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Laser Induced Fluorimetry IV. Determination of N-Methylcarbamates by 7-Chloro-4-Nitrosobenz-2-Oxa-1,3-Diazole

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A new sensitive fluorimetric method for the determination of N-methylcarbamates, a class of well known insecticides, based on the derivatization with 7-chloro-4-nitrosobenz-2-oxa-1,3-diazole (NBD-Cl) has been developed. Unreacted NBD-Cl was eluted ahead of derivatized carbamates from C-18 bonded column. An argon ion laser was used as an excitation source of chromatographic eluents and its fluorescence signal was monitored with optical multichannel analyzer. The detection limits of various carbamates were about 100 pg range and the working curves were linear to 10^4–10^5 nanogram ranges.

Introduction

Carbamate insecticides are of considerable interest since they are being used in many agricultural works as alternatives to the environmentally more hazardous halo-hydrocarbon insecticides. So, it is desirable to have a determination technique capable of separating and detecting low level residues of these compounds. The presently available routine methods of analysis of the carbamates are GC-MS[1], HPLC-MS[2], fluorimetry[3-5], absorption spectrophotometry[6], and phosphorimetry[7].

Most of the fluorimetric analyses of non-fluorescent chemicals were based on the derivatization reaction with fluorophores including dansyl chloride[8], o-phthalaldehyde[5] or 7-chloro-4-nitrosobenz-2-oxa-1,3-diazole (NBD-Cl)[3,9,10]. Out of these chemicals, NBD-Cl is considered superior to others because it does not form fluorescent derivatives with phenols, thiols, alcohols nor anilines but reacts well with alkylamines to yield highly fluorescent product. And the product alkylamine with NBD-Cl in a polar organic solvent. Therefore, we report a new sensitive fluorimetric method for the determination of N-methyl carbamate based on the derivatization with NBD-Cl using an argon ion laser and an optical multichannel analyzer.

Experimental

Reagents and Solutions. 7-Chloro-4-nitrosobenz-2-oxa-1,3-diazole(NBD-Cl) was obtained from Sigma Chemical Company (St. Louis, MO.) and used without further purification. The N-methylcarbamates employed in this study were Sevin (1-naphthyl-N-methylcarbamate, carbaryl) from Samkong Corp. (Suwon, Korea), Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) from Samkong Corp., and Baygon (2-isopropoxyphenyl-N-methylcarbamate) from Polyscience Corp. (Niles, Ill.) and they were used as received. A 1.0% solution of NBD-Cl was prepared in methyl isobutyl ketone (MIBK). Stock solutions of

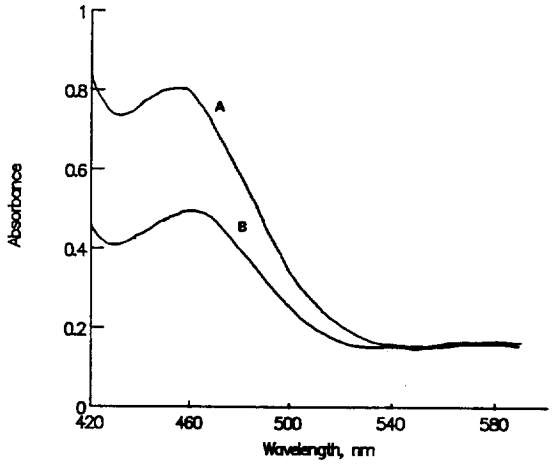

Figure 1. UV/Visible spectra of derivatized N-Methylcarbamate and blank; A: Derivatized product of Baygon (120 ppm) with NBD-Cl (1% in MIBK); B: Blank (1% NBD-Cl in MIBK).

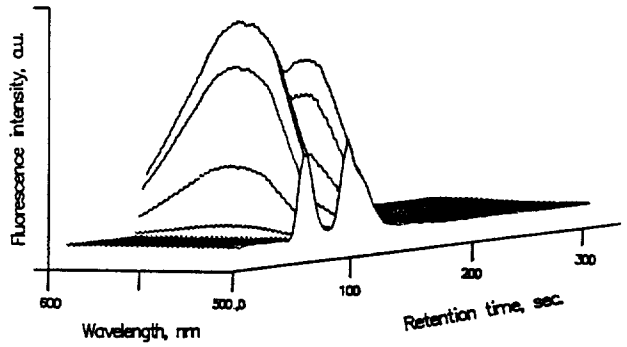

Figure 2. Three dimensional chromatogram of Sevin (injected quantity = 18 ng). See the text for other conditions.

dred microliters of N-methylcarbamates were transferred to a 5-m*l* test tube with a 100-μl Hamilton syringe, and the solvent was evaporated in a water bath kept at 40 °C. One milliliter of 0.1 M $NaHCO_3$ solution was added to the residue followed by the careful addition of an equal volume of NBD-Cl solution. The test tube was loosely stoppered, heated at 80 °C for 30 min, and cooled to room temperature. One 10 μl aliquot of the MIBK phase (top layer) was used for injection.

Chromatography. In order to choose a proper mobile phase, thin-layer plates were prepared by dipping the commercial TLC plates (Silica gel 60 F_{254}, Merck, Darmstadt, F.R.G.) into the 10% solution of liquid paraffin in hexane to coat silica with paraffin. Composition of the mobile phase was determined based on the TLC separation data which gave a suitable R_f value. The mobile phase was consisted of water (double-distilled)-acetonitrile-acetic acid (70 + 30 + 0.5). Reverse phase phase separation was carried out on a 30 cm × 3.9 mm id C-18 μ-Bondapak column (Waters Associates) at ambient temperature. The flow rate of mobile phase was 1 m*l*/min and it was degassed and filtered through Millipore membrane filtration equipment.

Fluorimetric Detection of HPLC Eluent. The excitation source for fluorimetric detection was 488 nm single line of an argon ion laser (Spectra Physics, model 164-05) and its output power was 100 mW. The signal accumulation number of the optical multichannel analyzer (OMA, E.G. & G. Princeton Applied Research Co., 1205A) was 90. Therefore, the fluorescence spectra of eluent were obtained at every 3 seconds in the wavelength window from 500 nm to 600 nm. The configuration of the experimental set-up and other details were published elsewhere.[10,11]

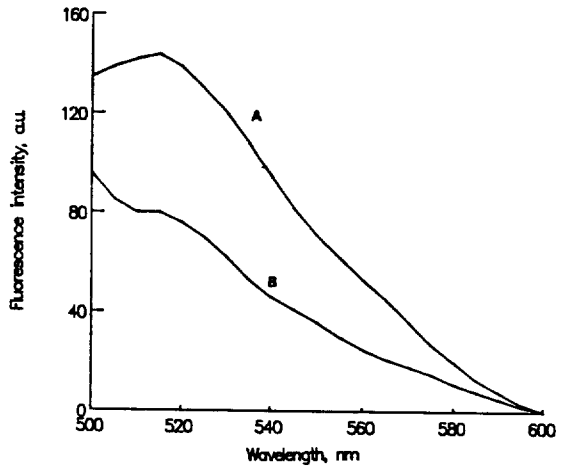

Figure 3. Fluorescence spectra of sample after column separation; A: Baygon (120 ppm) derivative; B: NBD-Cl blank. Spectra were obtained with Farrand MK-1 spectrofluorimeter. Excitation wavelength = 480 nm.

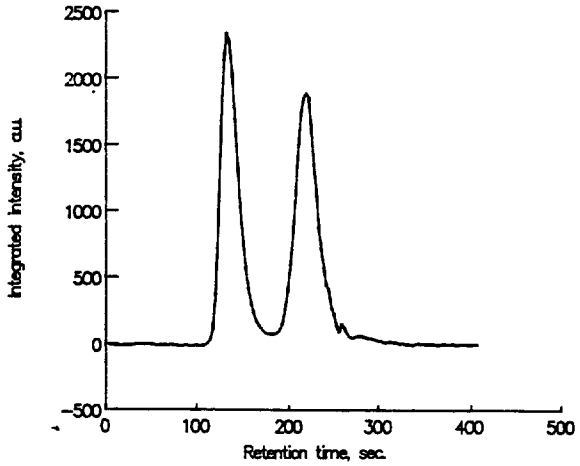

Figure 4. Chromatogram of Baygon by channel integration. Flow rate = 1 m*l*/min., Injected sample = 120 ng.

Results and Discussion

Figure 1 shows UV/visible absorption spectra of the derivatized product and blank mixture. It shows an increase of absorbance at absorption maxima around 460 nm which is quite suitable for the fluorescence detection by excitation of argon ion laser at 488 nm. A reaction time of 30 min at 80 °C seemed long enough for hydrolysis and coupling of alkylamine with NBD-Cl. Since NBD-Cl itself also absorbed at absorption maximum and fluoresced, it was necessary to separate the unreacted NBD-Cl from the derivatives. Figure 2 shows a three dimensional chromatogram of the reaction product (Sevin derivative) and the blank. Each spectrum was obtained at 3-sec interval. The first peak was due to unreacted NBD-Cl and the second peak was Sevin derivative. The fluorescence spectra of the unreacted NBD-Cl and the NBD

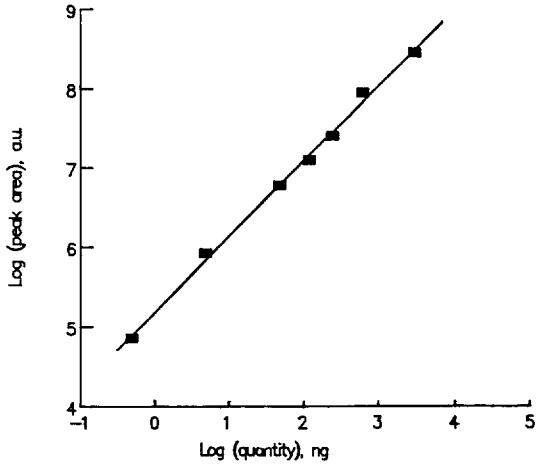

Figure 5. Analytical curve of Baygon.

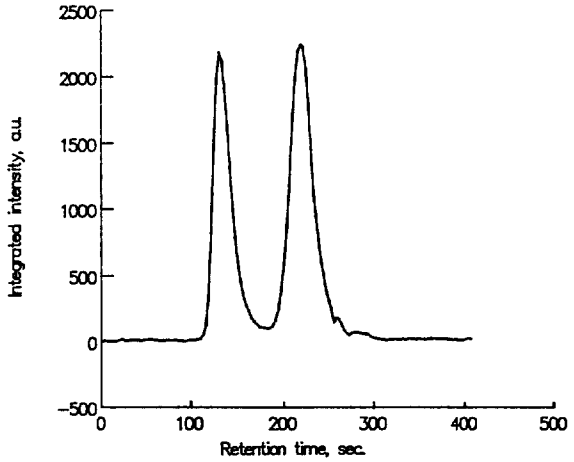

Figure 6. Chromatogram of Carbofuran by channel integration. Flow rate = 1 m*l*/min., Injected sample = 170 ng.

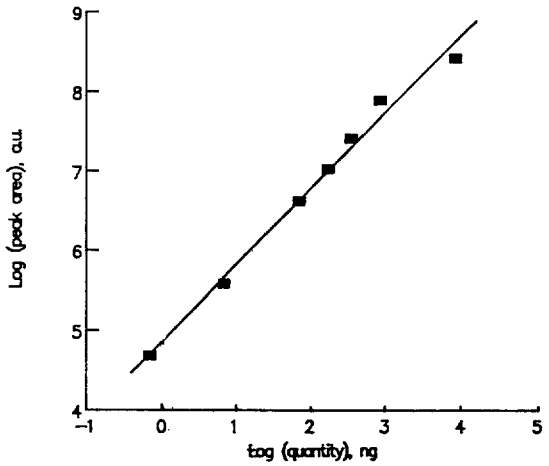

Figure 7. Analytical curve of Carbofuran.

derivative are shown in Figure 3. These were obtained with a conventional spectrofluorimeter after column elution. Lack of monochromaticity of the xenon lamp caused a strong scattering signal especially in the background and the resulting spectra showed lower fluorescence intensity.

We have report that the use of laser as an excitation source and the optical signal detection with an OMA by channel integration technique improved the sensitivity and the detection limit of the fluorimetric detection system[10,11]. Figure 4 shows the chromatogram of Baygon obtained by channel integration technique. The analytical curve of Baygon is shown in Figure 5. It was linear from 10 μ-gram down to subnanogram of Baygon with a slope close to unity. Other N-methylcarbamates showed a similar chromatographic behavior because the same NBD-methylamine would be formed from any N-methylcarbamates in MIBK layer. Similar figures of other carbamates are presented in Figure 6 through 9. The slope of analytical curves, correlation coefficients and the limit of detection of various carbamates are summarized in Table 1. The limits of detection were calculated by the method described by Knoll[12].

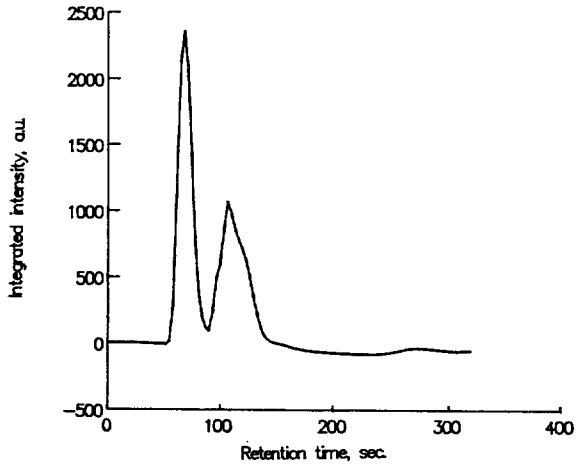

Figure 8. Chromatogram of Sevin by channel integration. Flow rate = 2 m*l*/min., Injected sample = 20 ng.

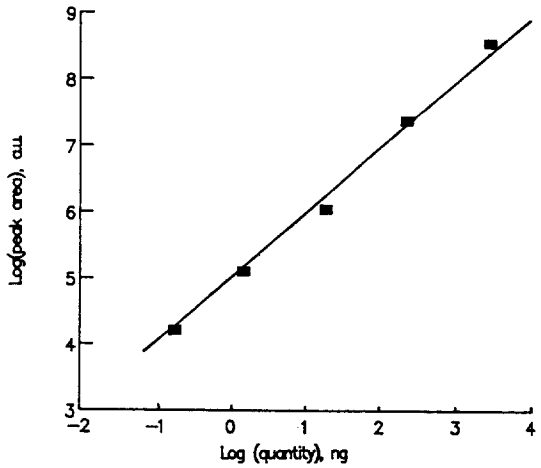

Figure 9. Analytical curve of Sevin.

The detection limit of carbamates and their analogues by various methods were reported. The detection limit obtained for ethyl carbamate by GC with Hall electrolytic conductivity detector was 10–20 ppb[1]. While that for 1-naphthyl N-methyl carbamate (carbaryl) by solid-surface room temperature phosphorimetry (SSRTP) was 800 pg[13]. Campiglia *et al.* improved the SSRTP technique and reported that 500 pg of carbaryl could be detected[7]. And the detection limit of the carbamates with HPLC-fluorimetry was 0.05 to 1 ppm[5].

Table 1. Summary of Analytical Curves of N-methylcarbamates

carbamates	slope[a]	R square[b]	LOD[c](pg)
Sevin[d]	0.98	0.9665	130
Carbofuran[e]	0.96	0.9885	300[f]
Baygon[g]	0.95	0.9962	140

[a]Slope from the analytical curves. [b]Correlation coefficients. [c]Limits of detection. [d]1-Naphthyl-N-methylcarbamate. [e]2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate. [f]The original sample assay was shown as 75%. [g]2-Isopropoxyphenyl-N-methylcarbamate.

In this study, we propose another micro-determination method of N-methyl carbamates which is simpler and more sensitive than the conventional fluorimetric determination of the post-column derivatives with OPA (o-phthalaldehyde)[14]. The presence of other carbamates such as N,N-dimethylcarbamates certainly will interfere the measurement but the quantification of N-methyl carbamate can be made without any problem because they exhibit different retention behavior[3]. The main limitation of this method is no capability of differentiating N-methyl carbamates in the mixture. Only the total amount will be provided.

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Polarographic Behavior of Cadmium-Tartrate Complexes in Weak Acid and Alkaline Media

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The electrochemical behavior of cadmium(II) in tartrate solution has been studied over the pH range of 6 to 13.6 in order to explain the phenomena of the changes in limiting current depending on the pH. The polarographic limiting current showed a constant value up to pH of 7.8, after which it decreased sharply to show a minimum at pH between 11 and 12. The limiting current, then, increased again with increasing pH. The number of peaks in cyclic voltammogram was 1 to 3 depending on the pH of the solution. Two other voltammetric peaks could be observed when the main reduction peak diminished. The decrease of limiting current at $7.5<pH<9$ was explained as the formation of complex $Cd(C_4H_3O_6)^-$. The increase of limiting current at strong alkaline solution, however, was due to the complex $Cd(Tart)_2(OH)_2^{4-}$.

Introduction

Lingane[1] first reported the limiting current obtained during the reduction of cadmium in basic tartrate supporting electrolyte was unusually small. Koh[2] observed that the limiting current and half-wave potential were constant up to pH 7.8, then the limiting current decreased sharply when pH was greater than 7.8 and the half-wave potential shifted to more negative potential as the pH exceeded 8.2. The limiting current was minimum at pH 11.2-11.4, then the current increased as pH was raised. He suggested that the process responsible for the decrease of the current in the pH between