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DETERMINATION OF SOME CARBAMATE PESTICIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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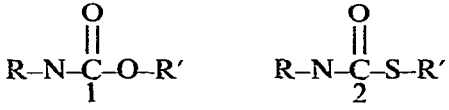
SUMMARY

An amperometric detector was used to determine some carbamate pesticides after a reversed-phase high-performance liquid chromatographic separation. Detection limits were determined which compared favorably with those obtained with other detectors. Dual detection, where the amperometric detector was in series with an ultraviolet detector, was employed to quantify pesticides that were not resolved on the chromatographic column.

INTRODUCTION

In recent years carbamate pesticides have gained a measure of popularity due to their pest specificity and low mammalian toxicity, hence methods to characterize their presence in the environment have been developed. As shown below, these pesticides may feature an oxygen atom (1) or a sulfur atom (2) adjacent to the carbamate function.



Direct analysis by gas chromatography has proved to be difficult due to the thermal instability of these pesticides, as many of the non-aromatic N-substituted and aromatic N-substituted carbamates tend to undergo thermal hydrolysis[1–3]. Because of the milder separation conditions, analysis by high-performance liquid chromatography (HPLC) has been more successful in maintaining the carbamate functionality of these compounds.

Sparacino and Hines[4] pursued an extensive study of some thirty carbamate

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pesticides, characterizing the elution order of these pesticides with different combinations of stationary and mobile phases and determining detection limits using an ultraviolet (UV) detector. Variable- or fixed-wavelength UV[4–14] and fluorescence[15–18] detection systems have also been used by many workers to detect carbamate pesticides after elution from a liquid chromatographic column. Specific detectors such as a modified electron-capture detector[19] and a Coulson electrolytic conductivity detector[20] which respond to the heteroatom (Cl and Br and N or S) of the pesticide have been described.

Even though an electrochemical detector has been recommended for pesticide determinations[21], the carbamate group of pesticides has been neglected in favor of the thiophosphate group of pesticides[22–26] which can be reduced at a dropping-mercury-electrode HPLC detector. Metabolites of some carbamate pesticides, however, have been determined using the electrochemical detector in the oxidation mode[27,28].

As many of these carbamate pesticides are electroactive, it was desired to use a suitable electrochemical detector (ElCD) to monitor the effluent from an HPLC separation. The detector system that we have been using in our laboratory, which incorporates a wax-impregnated graphite (WIG) working electrode, would be suitable for such an analysis. By judicious choice of working electrode potential, it was possible to monitor the column effluent for carbamate pesticides. Moreover, by selecting this proper operating potential of the detector, we also were able to determine selectively one pesticide in the presence of another when the resolution prohibited adequate separation of various combinations as described previously[4].

In addition to establishing detection limits for these pesticides using the ElCD, combinations of pesticides with similar retention times were eluted and monitored using a UV detector and ElCD operated in series arrangement (UV detector first) with the ElCD potential set so as to detect one of the carbamates in the mixture while the UV detector would respond to both carbamates. Hence, pesticides previously reported as "unresolved" were readily characterized quantitatively.

EXPERIMENTAL

Reagents and solutions

Ammonium phosphate, dibasic ($NH_4H_2PO_4$); phosphoric acid and methanol (Fisher Scientific, Pittsburgh, PA, U.S.A.) were used as received. Distilled water was run through a mixed bed ion-exchange column before use to remove as many impurities as possible. Mobile phases consisted of various combinations of methanol and 0.02 *M* ammonium biphosphate ($NH_4H_2PO_4$) solution, where the pH of the latter was adjusted to 3.0 $\pm$ 0.1 with concentrated phosphoric acid before mixing with methanol.

Carbamate pesticides were obtained from Chem Service (West Chester, PA, U.S.A.) and were used as received without further purification. Stock solutions in the range of 0.6–1.1 mg/ml were prepared by dissolving the carbamate pesticide in methanol. For the cyclic voltammetric studies, the stock solutions were diluted two-fold by addition of 0.02 *M* $NH_4H_2PO_4$ (pH 3.0). Serial dilutions with methanol of the stock solutions were prepared for the separation and detection limit studies. Pesticide mixtures were prepared from the concentrated stock solutions and then diluted with methanol.

Instrumentation

The liquid chromatographic system and EICD have been described thoroughly elsewhere[29]. The analytical column consisted of a LiChrosorb RP-8, 10-μm stationary phase (Scientific Products, Cleveland, OH, U.S.A.) that was slurry packed into a stainless-steel column (250 × 3.2 mm I.D.). Cyclic voltammetric studies were performed using a Princeton Applied Research (PARC, Princeton, NJ, U.S.A.) Model 173 potentiostat with a Model 175 universal programmer and a Houston Omnigraphic (Houston Instruments Austin, TX, U.S.A.) x–y recorder. A Laboratory Data Control Spectromonitor III variable-wavelength UV detector (LDC, Riviera Beach, FA, U.S.A.) was used for the UV analysis when the two detectors were operated in series. The UV detector wass first in line after the analytical column and was followed by the EICD. This order of detectors was chosen so that the UV detector would monitor the carbamates directly rather than their electro-oxidation products.

Procedures

Cyclic voltammetric studies were performed on the individual pesticides at a scan rate of 5 mV/sec. The scan started at the lower potential (0.5 V) and swept to the upper limit (1.4 V) and returned to the original potential. The cyclic voltammograms are the result of one scan cycle. Generally, multiple cycles (*i.e.* >6) would be pursued before recording the voltammogram to ensure electrochemical equilibrium. Since the cyclic voltammetric results were used only to establish an operating potential of the working electrode for the liquid chromatographic analysis of the carbamate pesticides, the multiple cycle procedure was deemed unnecessary.

For chromatographic determinations, replicate 10-μl samples of each stock pesticide solution, as well as the diluted standard solutions, were injected onto the column through the Valco injection valve which was equipped with a 10-μl sample loop. All separations were pursued with isocratic elution conditions, however, the methanol concentration was varied for optimal separations of a single pesticide or pesticide mixture. Various methanol–buffer solutions were used to adjust the capacity factor, k', of each pesticide to a range of 1–6 when determining the detection limits.

The effect of detector operating potential was also shown by operating the detector at various potentials while separating the same mixture of pesticides. Potentials used were 1370, 1325 and 1300 mV *vs.* the saturated calomel electrode (SCE).

Solutions prepared for dual detection had known concentrations of both pesticides. Peak areas were determined by the cut and weigh method.

RESULTS AND DISCUSSION

The cyclic voltammetric studies, summarized in Fig. 1, indicated that a number of carbamates were active electrochemically while others were not when using the specified mobile phase. The aromatic carbamate pesticides may be subdivided into two groups: those characterized by significant electrochemical activity, *e.g.* barban and propham in Fig. 1a and b; and those with marginal activity, *e.g.* sevin (Fig. 1c). Physically, this implies lower limits of detection for the former group. The non-aromatic pesticides, *e.g.* avadex (Fig. 1d), displayed no electrochemical activity. Since the magnitude of electrochemical activity of organic molecules may be solvent de-

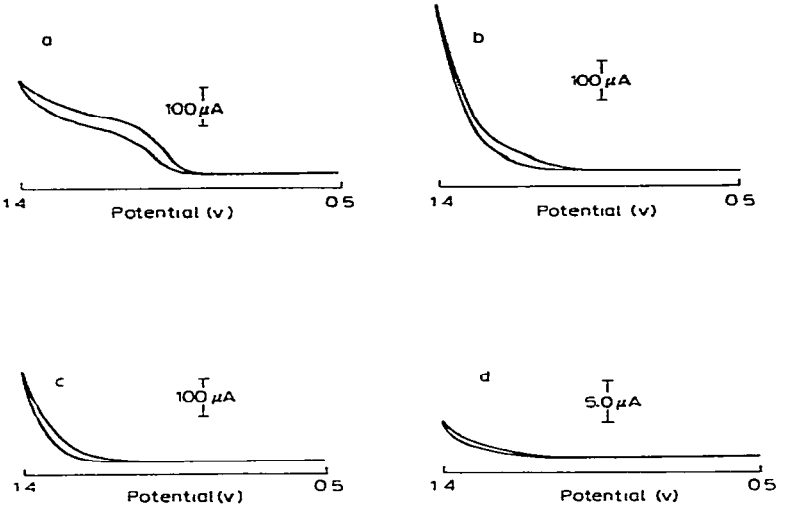


Fig. 1. Cyclic voltammograms of barban (a), prophan (b), sevin (c) and avadex (d); representing pronounced (a and b), moderate (c) and no electrochemical activity (d)

pendent, acetonitrile and acetone were considered as organic co-solvents in the mobile phase. Acetonitrile, which previously gave excellent results in the separation of carbamate pesticides[4], was particularly deleterious to the Plexiglas cell. The alcohol family was therefore chosen as the non-aqueous modifier of the mobile phase, and methanol was given preference due to its lower viscosity and therefore lower column backpressure.

Using the RP-8 reversed-phase system, the order of elution of the electroactive species, presented in Table I, was very similar to that previously reported for a C_{18} reversed-phase system[4]. Using mobile phase solutions of 50, 55 and 50% methanol, the capacity factor, k', was adjusted to a range of 1–6, and at that time detection limits for individual pesticides were determined and calibration curves for the serial dilution standards constructed. The results of the detection limit studies are summarized in Table II. The detection limits for a number of carbamate pesticides using the ElCD were on the same order of magnitude as the UV detection limits already

TABLE I
ELUTION ORDER OF ELECTROACTIVE CARBAMATE PESTICIDES

Common name	*Chemical name*
Benomyl	Methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate
Baygon	O-Isopropoxyphenylmethyl carbamate
Sevin	1-Naphthyl-N-methyl carbamate
Propham	Isopropyl-N-phenyl carbamate
Landrin	2,3,5-Trimethylphenylmethyl carbamate
	3,4,5-Trimethylphenylmethyl carbamate (1:4)
Mesurol	3,5-Dimethyl-4-(methylthio)phenylmethyl carbamate
Chloropropham	Isopropyl-N-(*m*-chlorophenyl) carbamate
Barban	4-chloro-2-butynyl-N-(3-chlorophenyl) carbamate

TABLE II
DETECTION LIMITS FOR ELECTROACTIVE CARBAMATE PESTICIDES

Pesticide	*Applied potential (mV)*	*Methanol (%, v/v)*	*k'*	*Mass detection limits (ng)*	
				HPLC-ElCD	*HPLC–UV** ****
Barban	1300	60	5 4	10	1.8 (206)
Baygon	1370	50	2.5	300	13 6 (200)
Benomyl	1325	50	0 9	6 0	2.8 (205)
Chloropropham	1310	60	4.4	2 5	1 0 (207)
Landrin	1370	55	3 4	1000	1.4 (207)
Mesurol	1310	60	3.6	1.0	1 2 (202)
Propham	1325	55	3.0	1.0	3.3 (199)
Sevin	1350	50	3.6	5 0	– –

* Determined for a 10-μl injection volume; to convert to concentration in ppm, multiply the mass detection limit by 0.1

** From ref. 4.

*** Optimal wavelength for detection in parenthesis

reported. These limits can vary, however, depending on the condition of the WIG electrode surface, and on placement of the WIG electrode relative to the SCE in the flow channel. Since there was not an absolute set distance between these two electrodes, their relative position varied, therefore changing cell volume slightly and consequently the detector response. Note that the detection limits refer to the actual mass in ng that was necessary to obtain a response from the ElCD. The concentration

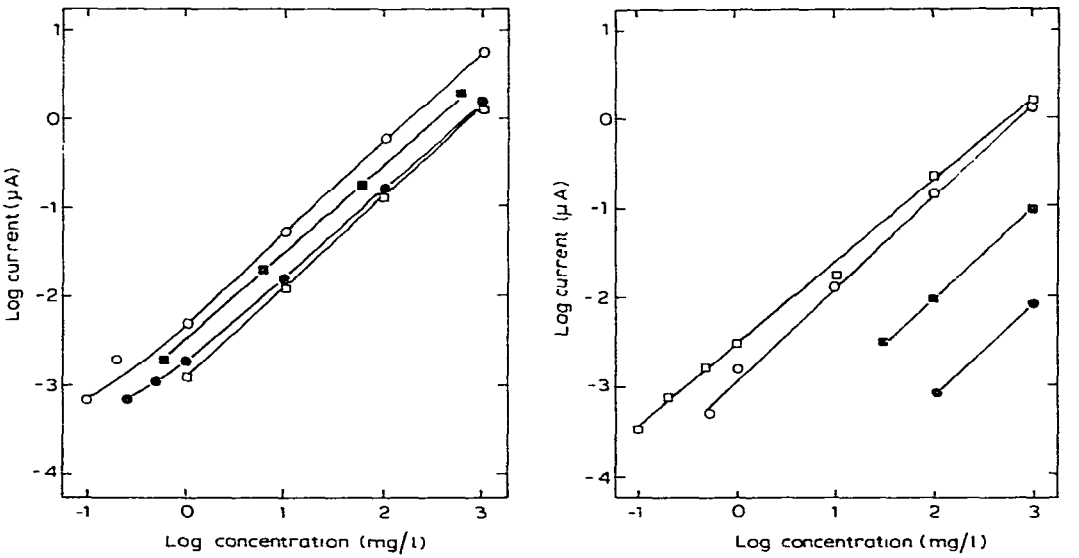


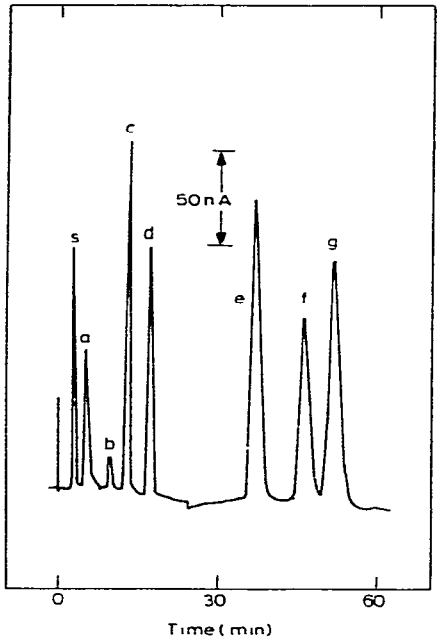
Fig. 2. Peak current *vs.* concentration plots of carbamate pesticides with aromatic part of molecule on the N-side of carbamate function. ○, Propham; ■, benomyl; □, barban; ●, chloropropham.

Fig. 3. Peak current *vs* concentration of plots of carbamate pesticides with aromatic part of molecule on the O-side of carbamate function ■, Baygon; ●, landrin; ○, sevin; □, mesurol.

detection limits generally fall in the low ppm range or in some cases the hundreds of ppb*.

Calibration plots of peak current *versus* concentration of the electroactive pesticides are shown in Figs. 2 and 3. The pesticides presented in Fig. 2 have the aromatic end of the molecule on the N-side of the carbamate function, while the calibration plots of Fig. 3 represent those carbamates with the aromatic end of the molecule at the O-side of the carbamate function. The plots of all the pesticides in both figures showed reasonable linearity throughout the concentration range studied. With the exception of baygon and landrin, both types of carbamates produced calibration plots with similar slopes and range of concentration over which the detector responded to the carbamate pesticide.

By changing the applied potential to the WIG working electrode, it was possible to note a considerable change in the detector response to a mixture of pesticides which were already determined to have considerable electrochemical activity. As shown in Fig. 4, at 1370 mV (*vs.* the SCE), all the pesticides in the mixture could be readily identified by their chromatographic peaks in the elution sequence. As the applied potential was lowered to 1325 mV *vs.* the SCE, there was a considerable shift in relative detector response to some of the pesticides (Fig. 5). The relative detector



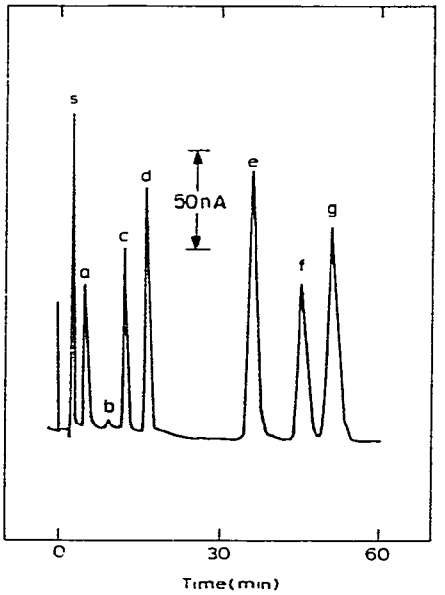


Fig. 4. Separation of carbamate pesticide mixture with ElCD at 1370 mV *vs* SCE Sample size, 10 μl; flow-rate, 0 6 ml/min. s = Solvent, a = 60 mg/l benomyl, b = 200 mg/l baygon, c = 100 mg/l sevin, d = 50 mg/l propham, e = 200 mg/l mesurol, f = 100 mg/l chloropropham, g = 200 mg/l barban. Mobile phase, methanol–0.02 *M* $NH_4H_2PO_4$ (1·1), pH 3 0.

Fig. 5. Separation of carbamate pesticide mixture with ElCD at 1325 mV *vs.* SCE. Same separation conditions and peak identification as Fig. 4.

* The American billion (10^9) is meant here.

sensitivity to baygon was reduced considerably as well as the response to sevin which also was reduced at this time. Finally, at 1300 mV *vs.* the SCE (Fig. 6), the detector does not respond to baygon at all, and the response to sevin has been reduced considerably both relative to other pesticides in the mixture and in terms of a real reduction in detector response to all pesticides in the mixture. This change in detector response to some carbamates could be taken advantage of when determining one pesticide in the presence of others, or when column resolution does not permit adequate separation of the desired pesticide from the interferences.

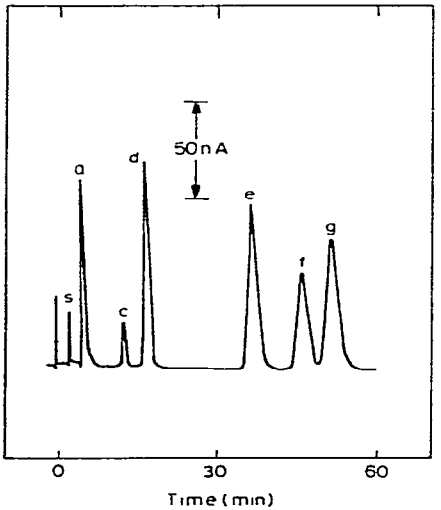


Fig 6 Separation of carbamate pesticide mixture with EICD at 1300 mV *vs* SCE Same separation conditions and peak identification as Fig 4.

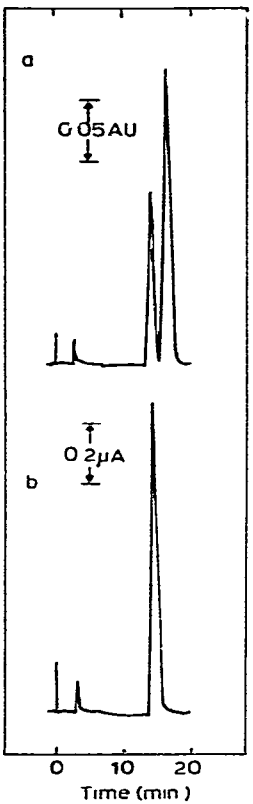
This concept of "selective detection" was utilized when analyzing pesticide combinations that were reported previously as incompletely resolved on a reversed-phase column[4]. A mixture of propham and landrin was separated and detected with a UV detector and an EICD in series. While both species are UV active, only propham is seen by the EICD (Fig. 7). More dramatic, however, are the chromatograms of a mixture of barban and captafol with dual detection. These species were not well resolved on the analytical column as shown by the UV chromatogram (Fig. 8a), where asymmetry is noted on the right side of the peak. Since barban is electrochemically active (whereas captafol is not), the electrochemical chromatogram shows only one symmetrical peak for barban. Hence, in this dual detection mode, quantitative information could be obtained as follows

The total area under the peak in Fig. 8a is proportional to the total concentrations of barban and captafol where

$$\text{area}_{\text{total}} = \text{area}_{\text{barban}} + \text{area}_{\text{captafol}} \tag{1}$$

Application of Beer's law leads to:

$$\text{area}_{\text{total}} = k_b a_b b c_b + k_c a_c b c_c \tag{2}$$



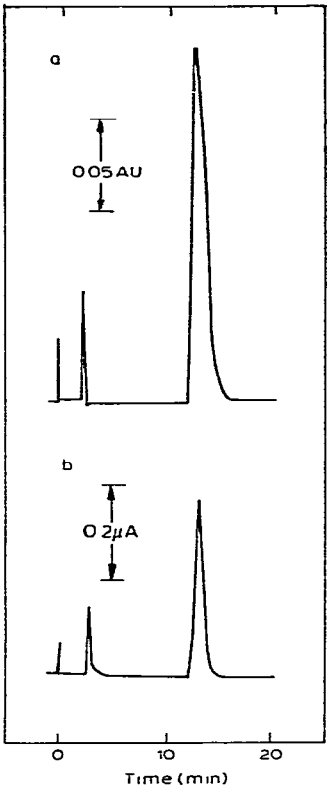


Fig. 7 Separation of 200 mg/l propham–508 mg/l landrin pesticide combination Sample size, 10 μl; flow-rate, 0.6 ml/min; mobile phase, methanol–0.02 *M* $NH_4H_2PO_4$ (1·1), pH 3 0 (a) UV detection at 220 nm, propham first peak, landrin second, (b) electrochemical detection at 1300 mV *vs.* SCE, propham only peak

Fig. 8. Separation of 204 mg/l barban–500 mg/l captafol pesticide mixture. Sample size, 10 μl; flow-rate, 0.6 ml/min; mobile phase, methanol–0 02 *M* $NH_4H_2PO_4$ (60.40), pH 3 0. (a) UV detection at 220 nm, (b) EC detection at 1300 mV *vs.* SCE.

where k_b and k_c are proportionality constants, a_b and a_c are the absorbtivities in mg^{-1} cm^{-1} and b is the pathlength in cm. The constants k_b, k_c, a_b, a_c and b may be obtained from experiments on stock solutions of barban and captafol. From the electrochemical chromatogram in Fig. 8a, the concentration of barban, c_b, is obtained directly. Knowing c_e, eqn. 2 can be rearranged and the concentration of captafol, c_c, de-

TABLE III
PERCENTAGE RECOVERY DATA FOR BARBAN–CAPTAFOL MIXTURES
Results for triplicate analysis.

Barban (ppm)	*Captafol (ppm)*	*Captafol recovered (ppm)*	*Recovery (%)*	*Relative standard deviation (%)*
50	500	424	85	20
250	500	447	89	10

termined. Results for two mixtures of barban and captafol (50 ppm; 500 ppm and 250 ppm; and 500 ppm, respectively) are summarized in Table III.

It is important to note that the precision in the latter determination is considerably better than the former. The peak areas for barban and captafol are nearly the same in the latter mixture which accounts for this improvement. Further, it is important to recognize this as a limitation of the dual detector approach, *i.e.* for best results, peak areas (or the detector response) should be comparable for both species.

CONCLUSION

Electrochemical detection has shown itself to be a useful detection method for some carbamate pesticides when separated by HPLC. Pesticides which were active electrochemically were characterized by detection limits comparable to other detectors, particularly the UV detector which is the most used mode of detection. When two detectors were used simultaneously in a series configuration, it was possible to detect selectively one pesticide only with the ElCD while the UV detector would respond to both pesticides in a binary mixture that was separated insufficiently by the HPLC column, thus facilitating both qualitative and quantitative characterization of the sample. However, the precision of such analysis may be poor, particularly if detector response factors are significantly different for the two solutes.

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