prove useful in the development of azadirachtin as a model compound for the chemical synthesis of a new class of insecticides.

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Feasibility Study of Constant Energy Synchronous Luminescence Spectrometry for Pesticide Determination: Application to Carbaryl, Naphthol, and Carbofuran

Leigh Ann Files[1] and James D. Winefordner*

Constant energy synchronous luminescence spectrometry (CESLS) at low temperature (77 K) is applied to pesticide determination. This method is inexpensive, selective, and sensitive. Limits of detection, linear dynamic ranges, and results obtained using a variety of scan parameters are given for carbaryl, naphthol, and carbofuran.

Carbamate pesticides are widely used to protect plants from insects (Haskell, 1985). Due to the toxicity of these compounds and the possibility of residual presence in the environment and crops, there is an obvious need for a sensitive and reliable method for determining them. A method for distinguishing pesticides from their hydrolysis products and/or metabolites could also have a wide variety of applications for studies involving optimizing application concentrations and times, as well as metabolism of pesticides by insects, animals, and humans.

Pesticide determination has been done by a wide variety of methods ranging from liquid chromatography followed

Department of Chemistry, University of Florida, Gainesville, Florida 32611.

[1]Present address: P.O. Box 655936, MS 147, Texas Instruments, Dallas, TX 75265.

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by mass spectrometry (Voyksner and Bursey, 1984) to room-temperature phosphorescence (Asafu-Adaye et al., 1985), UV detection (Sparacino and Hines, 1976), and a variety of fluorescence techniques (Argauer and Warthen, 1975; Guilbault and Sadar, 1969; Frei and Laurence, 1972; Larkin and Day, 1979). Fluorescence techniques are among the most sensitive because of the high fluorescence quantum yields of many pesticides (Larkin and Day, 1979). Generally luminescence detection follows separation methods such as HPLC (Argauer and Warthen, 1975) or TLC (Frei and Lawrence, 1972). Separation is usually required due to overlap in conventional luminescence spectra of the compounds of interest.

Recently, a new fluorescence technique has been established that is much more suited to mixture analysis. This method is constant energy synchronous luminescence (fluorescence and phosphorescence) (CESLS). CESLS involves scanning simultaneously the excitation and emission monochromators while maintaining a constant energy difference between them. The theory and advantages of this method have been described previously (Inman et al., 1986; Inman and Winefordner, 1982). Measurements at low temperature (77 K) have also been demonstrated to further enhance sensitivity and resolution (Kerkhoff et al., 1984).

The main advantages of CESLS at low temperature over conventional luminescence measurements, which are higher resolution and increased optical throughput, will be demonstrated in this paper. It will be shown that CESLS will allow analysis of compounds in a mixture without prior separation. This is a very attractive possibility since separation techniques generally increase the cost and time required for each analysis.

For this study carbaryl, naphthol (a hydrolysis product of carbaryl), and carbofuran were evaluated with respect to identification, limits of detection, and linear dynamic ranges. Band-passes and constant energy differences were varied to evaluate their effect upon spectra obtained.

EXPERIMENTAL SECTION

Instrumentation. The experimental setup used for this study is similar to one used previously (Kerkhoff et al., 1985). Changes were made and described in a more recent publication (Files et al., 1987). Two monochromators of moderate resolution were used, varying spectral band-passes from 1.5 to 4 nm. The scan rate was approximately 50 nm/min, allowing a constant energy scan from 250 to 350 nm to be collected in 2 min. The excitation monochromator was pulsed at a constant rate while the emission monochromator was pulsed at a variable and faster rate to maintain the desired constant energy difference. Scan rates were controlled by an Apple II pulse microcomputer through a versatile interface adapter (VIA, SY622). Spectra (relative fluorescence vs. excitation wavelength) were taken at low temperature (77 K) with the use of a liquid nitrogen Dewar system and round quartz sample tubes (3-mm i.d. and 5-mm o.d.).

Sample Preparation. Stock solutions of 100 ppm carbaryl, naphthol, and carbofuran (obtained from EPA, Research Triangle Park, NC) were made in reagent-grade ethanol (obtained from Aaper Alcohol & Chemical Co., Shelbyville, KY). These stock solutions were subsequently diluted in ethanol to form samples for calibration curves and to obtain spectra for demonstration purposes.

RESULTS AND DISCUSSION

Figures 1 and 2 show conventional excitation and emission luminescence spectra for carbaryl and naphthol, respectively. Spectra were taken at 77 K and were not corrected for instrumental response. These spectra will

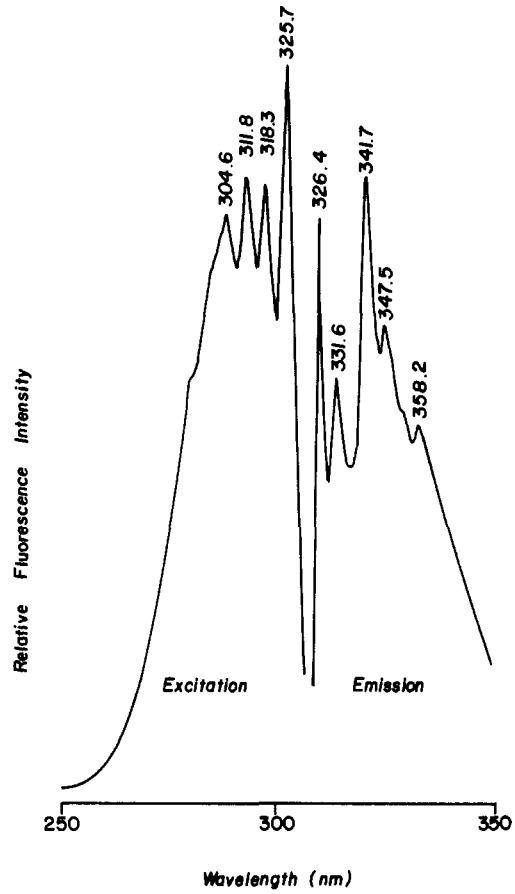

Figure 1. Excitation and emission scans of carbaryl. Prominent peaks are listed in Table I.

Table I. Calculated Energy Transitions (cm^{-1}) for Carbaryl

excitation peaks, nm	emission peaks, nm			
	315.7	320.7	330.5	335.5
273.2	4926	5413	6347	6792
283.1	3650	4137	5070	5515
294.3	2310	2797	3730	4176

Table II. Calculated Energy Transitions (cm^{-1}) for Naphthol

excitation peaks, nm	emission peaks, nm				
	326.4	331.6	341.7	347.5	358.2
304.6	2193	2673	3565	4053	4913
311.8	1435	1915	2806	3295	4154
318.2	780	1260	2152	2640	3500
325.7	66	547	1438	1927	2786

be used here to demonstrate the results one can expect with constant energy scanning and variations in band-passes.

From conventional luminescence excitation and emission spectra of the three compounds, tables of possible energy differences between each excitation and emission pair were calculated from the expression

$$\Delta\nu = (1/\lambda_{ex} - 1/\lambda_{em}) \times 10^7$$

where λ_{ex} = excitation wavelength (nm), λ_{em} = emission wavelength (nm) and $\Delta\nu$ = constant energy differences between excitation and emission monochromator outputs (cm^{-1}).

Tables I and II contain results of these calculations for carbaryl and naphthol, respectively. On the basis of these calculations, constant energy scans were obtained at a variety of energy differences. For example, naphthol has three transitions with an overall vibrational energy loss

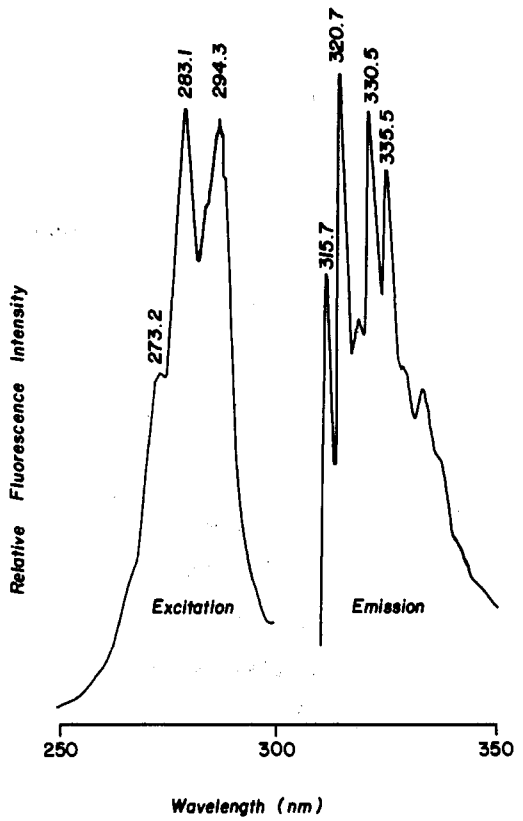

Figure 2. Excitation and emission scans of naphthol. Prominent peaks are listed in Table II.

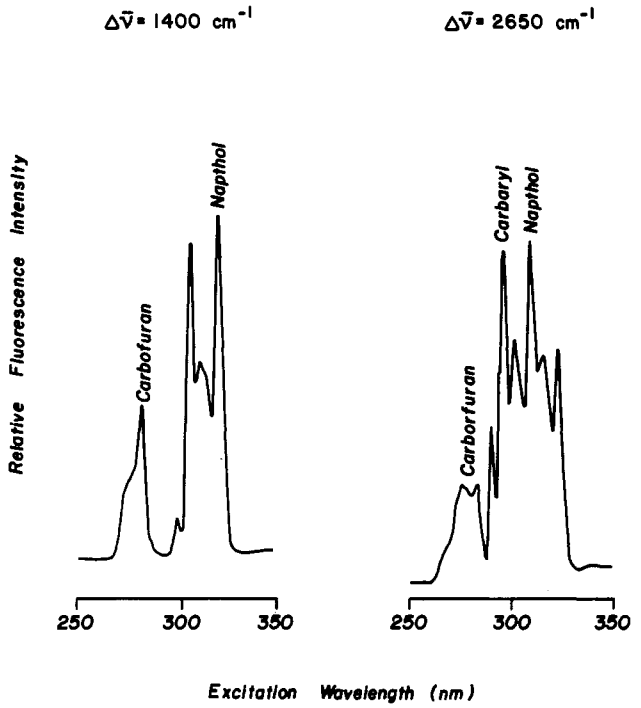

Figure 3. Constant energy scans of a mixture of carbaryl, naphthol, and carbofuran with $\Delta\nu$ = 1400 and 2650 cm^{-1}. Excitation and emission band-passes 1.5 nm.

close to 1400 cm^{-1} (see Table II). Tables I and II for naphthol and carbaryl give the energy differences between excitation and emission peaks (cm^{-1}); an energy difference of 2650 cm^{-1} is a compromise for identification of both compounds as can be seen by the values in Tables I and II. Carbofuran could be determined at a wide range of constant energy differences as illustrated in Figure 3, which includes constant energy synchronous luminescence scans

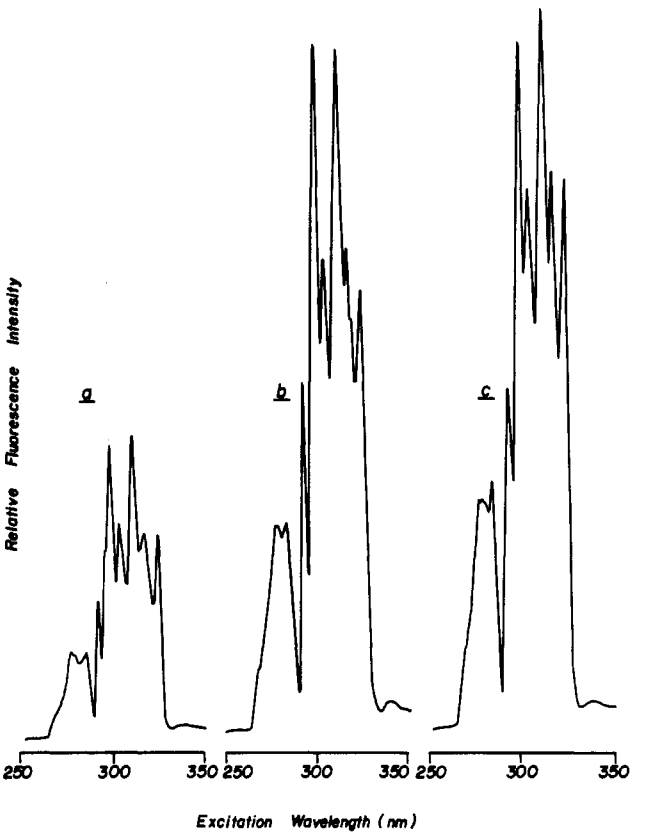

Figure 4. Constant energy scans of a mixture of carbaryl, naphthol, and carbofuran: (a) band-passes of 1.5 nm on both monochromators; (b) excitation band-pass of 2.5 nm and emission band-pass of 1.5 nm; (c) excitation band-pass of 1.5 nm and emission band-pass of 2.5 nm. The value of $\Delta\nu$ for these spectra is 2650 cm^{-1}.

of a mixture of 4 ppm carbaryl, 4 ppm carbofuran, and 2 ppm naphthol at both $\Delta\nu$ = 1400 and 2650 cm^{-1}. Also, this figure demonstrates the advantage of using several constant energy scans for confirmation of identification. Peaks used for identification and limit for detection measurements are labeled. These scans were obtained with 1.5-nm band-passes on the excitation and emission monochromators. Concentrations were calculated on the basis of peak heights and comparison to standard calibration curves. The mixture was run in triplicate, and peak heights were averaged to minimize variation in signal intensity due to effects such as solvent cracking and changes in sample tube alignment. Values detected in this manner gave results within 13% of the true value for all three compounds; the accuracy was primarily determined by a precision of ~10% rsd for replicates. Precision and accuracy could be improved considerably by improved sample tube alignment methods and solvents that form clear glasses. In conventional luminescence, the accuracy and precision of such measurements are dependent on the relative concentrations (thus the relative luminescence signals) of the compounds being quantitated, which influences spectral overlap and therefore random and systematic errors. The excellent resolution obtained with CESLS as shown in Figure 3 minimizes this problem.

Theory presented earlier by Inman et al. (1986) indicated that it should be possible to maintain good resolution while increasing signals by opening either the excitation or emission monochromator and keeping the other narrow. Therefore, it was decided to evaluate these same compounds under varying conditions. Table III gives limits of detection obtained for the three compounds at a variety of band-passes and energy differences. Linear dynamic

Table III. Limits of Detection (LOD)[a] for Compounds with Variable Parameter

compound	$\Delta\nu$, cm^{-1}	band-passes[b]	LOD, ppb
carbaryl	2650	1.5, 1.5	24
	2650	4.0, 1.5	10
	2650	1.5, 4.0	13
naphthol	1400	1.5, 1.5	6
	2650	1.5, 1.5	7
	2650	4.0, 1.5	2
	2650	1.5, 4.0	3
carbofuran	1400	1.5, 1.5	20
	2650	1.5, 1.5	45
	2650	4.0, 1.5	13
	2650	1.5, 4.0	18

[a] LOD defined as concentration (ppb) giving a signal to noise ratio of 3. [b] Band-passes for excitation, emission monochromators in nanometers. *Note*: Linear dynamic ranges were approximately 3.5 orders of magnitude extending from the limits of detection for all conditions and compounds.

ranges were approximately 3.5 orders of magnitude for the three compounds under all conditions.

Figure 4 shows the effect of varying band-passes on the excitation and emission monochromators for the three-component mixture. For scan (a) both monochromator band-passes are 1.5 nm; for (b) the excitation band-pass is 2.5 nm and the emission band-pass is 1.5 nm; and for scan (c) the excitation band-pass is 1.5 nm and the emission band-pass is 2.5 nm. It can be seen that, for these variations in band-passes, signal intensities are increased while resolution is maintained for identification and quantitation purposes. If the discrepancy between band-passes becomes larger than 1.5–2.5 nm, then a *slight* resolution loss can occur particularly for narrow excitation peaks when a wide excitation band-pass is used or for narrow emission peaks when a wide emission band-pass is used; larger band-passes will, of course, increase optical throughput and thus signal levels. Optimum parameter selection will be determined by the complexity of the mixture being studied, the specific components being determined, and the concentration of the analytes. Depending on the application, one may trade resolution for increased sensitivity or vice versa.

In conclusion, we have demonstrated the sensitivity and selectivity of CESLS, an inexpensive and reliable method, for measurement of pesticides and hope that in the future this technique will find wide applicability to studies involving pesticides as well as other complex mixtures where physical separations may be avoided.

Registry No. Carbaryl, 63-25-2; naphthol, 90-15-3; carbofuran, 1563-66-2.

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Chloroacetanilide Herbicide Selectivity: Analysis of Glutathione and Homoglutathione in Tolerant, Susceptible, and Safened Seedlings

E. J. Breaux,*[1] James E. Patanella, and Ernest F. Sanders

The basis for selective phytotoxicity is often the lack of metabolic deactivation in susceptible plants. For example, the selective chloroacetanilide herbicides alachlor, acetochlor, and metolachlor are metabolized less readily by susceptible weeds such as barnyardgrass than by tolerant corn seedlings. Chloroacetanilide herbicide tolerance is due to conjugation with glutathione (GSH; glutamylcysteinylglycine) or homoglutathione (hGSH; glutamylcysteinyl-β-alanine). New analytical methods were developed and used to analyze these tripeptide thiols in plants. These methods are based on the selective derivatization of these detoxification thiols with radiochemically labeled maleimides such as *N*-ethylmaleimide. The maleimide adduct derivatives were then separated by reversed-phase high-performance liquid chromatography (RP HPLC) and quantitated with the aid of a radiochemical HPLC detector. By these new methods it was found that chloroacetanilide herbicide tolerance was related to the seedling detoxification thiol content. It was also found that the herbicide safener flurazole caused the level of GSH to increase in the shoots of treated corn and sorghum.

Chloroacetanilide herbicides are widely used for the control of grass and some problem broad-leafed weeds in a variety of major crops such as corn and soybeans (Beste, 1983). It has been reported that the biochemical basis for selectivity is the metabolic detoxification of these herbicides by conjugation with either glutathione (Lamoureux et al., 1971) or homoglutathione (Breaux, 1986) in tolerant plants. Glutathione or homoglutathione conjugation is also involved in the detoxification of the chloroacetanilide herbicide acetochlor in susceptible crops and weeds (Breaux and Patanella, 1985). This herbicide was found to be metabolized more rapidly in the tolerant plants to

Monsanto Agricultural Company, St. Louis, Missouri 63167.
[1] Present address: Rhone-Poulenc Agrochimie, 14-20 Rue Pierre Boizet, BP 9163, Lyon Cedex 1, France.

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