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Note

Determination of carbamates by high-performance liquid chromatography with electrochemical detection using pulsed-potential cleaning

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Carbamate pesticides have come into common use worldwide in recent years. Thus analytical methods for carbamate pesticides are of interest in evaluating contamination of water and agricultural products.

The general interest in analytical methods for carbamate pesticides is evident from the extensive literature. Most authors conclude that gas chromatographic (GC) methods are basically unsuitable since the carbamates are generally unstable at the elevated temperatures needed for GC analysis. Interest has therefore turned to high-performance liquid chromatography (HPLC). The most common detector used with HPLC is the UV detector, and a number of articles discuss analysis for carbamates using HPLC with UV detection. The UV detector, however, is not as sensitive as desired for carbamates. Limits of detection are usually claimed to be in the low nanogram range, although, in a few cases, compounds are claimed to be detectable down to the mid-picogram range[1]. Somewhat lower limits of detection are claimed for post-column derivatization with fluorogenic agents[2,3].

In recent years, electrochemical detectors have been developed and applied to many classes of analytes. For some classes of analytes, electrochemical detectors have proved to be more sensitive that UV detectors. Previous attempts to apply electrochemical detection to carbamates have been successful in a few cases in which the specific carbamate pesticide also contains functional groups such as aromatic amines which can be oxidized readily (*e.g.* Aminocarb[4–7]). Mayer and Greenberg[8] report the detection of eight carbamates using a flow cell with a wax-impregnated graphite electrode. They worked at the positive potential limit for this electrode and obtained limits of detection below 5 ng for only three of the carbamates.

The purpose of this investigation was to apply a newly developed instrument and flow cell to the electrochemical detection of the common carbamate pesticides. The instrument was developed in the School of Chemistry of the Georgia Institute of Technology under a cooperative agreement with the U.S. Environmental Protection Agency[9–11]. The flow cell has a wall-jet configuration and a platinum working electrode. It is a prototype of a new cell under development by Hewlett-Packard.

One of the biggest problems in the use of electrochemical detectors is maintaining a clean electrode surface. Electrochemical detectors are reaction detectors, and reaction products tend to accumulate on the surface of the electrode, where they block the surface and lead to a deterioration of response. The most commonly used

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electrodes are made of carbon, and these must be cleaned and polished periodically. Several groups of workers have reported that electrodes can be cleaned *in situ* by periodically pulsing the electrode to extreme potentials[12–17]. In addition, Johnson[18] has reported that platinum electrodes cleaned in this manner are more active than other electrodes and that groups of compounds previously thought to be undetectable can now be detected. This approach was applied in this study to the detection of carbamate pesticides.

In this study a computer-controlled instrument is used. One advantage of such instrumentation is the ability to use the instrument in a wide range of modes by changing the computer software for control and data acquisition. Since the d.c. mode with cleaning pulses was a mode previously unused with this instrument, it was necessary to write a new computer program for this purpose. In addition, it was necessary to include programming to control an instrumental current offset and thus compensate for the high background currents at the extreme positive electrode potentials necessary for the oxidation of the carbamates. This allowed the instrument to be operated at a much higher gain setting and resulted in significant improvements in sensitivity and limits of detection.

EXPERIMENTAL

The instrument has been described in detail[10]. The isocratic HPLC system was the same as before[9], except that a Zorbax ODS column, 150 × 4.6 mm I.D., was used. For most experiments a Spectra-Physics 8770 pump was used. For other experiments a Haskel pneumatic-amplifier pump (No. 26740), as modified by DuPont for their Model 830 chromatograph, was used.

The wall-jet detector cell is a prototype of a cell under development by Hewlett-Packard. The case of the cell is machined from high-density polyethylene. The working electrode is a platinum disk of 0.81 mm diameter, the counter electrode is a length of platinum wire, and the reference eletrode is silver–silver chloride. The effluent enters the cell and is directed perpendicularly to the center of the working electrode by means of a short length of fused silica tubing (57 μm I.D.).

The mobile phase was acetonitrile–acetate buffer, pH 5.50 (1:1). The mobile phase was filtered through a 0.45-μm filter before use. The flow-rate was 1.0 ml/min.

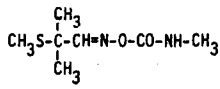
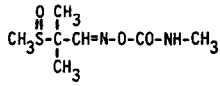
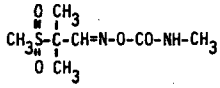
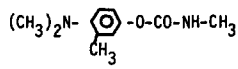
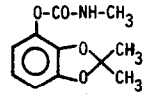
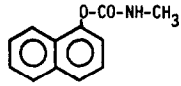
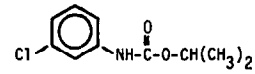
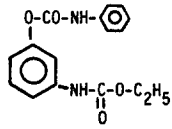
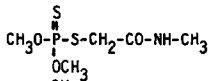
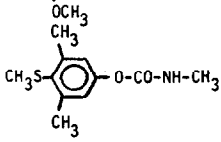
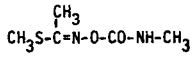
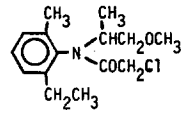
A selection of carbamate pesticides was obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC, U.S.A., and two others were obtained from the Toxicology Branch of the Centers for Disease Control in Atlanta, GA, U.S.A. The compounds studied, along with their structural formulas, are given in Table I. These compounds were used without further purification.

Preliminary chromatographic experiments were performed using a Chromatronix Model 220 UV detector and injections of approximately 500 ng of each carbamate. Without the availability of a gradient-elution system, it was necessary to compromise on a mobile-phase composition that resulted in acceptable retention and separation of most of the carbamates tested. The primary thrust of this work was the evaluation of the detector, and optimum chromatographic separation was considered to be of secondary importance. Table I summarizes the capacity factors obtained in this study. The multiple retention times indicate the impure condition of these commercial products.

TABLE I

CARRBAMATE PESTICIDES AND CAPACITY FACTORS

Column: Zorbax ODS 150 × 4.6 mm, I.D. Mobile phase: acetonitrile–acetate buffer, pH 5.50 (1:1). Values in parentheses indicate impurities.

Compound	*Structure*	*Capacity factors (k')*
Aldicarb		1.26
Aldicarb sulfoxide		0.11
Aldicarb sulfone		1.44 (0.38)
Aminocarb		1.66
Bendiocarb		1.82
Carbaryl		2.05
Chloropropham		6.48
Desmedipham		3.44 (0.68, 1.12, 2.36)
Dimethoate		2.66 (1.62, 6.11)
Methiocarb		4.24
Methomyl		0.56
Metolachlor		1.65

Preliminary electrochemical experiments were performed in a flow-injection mode without a chromatographic column in the system. Of particular interest were experiments investigating the variation of response with pH. As anticipated, low pH values resulted in decreased response. However, high pH values showed no improvement over neutral values and, in some cases, resulted in poorly defined response peaks on a time basis. Thus the usual range of pH values used with silica-based columns was satisfactory for the electrochemistry. These preliminary experiments also included variations in potential to establish minimum potentials needed for the oxidation of each carbamate. These preliminary experiments showed that all of the carbamates studied were detectable, although there was a considerable range of sensitivities and required potentials.

After these preliminary experiments, the electrochemical detector was connected to the effluent of the chromatographic column and a series of experiments was performed to determine the optimum conditions for the detection of each individual carbamate. The varying factors were the three potentials (reaction potential and first and second cleaning potentials) and the time delay between going to the reaction potential and the start of the current sampling period. Each cleaning pulse and the sampling period were set to 1/60th of a second. The composition and flow-rate of the mobile phase were kept constant as cited above for Table I. All injections were 10 μl containing approximately 180 ng of a carbamate.

The potential of the first pulse was not critical so long as it was greater than 2.0 V. A value of 3.0 V was used for all subsequent experiments. Any adsorbed reaction product is believed to be oxidized at this potential and then removed from the surface of the electrode by the force of the flow stream.

The role of the second cleaning pulse is less certain. If this potential is too negative, hydrogen gas is evolved during the cleaning pulse and large, erratic background currents are observed. If this potential is too positive, the baseline increases during a chromatographic run and sensitivity decreases. A value of 0.5–0.7 V was found to be optimum and was used for all subsequent experiments.

RESULTS AND DISCUSSION

The optimum reaction potential varies with the carbamate being detected. The potential must be postive enough to drive the oxidation reaction. If too positive, performance decreases because the background current increases, and there is also a decrease in current response. Possibly this decrease results from a charge repulsion between the carbamate and the highly positive charge on the electrode. The most striking example is for Aminocarb in acidic solution where the protonated amine group would carry a postive charge. Optimum potentials ranged from a low of 1.3 V for Aminocarb to a high of 2.1 V for Bendiocarb. A value of 1.9 V was adopted for analysis of mixtures of carbamates.

The most surprising results in the optimization experiments came from studies of the time delay between setting the reaction potential and measuring the cell current. Early experiments showed a slowly decreasing background current following the potential jump from the second cleaning pulse to the reaction potential. This background current limited the current-amplifier setting that could be used with the instrument. It was assumed that the desired current response from the carbamate

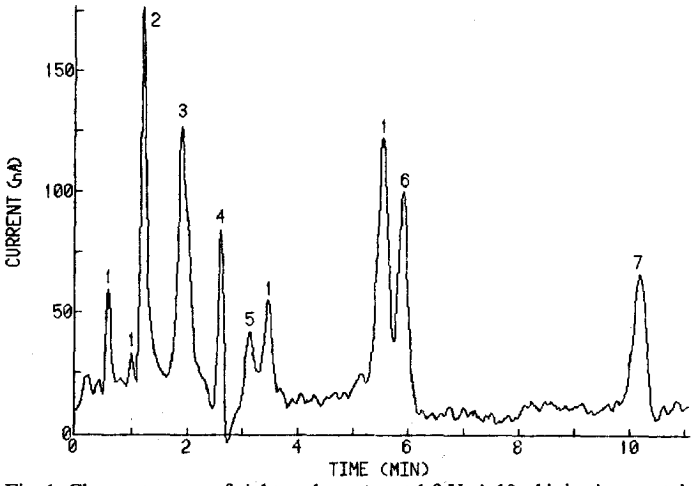


Fig. 1. Chromatogram of eight carbamates at 1.9 V. A 10-μl injection containing 10 ng of each carbamate. Peaks: 1 = Desmedipham; 2 = Aldicarb; 3 = Aminocarb and Bendiocarb; 4 = Carbaryl; 5 = Dimethoate; 6 = Methiocarb; 7 = Chlorpropham.

would also decrease with time after the potential jump, but that perhaps there might be some optimum delay time. The optimization experiments showed the expected decrease in background current and noise level as the delay time increased. However, most carbamates showed a significant increase in current response with increasing delay time. Thus a triple benefit resulted from increased delay time: decreased background which allowed higher current-gain settings, lower noise in the background current, and increased response from the carbamate. The negative aspect of increasing the delay time is that the frequency of sampling is decreased. In order to obtain enough sample points to define adequately the chromatographic peaks under these chromatographic conditions, a minimum sampling rate of one point per second was adopted.

The reason for the increased response with increased delay time is not certain. Johnson[19] suggests that the rate of the oxidation reaction might be proportional to the extent of the oxide layer on the platinum surface and that this layer grows at a relatively slow rate after going to the reaction potential. It is interesting to note that the one carbamate that does not show this increased response with delay time is Aminocarb, which is detected at much lower potentials by the oxidation of its aromatic amine group.

Fig. 1 shows the chromatogram of a mixture of eight carbamate pesticides, each one at 10 ng injected. In this chromatogram, which was run with an electrode potential of 1.9 V, peak number 3 is an unresolved doublet of Aminocarb and Bendiocarb. Fig. 2 is a repeat of Fig. 1 with the electrode potential at 1.3 V where Bendiocarb does not oxidize. An alternative way of resolving the chromatographic overlap would be to use a reduction detector that is sensitive to Bendiocarb but not Aminocarb[20].

Limits of detection for the carbamates vary with the compound, the potential, and the retention time. No attempt was made to estimate optimum limits of detection except for Aminocarb. However, under the conditions of Fig. 1, limits of detection

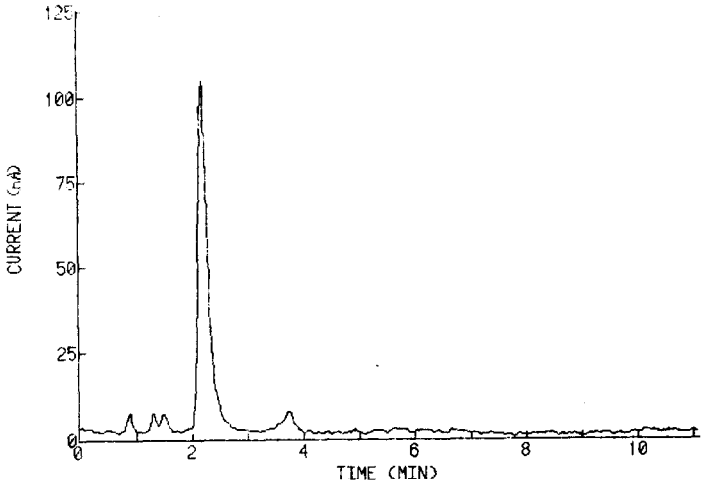


Fig. 2. Chromatogram of eight carbamates at 1.3 V. Repeat of injection of Fig. 1. The only significant peak is that of Aminocarb.

are in the order of 0.1 ng, which is approximately a factor of 10 better than for UV detection and comparable to the post-column derivitization with fluorogenic agents. Since the background current is lower at lower potentials, compounds that react at lower potentials have better limits of detection. Of the carbamates studied, Aminocarb is detected at the lowest potential, and limits of detection were measured for Aminocarb by itself.

Fig. 3 shows a chromatogram for 500 pg of Aminocarb with the electrode potential at 1.30 V. The limit of detection, calculated as three times the standard deviation of the baseline, is 20 pg. However, it is obvious that the baseline has a

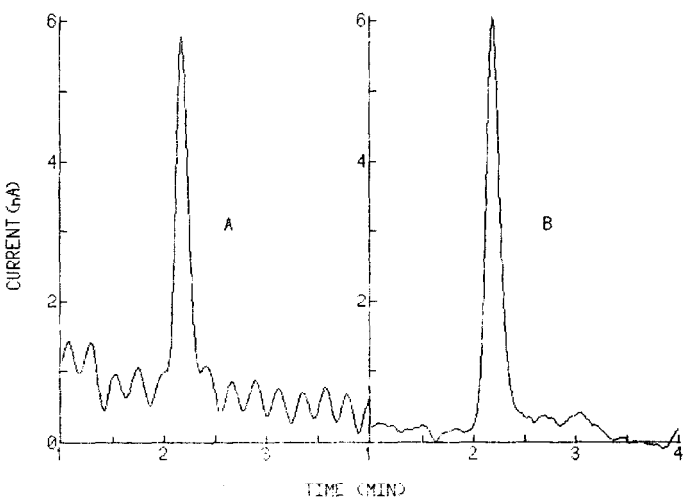


Fig. 3. (A) Chromatogram of aminocarb using Spectra-Physics pump. Potential 1.3 V. Injection is 0.5 ng. (B) Chromatogram of Aminocarb using Haskel pump. Repeat of Fig. 3a, except for pump.

regular noise signal, and further investigation revealed that the frequency of this noise signal is the same as the cycle time of the Spectra-Physics pump. This pump had been selected after comparison trials against other reciprocating piston pumps, and the pump noise had never been noted at higher levels of analyte. To clarify the situation, the chromatogram was repeated after substitution of an air-driven Haskel pump. It is obvious that the regular noise signal is no longer present. For this chromatogram, the limit of detection, as calculated before, is 5 pg, which is approximately an order of magnitude better than reported previously by any method.

REFERENCES

1 E. Grou, U. Rădulescu and A. Csama, *J. Chromatogr.*, 260 (1983) 502.
2 R. T. Krause, *J. Chromatogr.*, 185 (1979) 615.
3 L. Nondek, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.
4 M. Lanouette and R. K. Pike, *J. Chromatogr.*, 190 (1980) 208.
5 J. L. Anderson and D. J. Chesney, *Anal. Chem.*, 52 (1980) 2156.
6 J. L. Anderson, K. K. Whiten, J. D. Brewster, T.-Y. Ou and W. K. Nonidez, *Anal. Chem.*, 57 (1985) 1366.
7 G. Batley and B. Afgan, *J. Electroanal. Chem.*, 125 (1981) 437.
8 W. J. Mayer and M. S. Greenberg, *J. Chromatogr.*, 208 (1981) 295.
9 J. J. Scanion, P. A. Flaquer, G. W. Robinson, G. E. O'Brien and P. E. Sturrock, *Anal. Chim. Acta*, 158 (1984) 169.
10 P. A. Reardon, G. E. O'Brien and P. E. Sturrock, *Anal. Chim. Acta*, 162 (1984) 175.
11 M. B. Thomas, H. Msimanga and P. E. Sturrock, *Anal. Chim. Acta*, 174 (1985) 287.
12 B. Fleet and J. C. Little, *J. Chromatogr. Sci.*, 12 (1974) 747.
13 S. Hughes, P. L. Meschi and D. C. Johnson, *Anal. Chim. Acta*, 132 (1981) 1.
14 S. Hughes and D. C. Johnson, *Anal. Chim. Acta*, 132 (1981) 11.
15 S. Hughes and D. C. Johnson, *Anal. Chim. Acta*, 149 (1983) 1.
16 A. Fogg, M. Fernandez-Arciniega and R. Alonso, *Analyst (London)*, 110 (1985) 851.
17 A. Fogg, M. Fernandez-Arciniega and R. Alonzo, *Analyst (London)*, 110 (1985) 1201.
18 D. C. Johnson, *1984 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy*, paper No. 139.
19 D. C. Johnson, private communication.
20 M. L. Hitchman and S. Ramanathan, *Anal. Chim. Acta*, 157 (1984) 349.