary aromatic amine, two steps are necessary to obtain the fluorescent derivative: the hydrolysis of the carbamate in sodium

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Table I. Characteristics of the Analytical Methods

method	slope, cm ng^{-1} mL	intercept, cm	S_A, ng/mL	C_L, ng/mL	dynamic range, ng/mL	RSD,[a] %	error, %
A[b]	0.04[e]	0.08[f]	44.0	220	730–2000	4.19	3.71
B[c]	0.006	0.49	48.0	110	370–2000	4.95	4.43
C[d]	0.004	0.36	50.0	120	390–2000	5.15	4.64
A	0.06[e]	3.83[f]	19.0	51.5	172–400	8.45	7.54
B	0.05	1.52	10.0	25.2	84–400	5.06	4.53
C	0.03	1.17	10.3	30.0	100–400	5.02	4.49
A	0.42[e]	−4.36[f]	3.4	13.5	45–175	3.79	3.39
B	0.12	−0.27	2.5	7.2	24–175	2.93	2.62
C	0.07	0.08	4.6	12.8	42–175	5.24	4.69

[a] Relative standard deviation (RSD). [b] Normal spectrofluorometric method. [c] Synchronous first-derivative spectrofluorometric method. [d] Synchronous second-derivative spectrofluorometric method. [e] I_F ng^{-1} mL. [f] I_F.

hydroxide and the subsequent coupling of the liberated aniline to the fluorescamine in an appropriate buffer.

After a detailed absorptiometric and fluorometric study, a 1 M sodium hydroxide solution was found to be the most suitable for the liberation of the aniline. The hydrolysis is complete in 40 min at 100 °C. This is considerably below previously established hydrolysis times (*2*). In this solution, the coupling of the aniline to fluorescamine takes place instantaneously, but it is necessary to reduce the hydrolysis solution pH to the optimum value before the fluorescamine reaction will give any significant yield of derivatives.

We examined the effects of the pH on derivative formation in the media and the relative fluorescence intensity of the IPC derivative. In the range of pH 3.5–4.2, the variations were minimum, and pH values greater than 6.0 produced very little fluorescence. Optimum pH was maintained by using a pH 4.0 potassium phthalate buffer.

The amount of fluorescamine used was found to influence the results. Approximately a 5-fold reagent excess is required for complete formation of the derivative. The order of mixing of the aniline, buffer, water, and fluorescamine to obtain the derivative was examined. No appreciative changes were observed, so the sequence aniline, buffer, fluorescamine, and water was chosen for the present study.

Fluorescence Phenomena. The fluorescence characteristics of the IPC derivatives are shown in Figure 1a. The main fluorophor formed is an acidic compound. The possibility of enhancing the sensitivity of the fluorescamine derivative by using different organic solvents to extract the fluorophor was investigated. Ethyl acetate gave the best results. The other solvents used were cyclohexane, hexane, chloroform, and toluene. As previously indicated (*12*), the fluorophor is quantitatively extractable at pH 5.0–5.5, so the reaction was carried out at the optimal reactivity. The fluorophor was extracted directly into ethyl acetate, and fluorescence was measured at the respective emission and excitation maxima.

When the fluorophor was extracted with ethyl acetate, a hypsochromic shift of the emission band was observed (Figure 1a) and the sensitivity of the method was significantly improved by this procedure (Table I). Derivative fluorescence was found to be strong in solvents with medium dielectric constant values like methanol, propanol, ethanol, and water but was extremely weak in dimethylformamide (ϵ = 36.71); consequently, the aqueous buffer medium was chosen for the experimental work.

Synchronous Derivative Spectrofluorometry. Instrumental Parameters. The most appropriate parameters to register both synchronous and derivative spectra for the procedure with and without solvent extraction were selected.

Various synchronous spectra at different scanning intervals ($\lambda_{em} - \lambda_{ex} = \Delta\lambda$) were recorded. The best results were $\Delta\lambda$ = 100 nm (without extraction) and $\Delta\lambda$ = 83 nm (with extraction). To register both synchronous first and second derivatives, a combination of 120 nm/min scan speed, 3-s time constant,

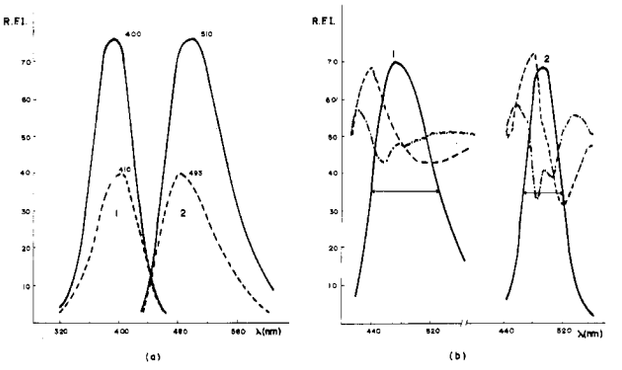

Figure 1. (a) Excitation (1) and emission (2) spectra of the fluorescamine derivatives: (—) without extraction (10 μg/mL), sensitivity 0.3–2, slits 10–10; (- - -) with extraction (2 μg/mL), sensitivity 0.1–3, slits 10–10. (b) (1) Fluorescence emission spectrum of IPC (2 μg/mL) and its first and second derivative; (2) synchronous spectrum of the same solution ($\Delta\lambda$ = 100 nm) and its first and second derivative. The double-headed arrows show the reduction of bandwidth (sensitivity 1–3, slits 10–10).

and $\Delta\lambda'$ = 10 nm gave the best conditions.

The reduction of the peak half-width and the position of the synchronous maxima for both procedures were calculated (*13*). A reduction of $\Delta\lambda_{s,em}$ from 94 to 50 nm (without extraction) and from 87 to 54 nm (with extraction) was observed. It is important to note that the effectiveness of derivative spectroscopy is a function of the bandwidth of the normal spectrum. The spectra with large bandwidths will yield more intense derivative signals than spectra with narrow bandwidths. This point is well illustrated in Figure 1b.

Quantitative Analysis. Standard curves were constructed by analyzing a series of samples of known IPC concentration. The results of the different methods are given in Table I. The detector response was linear over the IPC concentration range of 24–2000 ng/mL. Linear least-squares regression analysis gave lines with intercepts that were not statistically different from zero.

To determine the error inherent in the derivatization reaction step, intraassay precision was determined by analyzing a set of seven replicate samples that contained 1.0 μg/mL and were prepared from the same hydrolyzed solution. On the other hand, to evaluate the error of the hydrolysis reaction, interassay precision was determined by analyzing seven aliquots of a 1.0 μg/mL sample from individual hydrolyzed solutions. Both determinations were performed by normal fluorescence methods. The intraassay relative standard deviation and error were 4.1% and 3.71%, respectively. The interassay relative standard deviation and error were 2.01% and 1.80%, respectively, which indicates that the higher percentage of error is caused by the hydrolysis reaction, as may be expected.

The limit of detection, C_L, and quantitation, C_Q, used as the lowest values of the linear dynamic range for all the

Table II. Interference Study

interferent	ratio, IPC:interferent	% recovery: 1 μg/mL IPC A	1 μg/mL IPC B	1 μg/mL IPC C	0.2 μg/mL IPC A	0.2 μg/mL IPC B	0.2 μg/mL IPC C	0.088[a] μg/mL IPC A	0.088[a] μg/mL IPC B	0.088[a] μg/mL IPC C
NAA	1:20	113.8	108.5	94.0			105.5			
	1:10							115.3	91.3	115.4
NAA + 2,4-D	1:25:100					112.0	93.0			
	1:50:200							98.6	97.2	112.3
2,4-D	1:20	107.3	110.5	91.0	123.0	99.8	82.5			
	1:10							103.2	92.5	115.4
2,4-D + flur	1:20:10			117.7						
	1:200:100							113.2	102.0	112.4
flurecol	1:20	116.0	114.7	103.5	126.0	94.8	82.5			
	1:10							105.1	95.0	100.2
flur + colch	1:10:10			111.1						
	1:50:25					99.3	95.0			
	1:100:50							106.5	92.5	120.4
colchicine	1:20				126.0	101.0	80.5			
	1:10	109.8	104.3	91.0				103.2	92.5	100.2
dimethoate	1:20	103.5	102.2	85.0	126.0	102.5	80.5			
	1:10							112.2	95.0	96.1
NAA + dimeth	1:20:20		117.7							
	1:25:50				101.8	101.3				
	1:50:100							109.2	102.0	112.3
flur + dime + colchicine	1:10:20:10		107.0	101.0						
2,4-D + flur + dimethoate	1:20:10:20	115.9	107.0	117.7						

[a] Method with extraction procedure.

Table III. Application to Wellwater Samples

concn of IPC added, ng/mL	vol water taken, mL	% recovery[a] A	B	C
200	1	103.15	93.25	82.65
	2	111.50	94.90	80.50
300	1	103.23	95.17	90.57
	2	149.55	93.66	106.43
400	1	118.52	105.90	96.67
	2	147.75	108.25	96.50

[a] Mean of three determinations.

methods are reported by the definitions accepted by IUPAC (*14*). To compare all the methods in a coherent units system, we give a new definition of analytical sensitivity, s_A, as $s_A = s_S/m$, where s_S is the standard deviation of the analytical signals at a particular concentration and m is the slope of the calibration curve. With this definition, both s_S and m of the analytical procedure are introduced, and this value, s_A, can be expressed in concentration units, which is easily comparable, irrespective of the nature of the analytical signal.

The reproducibility of the methods was determined by simultaneously performing the labeling reaction on seven hydrolyzed IPC samples of equal concentration. At concentration levels ranging from 0.088 to 1.0 μg/mL the data were found to vary from 2.01 to 5.06% relative standard deviation with an average of 4.05% (see Table I).

Interference Study. The effect of other pesticides on the determination of 1.0, 0.2, and 0.088 μg/mL of IPC by the different methods proposed was studied, in particular the plant growth regulators naphthaleneacetic acid (NAA) and colchicine, the herbicides (2,4-dichlorophenoxy)acetic acid (2,4-D) and flurecol, and the insecticide dimethoate.

The results are shown in Table II. The criterion for interference was a deviation of the fluorescence intensity or derivative value of $\bar{x} \mp ts_A$, where $\bar{x}$ is the mean value of the IPC alone, s_A is the standard deviation of the analytical signal, and the "Student's t" test is the value for seven measurements.

The specificity of the methods was checked in relation to the five pesticides listed in Table II. The values indicate that most of the mixtures examined gave positive interferences when tested by the three methods. Generally, at the different levels of IPC concentration, the recoveries are lower in changing from normal to second derivative, except on the extraction method where differences were observed. It is also observed that for high concentrations of IPC, the normal fluorescence method is best because of the wider tolerance associated with the imprecision and lack of sensitivity ($>s_A$). For lower concentrations of IPC, the best markedly superior results are obtained with synchronous first-derivative methods (with or without extraction), which demonstrates the higher sensitivity and selectivity of the derivative methods.

Application to Natural Samples. Results of the analysis of local wellwater samples by the standard addition method are shown in Table III. The graphs are linear for both 1.0- and 2.0-mL water samples, indicating the persistence of the relation between relative fluorescence intensity or derivative value (cm) on the concentration of IPC. On the other hand, for the normal spectrofluorometric methods, the increase in slope as the sample volume increases reveals the existence of a matrix effect for high water volumes, which is conveniently side-stepped by application of the synchronous first- and second-derivative method where no changes in the slopes may be seen.

CONCLUSION

The usefulness of fluorescamine as a labeling reagent is extended to the quantitation of compounds containing secondary amino groups if they are previously hydrolyzed to a primary amino group. The method is simple, rapid, and reproducible. Combination of the fluorogenic labeling with the sensitivity of the synchronous derivative technique, enhanced by the organic solvent extraction, gives analytical results comparable to those obtained by thin-layer chromatography. It is a convenient and simple means of analysis of pesticide residues when sophisticated and expensive instrumentation is not available.

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Negative Ion Mobility Spectrometry for Selected Inorganic Pollutant Gases and Gas Mixtures in Air

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Spectra for inorganic gases common to combustion emissions were obtained in air by using negative ion mobility spectrometry (NIMS). Gases were characterized individually from 10 to over 4000 ppb in air and in two binary mixtures of SO_2/NO_2 and HCl/SO_2. Spectra for HCl and SO_2 were distinguished by single intense peaks at reduced mobilities (K_0) of 2.98 $cm^2/(V\ s)$ and 2.30 $cm^2/(V\ s)$, respectively. The product ion for NO_2 had a K_0 of 2.80 $cm^2/(V\ s)$ as determined using binary mixture studies and was unresolved otherwise from reactant ions. In contrast, NIMS spectra for H_2S showed three peaks that were concentration dependent and had mobility values of 2.38, 2.28, and 1.70 $cm^2/(V\ s)$. Peak currents for H_2S ranged from 10 to 50 pA and the estimated limit of detection (LOD) was 10 ppb. Peak currents for SO_2 ranged from 15 to 65 pA and the estimated LOD was 2 ppb. The range of peak currents for HCl was comparatively large at 140–215 pA and estimated LOD was <1 ppb. At constant concentration for HCl at 60 ppb, SO_2 was added from 100 to 900 ppb. Competitive ionization was observed in the order of $HCl > NO_2 > SO_2$.

An environmental aspect in the operation of coal-fired power plants, municipal incinerators, and other combustion-based units is production of large amounts of inorganic waste gases, including SO_2, NO_2, HCl, CO, and CO_2. In addition to corrosion of equipment from acids, release of these gases into atmospheric environments has also been associated with other deleterious effects, such as acid deposition and respiratory irritation in humans. Subsequent to the Clean Air Act of 1968, monitoring of gaseous emissions for inorganic gases from stacks as point sources has been practiced nationally. Presently, at least 10 methods have been developed for sensing such pollutants and include wet chemical (*1*), electrochemical (*2*, *3*), spectroscopic (*4*, *5*), and chemiluminescence (*6*) methods. When evaluated in terms of speed, sensitivity, selectivity, continuous operation, automation, and low cost, these approaches all have certain limitations. For example, chemiluminescence detection of SO_2 may have interference from other sulfur-containing compounds (*6*), while peroxyacetyl nitrate (a common photochemical pollutant) can greatly interfere in the detection for NO_2. Electrochemical sensors for these gases have been reviewed in detail (*2*, *3*) but, despite promise, are limited by specificity, the number of sensors needed, and maintenance demands. Instrumentation for optical spectroscopic techniques, including Fourier transform infrared spectrometry, with better specificity and speed can be expensive, and inexpensive wet chemical and adsorbent methods are slow and labor intensive. Additionally, a more serious limitation for wet chemical techniques is the time-averaged or integrating nature of such sensors.

Ion mobility spectrometry (IMS) is an atmospheric pressure based technique that has been used almost exclusively in prior applications for separation and detection of organic compounds in the vapor phase. Reactant ions which are produced from β emission into a supporting gas (N_2, air) react with analyte through ion/molecule collisions and product ions are produced. Although formation of positive product ions through proton (or NH_4^+ and NO^+) exchanges has been more generally used, negative product ions can also be formed through electron attachment and other mechanisms (*7*, *8*). Thus, negative ion mobility spectrometry (NIMS) will result in a mobility spectrum for separation and detection of only negative product ions. These reactions are especially useful for compounds containing atoms with high electron affinity such as halogens, oxygen, and nitrogen. Since compounds that do not attract or hold protons may show some sensitivity as negative ions, NIMS has advantages of selectivity and possible extension to applications with inorganic gases not usually associated with IMS. Although inorganic gases have been characterized by use of atmospheric pressure ionization mass spectrometry (*9*, *10*), and NIMS spectra for a few of these gases have been reported (*11*), response of NIMS toward such compounds has been unexplored.

Work described below is directed at two aspects of use of IMS in sensing of atmospheric environmental pollutants: (1) possible interferences from inorganic gases in NIMS detection of organic compounds, and (2) direct multiple analysis of emissions for inorganic gases. Should IMS be used for in-stack monitoring of organic compounds such as polycyclic aromatic hydrocarbons or polychlorinated biphenyls, inorganic gases may certainly be major chemical interferences. Thus, knowledge of IMS behavior for HCl, SO_x, NO_x, and others will provide background information on feasibility for using NIMS

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