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# HETEROGENEOUS CATALYTIC POST-COLUMN REACTION DETECTORS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY APPLICATION TO N-METHYLCARBAMATES

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## SUMMARY

The potential of solid-phase, and especially catalytic, reaction detectors in high-performance liquid chromatography is discussed. A post-column reactor, packed with Aminex A-28, a tetraalkylammonium anion exchanger, is used for the catalytic hydrolysis of N-methylcarbamates. The liberated methylamine is labelled with *o*-phthalaldehyde (OPA) and the resulting fluorophore is detected on-line with a fluorescence detector. The use of a short (45-mm) catalyst bed packed with small catalyst particles ($d_p$, 9 $\mu$m), a sufficiently high temperature (100–120°C) and minimal dilution of the reactor effluent with OPA reagent (3%) efficiently suppresses band broadening and allows the detection of sub-nanogram quantities of several carbamate pesticides. The repeatability is better than 2% relative S.D. ($n = 6$), and calibration curves are linear over at least two orders of magnitude. The method has been applied to the analysis of river-water samples.

## INTRODUCTION

Post-column reaction detectors are now quite widely accepted for selective and sensitive detection in column high-performance liquid chromatography (HPLC)[1,2]. They still possess some shortcomings, however, such as undue additional band broadening and, often, the absence of suitable reagent pumping systems. Band broadening is at least partly related to the residence time of the analyte zone in the reactor and, hence, to the kinetics of the reaction. The situation can therefore be improved by increasing the rate of reaction. The post-column reagent addition can occasionally be obviated by, *e.g.*, adding the reagent to the mobile phase or by using photochemical reactions[3,4] or solid-phase reactors[5]. The use of solid-phase reactions can be viewed as an extension of the packed-bed reactor principle with the new aspect that now the packing material in the reactor directly participates in the reaction, either as a reagent source or as a heterogeneous catalyst. The latter alternative is especially attractive: provided no catalyst deactivation or poisoning takes place, no depletion

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of the reactor bed will occur. The reagent or catalyst can be present in such a reactor either as a solid, or physically coated or chemically bonded into an inert matrix, such as silica, alumina or a polymer.

The concept of solid-phase reactors offers interesting possibilities and advantages such as:

(i) Elimination of instrumentation, such as reagent pumps and mixing tees and, hence, the reduction of cost and the absence of mixing problems and flow pulsations, etc.

(ii) Possibility of working at relatively high temperatures and pressures.

(iii) Possibility of working with reagents and catalysts which would otherwise interfere in the detection process.

(iv) Good compatibility with a variety of mobile phases for normal- and reversed-phase chromatography.

(v) Good automation and miniaturization potential.

Besides, derivatization in solid-phase reactors, may well be more rapid and more specific than in solution with the same reagents.

Applications of this concept have recently been reviewed by Krull and Lankmayr[5], and a specific application to the polymeric reduction of carbonyl compounds has been proposed[6]. Another area is the use of immobilized enzymes for various types of solid-phase enzymatic reactions, as recently reviewed by Bowers and Bostick[7].

The use of heterogeneous catalysis in connection with solid-phase reactors is less widespread. Catalytic hydrolysis of urea herbicides on active silica has been used and investigated in a pre-chromatographic mode in our group[8,9]. Vrátný *et al.*[10] have recently reported the catalytic hydrolysis of disaccharides prior to their detection. A more detailed study of band-broadening aspects of such catalytic reactors has recently been carried out by our group[11] with two model systems, *viz.*, the catalytic retroaldolization of diacetone alcohol on alumina, and the catalytic hydrolysis of 1-naphthyl-N-methylcarbamate (Carbaryl) on anion exchangers. In this study, a simple model, valid for first-order reactions, has been used to describe the relationship between the band broadening due to the solid-phase reaction —the so-called* reaction band broadening $\sigma_r$— and parameters such as the rate constant of the reaction and capacity factors for reactant and product (*cf*, ref. 11 and below). The relationship appears to have fairly general validity for solid-phase reactions with first-order kinetics.

The model system with Carbaryl is also of considerable practical interest as a technique for monitoring carbamate pesticides in the environment selectively and sensitively. The method is actually an extension of a post-column fluorogenic technique, proposed by Moye *et al.*[12] for the residue analysis of N-methylcarbamates by HPLC. This technique involves a base-catalysed hydrolysis of the separated carbamates as the first step. In the second step the alkylamines produced are allowed to react with *o*-phthalaldehyde (OPA) to produce highly fluorescent products. Krause[13] subsequently studied this technique in more detail and proposed some further im-

* As outlined in our previous paper[11], the total band broadening, $\sigma_t$, in a system involving the use of a solid-phase reactor can be expressed as $\sigma_t^2 = \sigma_r^2 + \sigma_n^2$, where $\sigma_r$ and $\sigma_n$ are the band broadening contributions of reaction and non-reaction (*i.e.*, all further) band broadening, respectively.

provements. A solution of sodium hydroxide was the base catalyst and a 3 m × 0.5 mm I.D. coiled capillary, kept at 80°C, was used as a reactor for the first step. The relative dilution of the column effluent by adding the base and the OPA amounted to 66% (ref. 13) and 30% (ref. 12). Thus, the band broadening attributable to the reaction detector was not only caused by axial dispersion in the reactor coils and dispersion in mixing tees and connections, but also by a noticeable dilution. In addition, with the slowly hydrolyzing carbamates, only a small fraction was converted into the alkylamine.

From this it is obvious that the use of heterogeneous catalysis for the first step can lead to considerable improvement of this method by reducing band broadening —thus improving the sensitivity— and simplifying the entire detector system. The possibility of using basic ion exchangers, such as Amberlite GC-400 or Aminex A-28, as a catalyst for this type of compounds has been demonstrated in our previous work[11]. However, Carbaryl, used as a model system, hydrolyses very rapidly in comparison to other carbamates of interest[12]. It was the aim of this study, therefore, to test the previously described reactor for the hydrolysis of a broader range of N-methylcarbamates —some less reactive than Carbaryl— Aminex A-28 being the obvious choice as the catalyst, because of its small particle size (9 $\mu$m). These data will also permit a further test of the model referred to above. Finally, the feasibility of the above detection system was tested for the analysis of real samples.

## THEORETICAL

The reaction mechanism proposed for the catalytic hydrolysis of N-alkylcarbamates[14] is:

$$R_1\text{–O–}\underset{\underset{\text{O}}{\|}}{\text{C}}\text{–NH–}R_2 \xrightarrow{\text{base}} R_1\text{–OH} + R_2\text{–N=C=O} \quad (1)$$

$$R_2\text{–N=C=O} + H_2O \rightarrow R_2\text{–NH}_2 + CO_2 \quad (2)$$

This mechanism involves a base-catalyzed splitting of the reactant to phenol and isocyanate in the first step (eqn. 1). The isocyanate is rapidly decomposed to an alkylamine in the second step (eqn. 2). In the present study, $R_2$ is invariably $CH_3$.

Recently, we have discussed[11] band broadening in solid-phase reactors as a result of the chemical reaction and the separation of reaction product (P) from injected reactant (R). For a reaction with first-order kinetics, such as the catalytic hydrolysis of N-alkylcarbamates, this so-called reaction band broadening, $\varphi_r$, depends on the capacity factors of P and R, $k'_P$ and $k'_R$, respectively, in the reactor and the rate constant, $k_r$, according to

$$\varphi_r = \sqrt{0.125 \ln 2}\,(1 - k'_P/k'_R)/k_r \quad (3)$$

where $k'_P < k'_R$ has been taken as an example.

Usually, the chromatographic conditions in the analytical HPLC column will determine the composition of the carrier stream entering the reactor and, thus, the

values of $k'_P$ and $k'_R$. In other words, the best way to reduce $\sigma_r$ is to increase $k_r$ by using a sufficiently active catalyst and appropriate reaction temperature. It has also been shown[11] that mass-transport problems, which may hamper the chemical reaction and, thus, increase $\sigma_r$, are eliminated if sufficiently small catalyst particles are used. For the rest, it is evident that increasing the reaction rate (shorter reactor bed) and decreasing the catalyst particle size will also reduce the normal type of band broadening occurring in any packed-bed reactor.

## EXPERIMENTAL

### *Chemicals*

Demineralized distilled water and analytical grade methanol (J. Baker, Deventer, The Netherlands) were used for the preparation of the mobile phase. OPA reagent was prepared freshly before measurement from 100 ml of 0.05 *M* aqueous borate buffer (pH 9.1), 0.1 ml of mercaptoethanol (E. Merck, Darmstadt, F.R.G.) and 0.1 g of *o*-phthalaldehyde (Merck). The mobile phase and OPA reagent were carefully degassed under vacuum prior to use. A stream of helium was bubbled through the mobile phase to prevent air from dissolving; no bubbles were observed in the detector cell, even at reaction temperatures of about 120°C.

Carbamate standards (U.S. EPA, Research Triangle Park, NC, U.S.A.) (see Table II) were dissolved in pure methanol (1 mg/ml) and these stock solutions were diluted in mobile phase to the required concentrations before measurement. Carbaryl and other carbamates available as technical formulations (Table I) were suspended under sonication in pure methanol (0.1 g per 5 ml) and the insoluble additives were removed by filtering through a Millipore filter (0.5 $\mu$m). If defined concentrations of these compounds were required, methanol was evaporated and the solid residue was dissolved, after weighing, in pure methanol (1 mg/ml).

The sample of Amstel river-water was filtered through a Millipore filter to remove microparticulate matter and was either directly analysed or first spiked with a known amount of pesticides.

### *Apparatus*

The HPLC apparatus and the post-column reaction detector are shown in Fig.

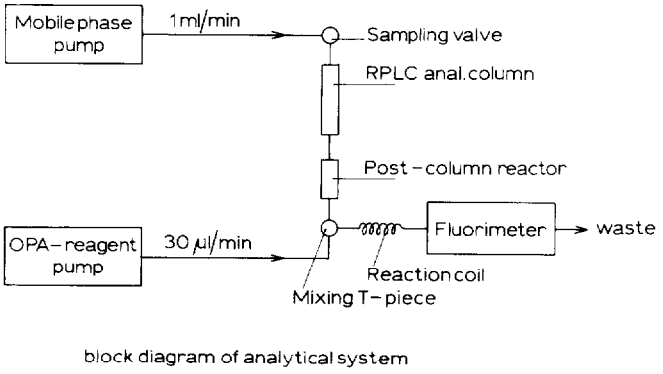



Fig. 1. Diagram of analytical system.

1. The catalytic reactor was made from 45 or 60 mm × 4 mm I.D. stainless-steel tubes, equipped with Swagelok couplings and 2-$\mu$m stainless-steel frits. The reactor was heated by means of a glass heating mantle, attached to a glycol heating bath (P. M. Tamson, Zoetermeer, The Netherlands), the temperature of which was maintained with a precision of 0.5°C.

Anion-exchange resin Aminex A-28 (Bio-Rad, Richmond, CA, U.S.A.) with tetraalkylammonium groups in acetate form and a particle size of 9 ± 2 $\mu$m was used. The material was swelled 24 h before packing in water–methanol (1:1), a mixture also used as slurry liquid. The reactor was packed by a slurry procedure at a constant flow-rate of 5 ml/min. After packing, the resin was heated at 120°C and flushed for 6 h with methanol–water (1:1) to remove labile anion-exchange groups.

The HPLC apparatus consisted of a reciprocating Altex 110 A pump with pulse dampener (Kontron, Zürich, Switzerland), a Valco (3000 p.s.i.) sampling valve with a 20-$\mu$l loop and a LC-55 (Perkin-Elmer, Norwalk, CT, U.S.A.) UV/visible spectrophotometer which was used in preliminary studies at 254 nm to investigate the hydrolysis reaction.

The OPA detection system consisted of a reactor, a low dead-volume mixing T-piece and 40 cm × 0.3 mm I.D. piece of PTFE capillary, coiled to a diameter of *ca.* 8 mm. The OPA reagent was added via a syringe pump, LD 13 A (Labotron Messtechnik, Gelling, F.R.G.), at a flow-rate of 30 $\mu$l/min. A Perkin-Elmer 204 A fluorescence spectrometer was used, which was operated at $\mu_{ex}$ = 340 nm and $\mu_{em}$ = 455 nm.

All chromatographic experiments were carried out under isocratic conditions on a 15 cm × 4.6 mm I.D. stainless-steel column, packed with 5-$\mu$m Spherisorb ODS, with methanol–water (1:1) as mobile phase.

## RESULTS AND DISCUSSION

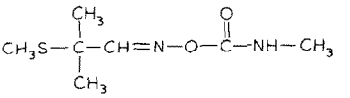
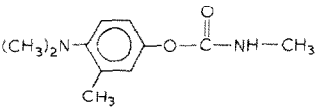
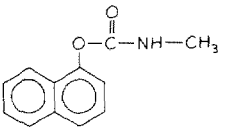
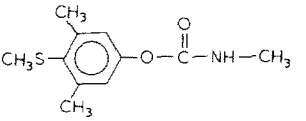
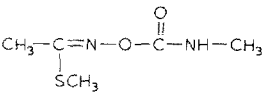
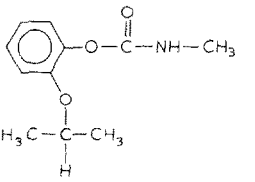
### *The hydrolysis reaction*

The catalytic hydrolysis of the selected carbamates (*cf.*, Table I) was followed chromatographically. Typical reaction chromatograms for Methomyl and Propoxur, obtained by means of UV detection at 254 nm, are shown in Figs. 2 and 3 for various reaction temperatures at a fixed residence time. According to Moye *et al.*[12], Methomyl is the least reactive of the test compounds, while Propoxur should perform rather similar to the highly reactive Carbaryl. The reaction chromatograms for Propoxur (Fig. 2) are in accordance with the reaction mechanism discussed above (eqns. 1 and 2). It is evident that, even at room temperature, Propoxur is partly decomposed to methyl isocyanate. At 57°C a good portion of the reactant is converted into the phenol, which is strongly retained by the anion exchanger, and the labile isocyanate has disappeared. The resulting alkylamine cannot be detected under the prevailing conditions. In this series of experiments, at 85°C (data not shown here) the hydrolysis of Propoxur is complete.

The relatively low reactivity of Methomyl as compared to Propoxur becomes clearly evident upon comparison of Figs. 2 and 3. The intermediate product (peak 2) is now predominant at reaction temperatures of between 40 and 80°C, and a trace amount of the phenol first shows up at the rather high temperature of 61°C. From an analytical viewpoint, however, it is more important to note that Methomyl is also

TABLE I

N-METHYLCARBAMATES USED AS MODEL COMPOUNDS

| *Common name** | *Structure* |
|---|---|
| Aldicarb (Temik, 99%; Aagrunol, Groningen, The Netherlands) |  |
| Aminocarb (Matacil, 50%; Bayer Nederland, Arnhem The Netherlands) |  |
| Carbaryl (Sevin, 50%; Union Carbide, Amsterdam The Netherlands) |  |
| Methiocarb (Mesurol, 50%; Bayer) |  |
| Methomyl (Lannate, 95%; Dupont de Nemours, 's Hertogenbosch, The Netherlands) |  |
| Propoxur (Undeen, 50%; Bayer) |  |

* Within brackets: trade-name, approximate wt. % of carbamate in technical formulation; manufacturer

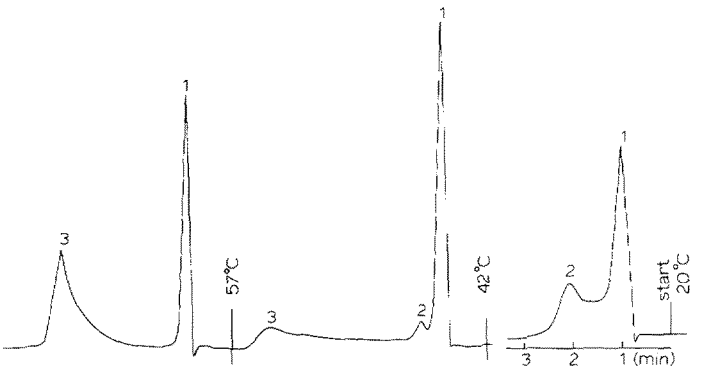



Fig. 2. Reaction chromatogram for the base-catalysed hydrolysis of Propoxur. Conditions: 60 × 4 mm bed, packed with Aminex A-28; carrier stream, methanol–water (1:1) at 0.5 ml/min; detection at 254 nm; injected amount, *ca.* 1 μg. Peaks: 1 = carbamate; 2 = probably isocyanate; 3 = phenol.

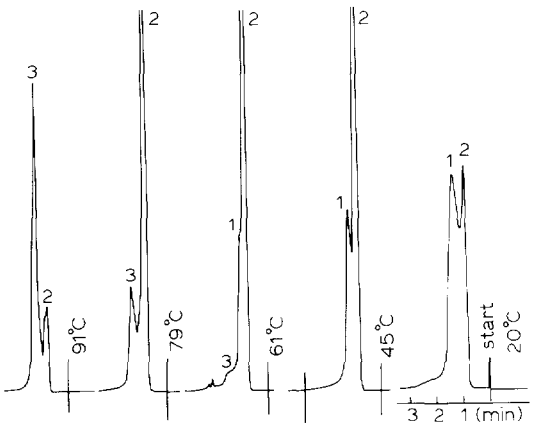



Fig. 3. Reaction chromatogram for the base-catalysed hydrolysis of Methomyl. For conditions and peak identification see Fig. 2.

completely hydrolyzed at a temperature of about 100°C, as will be illustrated in more detail in the next section.

*Detector performance*

In Fig. 4 the temperature dependence of the hydrolysis reaction for Carbaryl and Methomyl is recorded as a plot of peak area under the methylamine peak *versus* reaction temperature. It can be seen that both carbamates, which represent the extremes in terms of reactivity of the functional group tested, are completely hydrolyzed at about 100°C. With the given reactor geometry and flow-rate, this amounts to total conversion during a residence time of about 1 min.

In our earlier paper[11] we mentioned that the reaction temperature should be as high as possible in order to minimize reaction band broadening. In the present case, the upper limit is 120°C, the limiting factor being the thermal stability of the

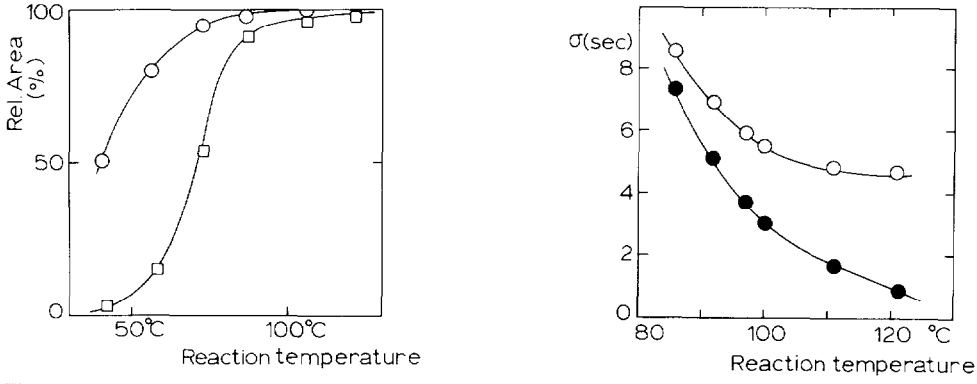



Fig. 4. Dependence of carbamate hydrolysis (measured as area of product peak relative to area for injection of standard amount of methylamine) on reaction temperature. Conditions: 60 × 4 mm bed, packed with Aminex A-28; carrier stream, methanol–water (1:1) at 1.0 ml/min; detection via fluorescence of OPA–methylamine reaction product. Injected amounts: 100 ng of Methomyl (□) or Carbaryl (O).

Fig. 5. Dependence of total band broadening ($\sigma_t$, ○) and reaction band broadening ($\sigma_r$ ●) on temperature for 20-ng injections of Methomyl.

catalyst resin. At high temperatures, decomposition of the catalyst causes a shift of the baseline and an increase of the detector noise. Another problem is the formation of bubbles in the detector cell at very high temperatures.

When all the above points are considered, the optimum temperature is found to be about 100°C. This conclusion is supported by Fig. 5, where the dependence of reaction band broadening, $\sigma_r$, and total band broadening, $\sigma_t$, on temperature is shown for Methomyl as test compound. Although $\sigma_r$ steadily decreases over the whole temperature range investigated, $\sigma_t$ remains essentially constant for $t > 100$°C. This is due to the fact that the non-reaction band broadening, $\sigma_n$, is only slightly dependent on temperature. As is seen from Table II, $\sigma_r$ at 100°C was 2–3 sec for all carbamates tested. Total band broadening at 100°C typically was about 5 sec (*cf.*, Fig. 5) and invariably was lower than 10 sec, which is the value reported by Moye *et al.*[12] for the band broadening in their reaction coils, mixing tees and detector cell at a flow-rate of 1 ml/min.

It should be pointed out that, in our system, $\sigma_t$ was markedly reduced by adding the OPA reagent in a more concentrated form but at only 30 µl/min. This corresponds to a mobile phase-to-reagent ratio of 100:3 or, in other words, to almost negligible dilution of the column effluent. Good mixing was assured by appropriate construction of the mixing unit and by using coiled reaction capillaries (see Fig. 1), which provide good radial mixing conditions.

*Analytical aspects*

All technical formulations gave roughly comparable detector responses in the reaction temperature range of 80–120°C. Because of the often uncertain content of active product in the commercial formulations, EPA standards were used for all quantitative work.

Detection limits, calculated for a signal-to-noise ratio of 2:1, and total retention volumes, $V_t$, are reported in Table II. Isocratic HPLC was carried out on a $C_{18}$-bonded silica with methanol–water (1:1) as mobile phase. For Aminocarb only a detection limit for direct injection into the reactor is given, since a different mobile phase would have been required to reduce the high retention of this compound.

TABLE II

TOTAL RETENTION VOLUME, $V_t$, REACTION BAND BROADENING, $\sigma_r$, AND DETECTION LIMIT OF PURE CARBAMATE STANDARDS

Conditions: hydrolysis at 100°C; flow-rate of HPLC effluent, 1 ml/min; flow-rate of OPA reagent, 30 µl/min; dead-volume of column + detector, 1.8 ml; for HPLC conditions and reactor dimensions, see text.

| *Carbamate* | $V_t$ *(ml)* | $\varphi_r$ *(sec)* | *Detection limit (ng)** | |
|---|---|---|---|---|
| Aldicarb | 3.2 | 2.5 | 0.10 | |
| Methomyl | 5.0 | 2.8 | 0.30 | (0.05) |
| Propoxur | 5.8 | 2.1 | 0.25 | |
| Carbaryl | 7.8 | 2.6 | 0.40 | |
| Methiocarb | 18.2 | 2.0 | 0.85 | |
| Aminocarb | > 50 | 1.9 | | (0.05) |

* Fluorescence detection of OPA–methylamine reaction product; values within brackets determined via direct injection into the reactor.

Repeatability, studied by repetitive injection of 2 ng of Methomyl into the reactor was found to be ± 2% relative S.D. ($n$ = 6).

The detector response was found to remain essentially unchanged, even after continuous use of the system for several days. Obviously, all reaction products, including the phenols (*cf.*, Figs. 2 and 3), are flushed from the catalyst bed: no deactivation or poisoning was ever observed, even after repeated injection of $\mu$g amounts of various carbamates.

As to linearity of response, a linear relationship between injected amount and signal intensity can be expected in the case of first-order kinetics. In this situation, the maximum concentration, $c_{max}$, of reaction product at the reactor outlet is[11]

$$c_{max} = n_p c_0 \left[\exp\left(\frac{\sqrt{\ln 2}}{8} \cdot \frac{t_{in}}{\sigma_r}\right) - 1\right] \quad (4)$$

where $c_0$ is the concentration of injected reactant, $n_p$ is the number of product molecules formed from one molecule of reactant and $t_{in}$ is the width of the square-wave input pulse of reactant. Because of the first-order kinetics found for the hydrolysis of 1-naphthyl-N-methylcarbamate, one can expect the same kinetic behaviour for the other carbamates.

In the present study, Methomyl and Propoxur were chosen as model compounds, and good linearity ($r$ = 0.9998) was indeed observed in both cases for injected amounts of between 2 and 20 ng. However, it must be stressed that a non-linear response is to be expected if the reaction order differs from unity.

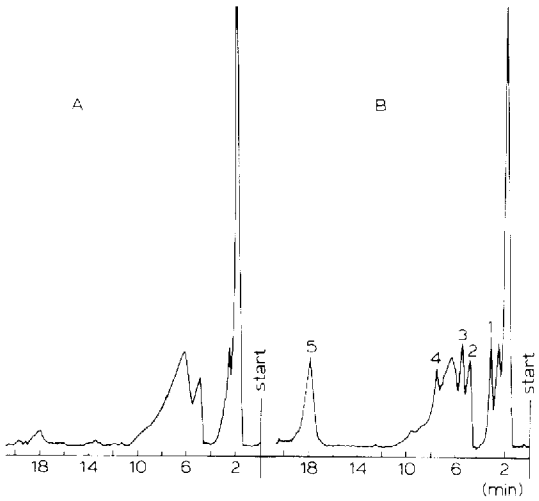



Fig. 6. Reaction chromatograms for non-spiked Amstel river-water (A) and Amstel river-water (B), spiked with 3 ng Aldicarb (1), 3 ng Methomyl (2), 5 ng Propoxur (3), 5 ng Carbaryl (4) and 10 ng Methiocarb (5). Conditions: 150 × 4.6 mm HPLC column, packed with 5-$\mu$m Spherisorb ODS; mobile phase, methanol–water (1:1) at 1.0 ml/min; 60 × 4.6 mm reactor, packed with Aminex A-28; reaction temperature, 100°C; OPA reagent flow-rate, 30 $\mu$l/min; detection with Perkin-Elmer Model 204 A fluorescence spectrometer; $\lambda_{ex}$ = 340 nm and $\lambda_{em}$ = 455 nm.

*Application*

The feasibility of the present approach was tested by injecting samples of tap-water and Amstel river-water. In Fig. 6, an example of such an analysis, which did not meet with any experimental problems, is given to illustrate the sensitivity and selectivity of the detection technique in the case of a surface water sample. The presence of OPA-reactive compounds in the non-spiked sample can possibly be attributed to naturally occurring amines.

## CONCLUSIONS

It has been shown that the post-column catalytic hydrolysis of N-alkylcarbamates can be put to good use for the detection of these compounds. Optimal reaction temperatures of around 100°C and residence times in the order of 1 min permit a complete hydrolysis of all the carbamates tested on a reactor bed packed with a strong anion exchanger of small particle size. The OPA reaction with the resulting amine provides a selectivity and sensitivity suitable for trace determinations of carbamate residues in real samples. Band broadening in the catalytic reaction detector is significantly lower than in previously reported post-column reactors[12,13], and consequently slightly better detection limits are observed. The reproducibility and linearity of response is such that the technique should be applicable to quantitative work. Future work will concentrate on further exploration of the application potential of the catalytic reactor detection principle, miniaturization and study of other types of such solid-phase derivatization reactors.

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