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Optimization of the postcolumn hydrolysis reaction on solid phases for the routine high-performance liquid chromatographic determination of N-methylcarbamate pesticides in food products

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SUMMARY

Various solid-phase materials were evaluated as catalysts for the postcolumn hydrolysis of N-methylcarbamate pesticides in a high-performance liquid chromatographic (HPLC) method for food products. Inexpensive magnesium oxide has been used for the first time in HPLC as a very efficient catalyst and as a good alternative for, *e.g.*, expensive anion-exchange resins. The reaction mechanism and kinetics were studied through measurement of the reaction band broadening, which depends on the stationary phase, mobile phase composition and flow-rate, and reaction temperature. Also, relationships between carbamate structure and reactivity were examined. The usefulness of the optimized postcolumn system was thoroughly tested by application to crop sample analysis during an extended period of time. The range of application of the solid-phase reaction was extended to 22 carbamate pesticides and ten of their metabolites. For crop sample analysis, limits of determination in the range 1–10 ppb were obtained.

INTRODUCTION

Postcolumn reaction systems in liquid chromatography[1] have attracted increased interest during the last decade as a means of converting compounds with unfavourable detection properties into derivatives which show enhanced sensitivity and often a higher selectivity towards such specific detectors as fluorescence and electrochemical detectors. For short reaction times, the tubular non-segmented reactors, preferably in the form of PTFE knitted tubes, are by far the most popular postcolumn reactors. However, solid-phase reactors, also called packed-bed reactors, can sometimes be substituted for tubular reactors. In the ideal case, the solid-phase material, normally packed in a short stainless-steel high-performance liquid chromatographic (HPLC) column, acts as a heterogeneous catalyst for the derivatization reaction. The main advantage is that the catalyst material is not consumed during the

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postcolumn reaction. Also, addition of reagent to the column effluent via an extra pump is obviated, thereby reducing the band broadening.

A good example of a possible substitution of a solid-phase reactor for a tubular non-segmented reactor is seen in the postcolumn hydrolysis reaction of N-methylcarbamate pesticides. In routine HPLC analysis for N-methylcarbamates in crops and water samples, the pesticides are first hydrolysed postcolumn in a tubular reactor by addition of a strong base (sodium hydroxide), to yield the common product methylamine, which is converted into a fluorescent derivative in a second, short reactor by addition of *o*-phthalaldehyde (OPA) reagent.

This method is widely used in the U.S.A.[2,3], where it is accepted as an official AOAC method. Recently, our group[4] improved the cleanup method for crop samples by the use of solid-phase extraction cartridges packed with aminopropyl-bonded silica phases. Further, the postcolumn tubular reaction system was optimized and its range of application was extended to include all 22 N-methylcarbamates and ten of their major metabolites. It was also realized that further improvement and/or simplification of the postcolumn reaction could be obtained by the inclusion of a solid-phase reactor acting as a heterogeneous catalyst for the base-catalysed hydrolysis.

Nondek *et al.*[5,6] have demonstrated that a strong anion exchanger, *e.g.*, Aminex A-28, is well suited to catalyse the hydrolysis of N-methylcarbamates when the catalyst bed is maintained at 100–120°C. The reaction band broadening, due to the hydrolysis process in the catalyst column, could be reduced in this way. In other words, the extra-column band broadening for the eluting peaks from the analytical column due to the postcolumn hydrolysis was reduced to negligible values. However, the use of catalytic hydrolysis on solid phases has been evaluated only for six well known carbamates, namely aldicarb, aminocarb, carbaryl, methiocarb, methomyl and propoxur[6]. Application of the complete HPLC method has only been reported for the determination of carbaryl in water samples[6,7].

It was the aim of this work to extend the application of the principle of solid-phase catalysis to a much wider group of N-methylcarbamates, namely 22 parent carbamates and ten of their major metabolites, mainly the sulphoxide and sulphone derivatives. The reaction methanism and kinetics were studied more closely with respect to the influence of mobile phase and stationary phase parameters and also the correlation with the chemical structure of the typical carbamate. Additionally, retention characteristics of reactants and products were determined and reaction band broadening could be indirectly measured. Finally, the total HPLC method was thoroughly tested over 2 years in real crop sample analysis for N-methylcarbamates. Examples of real residues found are presented.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, U.K.) and water for HPLC was purified with an Elgastat UHQ water-purification system (Elga, High Wycombe, U.K.). *o*-Phthalaldehyde (for fluorescence analysis), 2-mercaptoethanol and disodium tetraborate (anhydrous) were obtained from Merck (Darmstadt, F.R.G.)

The OPA reagent was prepared as follows: 2.1 g of disodium tetraborate were

placed in a 1-l volumetric flask and dissolved in purified water (*ca.* 500 ml) with heating. A 250-mg amount of *o*-phthalaldehyde was weighed and dissolved in 2 ml of acetonitrile and then added to the flask. Next, 0.25 ml of 2-mercaptoethanol was added and the contents of the flask were made up to volume with water. The mobile phase and OPA reagent were degassed under vacuum prior to use.

Carbamate standards (from the Environmental Protection Agency, Research Triangle Park, NC, U.S.A., or Promochem, Wesel, F.R.G.) were dissolved in pure acetonitrile (1 mg ml^{-1}) and these stock solutions were diluted with the mobile phase solvent to obtain the required concentrations for the HPLC measurements. All other solvents and reagents used in crop sample analysis were as described previously[4].

Apparatus

The HPLC apparatus consisted of a Kratos (Ramsey, NJ, U.S.A.) Model SF 400 pump, a Rheodyne (Berkeley, CA, U.S.A.) Model 7125 injection valve, provided with a 100-μl loop, a Merck LiChroCART 250 × 4.0 mm I.D. cartridge column packed with Supersphere RP-8 (4 μm) and a postcolumn reaction detection system. The postcolumn reactor consisted of a 50 × 4.0 mm I.D. stainless-steel column, packed with various solid phases, and equipped with Valco couplings and 2-μm stainless-steel frits. The reactor column was enclosed in a holder, fitted in a Kratos PCRS 520 postcolumn oven, which could be heated at temperatures up to 150°C. The reactor was packed via a slurry procedure using an HPLC pump with mobile phase solvent at a constant flow-rate of 5 ml min^{-1}. After packing, the reactor column was conditioned at a flow-rate of 0.8 ml min^{-1} and heated at the maximum working temperature for several hours or until reproducible hydrolysis was observed.

The following solid-phase materials were used: (1) alumina N 7-12, neutral (7–12 μm) for HPLC from ICN Biochemicals (Eschwege, F.R.G.); (2) alumina 90, basic (63–200 μm) for column chromatography from Merck; (3) poly-4-vinylpyridine (Reillex 425, 300–900 μm) from Reilly Tar & Chemical (Indianapolis, IN, U.S.A.); (4) anion exchange resin Aminex A-27 (15 $\pm$ 2 μm) with tetraalkylammonium groups in the acetate form, from Bio-Rad Labs. (Richmond, CA, U.S.A.); and (5) magnesium oxide heavy (5–100 μm) from BDH (Poole, U.K.). The ion-exchange resin was allowed to swell in the mobile phase solvent for 24 h before packing the reactor column.

After the catalytic hydrolysis reactor, OPA reagent (0.20 ml min^{-1}) was added to the eluent via a low-dead-volume T-piece (vortex mixer, Kratos). The reaction of methylamine, formed during the hydrolysis of carbamates, with OPA reagent took place in the 20 cm × 0.10 mm I.D. PTFE connection capillary to a Merck–Hitachi (Tokyo, Japan) Model F-1000 double-monochromator fluorescence detector, which was tuned at excitation and emission wavelengths of 340 and 455 nm, respectively. Downstream of the detector cell a back-pressure regulator (*ca.* 10 bar), obtained from Beckman (San Ramon, CA, U.S.A.), was installed to prevent boiling of the mobile phase owing to high temperatures used in the reactor.

For the reaction band-broadening measurements (on both the ion-exchange resin and magnesium oxide reactor column), a Kratos Model 757 UV detector was used with detection at 254 nm, while the analytical column was disconnected. Reaction band-broadening studies were also executed by measurement of methylamine via OPA derivatization and fluorescence detection.

Chromatographic runs were performed isocratically with acetonitrile–water

TABLE I

NAMES, RETENTION TIMES AND OPTIMUM HYDROLYSIS TEMPERATURES OF N-METHYLCARBAMATES ON AMINEX A-27 AND MAGNESIUM OXIDE

Aromatic carbamates				*Aliphatic carbamates*			
Carbamate	t_R *on Aminex (s)*	*Temperature of complete hydrolysis (°C)*		*Carbamate*	t_R *on Aminex (s)*	*Temperature of complete hydrolysis (°C)*	
		Aminex	*MgO*			*Aminex*	*MgO*
Carbofuran	50	100	120	Oxamyl	40	130	120
3-Hydroxycarbofuran	45	100	120	Methomyl	45	>140	>140
3-Ketocarbofuran	42	100	120	Butocarboxim	45	>140	>140
Propoxur	50	100	120	Butocarboxim sulphoxide	34	140	140
Bendiocarb	50	100	120	Butoxycarboxim	42	130	140
Dioxacarb	50	90	110	Aldicarb	45	>140	>140
Ethiofencarb	65	100	120	Aldicarb sulphoxide	34	120	130
Isoprocarb	68	90	120	Aldicarb sulphone	40	120	130
Landrin	78	100	120	Thiofanox	47	>140	>140
Carbaryl	115	90	110	Thiofanox sulphoxide	34	140	140
Carbanolate	n.d.	90	110	Thiofanox sulphone	42	130	140
Methiocarb	115	90	110				
Methiocarb sulphoxide	45	100	110				
Methiocarb sulphone	100	90	110				

(35:65) as the mobile phase at a flow-rate of 0.80 ml min^{-1}. With the reaction-mechanistic experiments the latter solvent, methanol–water (40:60) and pure water were used. Chromatograms were recorded on a Kipp (Delft, The Netherlands) BD 41 recorder.

Methods

Crop sample analysis was carried out as described previously[4] with the improved cleanup method for the HPLC analysis of N-methylcarbamates. Briefly, it consisted of an acetone homogenization/extraction of the chopped sample and subsequent extraction with light petroleum and dichloromethane. An aliquot of the sample extract was evaporated, redissolved in dichloromethane and cleaned up with aminopropyl-bonded silica cartridges. Further details can be obtained elsewhere[4].

RESULTS AND DISCUSSION

Theory

Heterogeneous catalytic hydrolysis of N-methylcarbamates on a solid phase was first studied by Nondek *et al.*[5] using the hydrolysis of carbaryl on an anion exchanger as a model system. In a subsequent paper, Nondek *et al.*[6] studied five more carbamates. They derived some equations for the band broadening due to the solid-phase reaction, the so-called reaction band broadening σ_r, as related to the rate constant of the reaction, k_r, and the capacity factors for reactant and product, k'_R and k'_P:

$$\sigma_r = \frac{c(1 - k'_P/k'_R)}{k_r} \tag{1}$$

where c is a constant. This relationship holds for solid-phase reactions with first-order kinetics, which appeared to be the case in their study of the carbamate hydrolysis. The reaction band broadening could be described by the equation

$$\sigma_r^2 = \sigma_t^2 - \sigma_n^2 \tag{2}$$

where σ_t is the total band broadening caused by the solid-phase reactor and σ_n is the non-reaction band broadening. σ_r can be calculated by measuring the variance of the product peak for the separate injections of product and reactant:

$$\sigma_r^2 = (\sigma_t^2)_{prod} - (\sigma_n^2)_{prod} \tag{3}$$

Solid-phase materials

Included in our routine analysis for the determination of N-methylcarbamates in crop samples[4] are a total of 22 carbamates and ten of their metabolites. This study focused on the application of the solid-phase catalytic hydrolysis for all these compounds. A possible relationship between molecular structure and rate of hydrolysis was determined for five different solid-phase materials. Retention times of the reactant molecules, the parent carbamate and, if possible, the available hydrolysis products were determined. Also, the temperature at which the optimum response of product could be observed was established.

The results for Aminex A-27, a strong-anion exchange resin, and magnesium oxide (MgO) are summarized in Table I. MgO, although never used before in typical HPLC applications, was nevertheless tried, because it is known in organic chemistry as a strongly basic catalyst in batch reactions. In addition, it is a much cheaper and more rigid material for column chromatography than the resin-based anion exchanger Aminex A-27. In practice, MgO fulfilled its expectations and requirements, namely efficiently converting all N-methylcarbamates into their hydrolysis products.

Apart from MgO, aluminium oxide (Al_2O_3) was also studied in two different forms, HPLC grade (neutral Al_2O_3 of particle size 7–12 μm) and classical column chromatographic grade, (basic Al_2O_3 of particle size 70–200 μm). Both forms of Al_2O_3 showed clearly inferior catalytic properties to Aminex A-27 and MgO, which may be explained by the fact that Al_2O_3 is known to act as a weaker basic catalyst and/or anion exchanger. Even at temperatures as high as 150°C, the easiest to catalyse carbamates were hydrolysed by not more than 40%. The same result was obtained with another weakly basic catalyst known from organic catalytic reactions, namely polyvinylpyridine in the form of polymeric beads of particle size 300–900 μm.

The main conclusion that can be drawn from these experiments with five basic catalysts is that the degree of basicity of the catalysts is the predominant factor determining the catalytic activity in the hydrolysis reaction. Although a decrease in particle size increases the degree of catalysis, as was also shown by Nondek *et al.*[5] and Kun She *et al.*[7] for carbaryl, the degree of basicity plays a much more important role than particle size. Neutral Al_2O_3 (7–12 μm) was even less catalytic than basic Al_2O_3 (70–200 μm), which was much less catalytic than 5–100 μm MgO (strongly basic) or 15 μm Aminex A-27 (strong anion exchanger).

More extensive research on the exact mechanism of the catalytic behaviour of the various solid-phase materials was confined to MgO and Aminex A-27. Measurements of reaction band broadening were useless on the Al_2O_3 and polyvinylpyridine phases, because of the low degree of hydrolysis, even at very high reactor temperatures.

Aminex A-27. A closer look at the Aminex A-27 results in Table I reveals that a rough relationship can be discerned between the molecular structure of the carbamate pesticide on the one hand and retention time on Aminex and temperature of maximum response of product on the other. The carbamates may be divided in two typical groups, namely aromatic carbamates and aliphatic carbamates (oxime carbamates). The aromatic carbamates, with retention times (t_R) between 45 and 115 s, invariably have their maximum product response at 90–100°C, whereas the aliphatic carbamates, with distinctly shorter retention times (t_R = 34–47 s) require temperatures of at least 120°C for maximum peak response of the products. The parent carbamates methomyl, butocarboxim, aldicarb and thiofanox even require a temperature slightly above 140°C for complete conversion.

Although Nondek *et al.*[5] had suggested that a certain retention of the parent carbamates on the solid-phase material would be required to undergo catalytic hydrolysis, it can be concluded from Table I that the ease of hydrolysis is definitely not linearly related to the retention on the solid phase.

The assumed reaction equations for the carbamate hydrolysis[6] are as follows:

$$ROC(O)NHCH_3 \rightarrow ROH + CH_3N{=}C{=}O \quad (4)$$

$$CH_3N{=}C{=}O + H_2O \rightarrow CH_3NH_2 + CO_2 \quad (5)$$

By subsequent injection and comparison of retention times of the available standards of the hydrolysis products ROH (oxamyl oxime, methomyl oxime and carbofuranphenol) and the hydrolysed parent compounds, it has been proved that these products are indeed formed.

The first step in the hydrolysis reaction is assumed to be a nucleophilic attack on the carbon atom of the ester group. The speed of this reaction step will be influenced by the fractional charge on the molecule, which will be (extra) induced by the solid-phase catalyst, thereby lowering the activation energy for the nucleophilic substitution. The differences observed in the hydrolysis rate between the various carbamates will probably be influenced by the substituents in the R group. Within the group of aliphatic carbamates, more sequences can be deduced from Table I and additional experiments. For the parent compounds, the hydrolysis rates decrease in the order oxamyl > methomyl > aldicarb > butocarboxim > thiofanox. Note that their respective retention times are 40, 46, 45, 45 and 47 s, which again are apparently not decisive. The hydrolysis rate sequence for the carbamates, which also have sulphoxide and sulphone oxidation products, namely aldicarb, butocarboxim, thiofanox and methiocarb, is invariably sulphone > sulphoxide > parent.

Finally, some general observations are of interest. The retention times of the hydrolysis products (ROH) on Aminex are always higher, than those of the parent compounds, *e.g.*, oxamyl oxime and oxamyl, 138 and 40 s; methomyl oxime and methomyl, 66 and 46 s; carbofuranphenol and carbofuran, 260 and 50 s; and naphthol and carbaryl, >2000 and 115 s. The methylamine, always formed in the hydrolysis reaction of N-methylcarbamates has a retention time of *ca.* 40 s. Methomyl has a strikingly high total band broadening in comparison with the other difficult to hydrolyse carbamates. No explanation can be given for this deviant behaviour.

When critically comparing the results of Nondek's group and this work, some striking differences are revealed. We have never observed any peak in the reaction product formation chromatograms other than those originating from the parent compound and the oxime or phenol. That is, we could not detect any intermediate isocyanate, an extra peak reported by Nondek *et al.* Although Nondek *et al.*[5,6] experienced decomposition of their Animex A-28 ion-exchange material above 120°C, in our study temperatures up to 150°C could be applied without noticeable deterioration of the phase. Surprisingly, the Aminex A-27 (15 $\pm$ 2 μm) used in this study required a *ca.* 20°C higher temperature than the Aminex A-28 (9 $\pm$ 2 μm) used by Nondek *et al.* The small difference in particle size can hardly have such an influence, taking into account the results of Nondek *et al.* when they compared Amberlite CG 400 (70 μm) with Aminex A-28 (9 μm).

There is no other conclusion, then, that the reaction kinetics and possibly also the mechanism appear to be different in our case. This may be caused by differing production batches of the Aminex, due to changed production methods.

Magnesium oxide. Although not commercially available in a small, narrow-ranged particle size as typical HPLC packings, "general-purpose reagent" MgO (particle size 5–100 μm) packed in a catalytic reactor showed surprisingly good catalytic activity. Compared with Aminex A-27, the reactor temperature needed to obtain the optimum product response averaged 20°C higher (see Table I). This could be explained by the differences in basicity and/or available particle sizes of both materials. It is to be hoped that in the near future better defined MgO with a narrow

particle size distribution will become commercially available for our special application. The retention times of all parent carbamates and those of the hydrolysis products (ROH) and methylamine appeared to be identical, namely 40 s. This again underlines the earlier statement that the absolute retention on the solid phase has no relationship with the ease of hydrolysis of the compound. Solute–catalyst interactions at the molecular level must be far more important. With respect to the reaction band-broadening (σ_r) measurements, a significant difference could be seen between the measurements via the methylamine or via the phenol/oxime product with fluorescence and UV detection, respectively. In the latter instance, σ_r appeared to be zero, which would suggest that a first-order reaction takes place (see also eqn. 1). However, with the fluorescence measurements, the peak widths of the hydrolysed reactant and methylamine standard injected differed. In addition, the peak width of the oxime or phenol product (as measured with UV detection) appeared to be equal to the peak width of the injected methylamine standard, but smaller than the peak width of the methylamine product from the injected (carbamate) reactant (as measured with

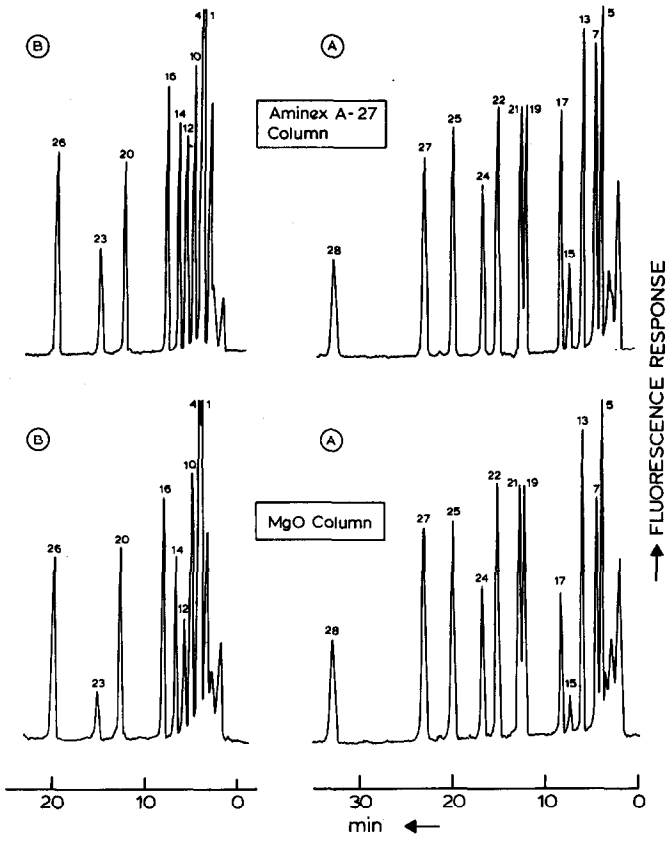

Fig. 1. Comparison of the catalytic hydrolysis efficiency of two mixtures (A and B) of N-methylcarbamate standards on two different solid-phase materials, Aminex A-27 and MgO, at a reactor temperature of 120°C. Mobile phase: acetonitrile–water (35:65); flow-rate, 0.8 ml min^{-1}; injected amounts, 2 ng of each compound. Numbers above the peaks correspond to those in Table III.

fluorescence detection). Apparently, the conversion of methyl isocyanate into methylamine is the rate-determining step, which can only be caused by a certain retention of the intermediate carbamic acid on the MgO. This results in the measured σ_r of methylamine product being unequal to zero. The overall reaction from carbamate to methylamine is thus not of first-order kinetics. Because these differences between σ_r values measured via UV or fluorescence detection did not occur on Aminex A-27, it might be concluded that, if not the reaction mechanism, at least the reaction kinetics of the carbamate hydrolysis are different on the two solid-phase materials. A typical example of chromatograms of two standard mixtures of carbamates hydrolysed on MgO and Aminex A-27 at 120°C is shown in Fig. 1. The absolute response of the aromatic carbamates is identical on both phases, because at 120°C complete hydrolysis has taken place, as can also be seen from Table I. The responses of the aliphatic carbamates, however, are lower with MgO than Animex. Note also that the peak width, at sufficiently high reaction temperatures, is almost identical on both phases. The slightly higher total chromatographic peak width of most carbamates on MgO is simply caused by the higher band broadening of methylamine on MgO compared with Aminex A-27, *e.g.*, 6.5 *versus* 2.6 s, at 120°C.

Reaction band-broadening measurements

Dependence of σ_r on mobile phase. Reaction band broadening, σ_r, on Aminex A-27 and MgO was measured via subsequent injections of the parent compound and oxime/phenol product, directly onto the reactor column, for the model compounds oxamyl, methomyl and carbofuran, followed by UV detection at 254 nm. σ_r could also be measured via subsequent injections of standard methylamine and parent compound, followed by OPA reaction and fluorescence detection.

When oxamyl was tested on Aminex at different flow-rates, σ_r increased when the flow-rate was increased. For, *e.g.*, flow-rates of 0.5 and 1.0 ml min^{-1} of acetonitrile–water (35:65), σ_r was 6.5 and 16 s, respectively. The flow dependence of σ_r means that, in this work, in contrast to the results of Nondek's group[5], the hydrolysis reaction is not first order. The same behaviour was also observed with MgO.

Another interesting feature of the catalytic hydrolysis could be seen when the mobile phase composition was changed. Pure water appeared to give more efficient catalysis and also lower σ_r values than acetonitrile–water (35:65). A methanol–water (40:60) mixture was even less efficient than acetonitrile–water. The influence of the mobile phase is more pronounced the lower the temperature. In practice, the optimum mobile phase is thus an acetonitrile–water mixture with as low as practical a flow-rate for the chromatographic separation of the complete mixture of carbamates.

Dependence of σ_r on retention temperature. In accordance with the expectations, a significant decrease in σ_r with increasing temperature was actually observed on both solid phases. As a typical example, in Table II the dependence of σ_r on reaction temperature is shown for the three model compounds on MgO and Aminex A-27. The absolute values of σ_r are typically higher on Aminex than on MgO. This effect is more apparent as the reaction temperature becomes lower than the temperature for complete hydrolysis. Apparently, σ_r is not necessarily directly related to the ease of hydrolysis. The lower σ_r value of oxamyl compared with the easier to hydrolyse carbofuran also supports this statement. Another example is given by the almost negligible σ_r value (<0.1 s) of methomyl hydrolysed on MgO at 120°C, where the extent of hydrolysis has not yet reached 100%.

TABLE II

DEPENDENCE OF THE CATALYTIC HYDROLYSIS REACTION BAND BROADENING (σ_r) ON REACTION TEMPERATURE ON AMINEX A-27 AND MAGNESIUM OXIDE SOLID PHASES FOR THREE N-METHYLCARBAMATES

Mobile phase, acetonitrile–water (35:65); flow-rate, 0.8 ml min^{-1}.

Solid-phase material	*Carbamate*	*Reaction band broadening, σ_r (s)*			
		80°C	*100°C*	*120°C*	*140°C*
Aminex A-27	Oxamyl	11.4	4.1	0	0
	Methomyl	n.d.	n.d.	8.3	0.8
	Carbofuran	19.1	6.9	0	0
MgO	Oxamyl	5.4	3.1	0	2.7
	Methomyl	6.9	3.8	0.4	0
	Carbofuran	8.1	4.9	0	0

Oxamyl shows a very typical hydrolysis behaviour (see Table II) on MgO. After σ_r has approached zero at 120°C, it increases again towards 2.7 s at 140°C. In the product formation analysis with fluorescence detection, an extra peak with a slightly different retention time indeed appears in the chromatogram. Probably a second reaction route, with a higher activation energy, becomes possible for oxamyl at such high reaction temperatures.

Typical chromatograms of a carbamate standard mixture, catalytical hydrolysed on MgO at various temperatures, are shown in Fig. 2. The decrease in peak width with increase in reactor temperature is clearly visible.

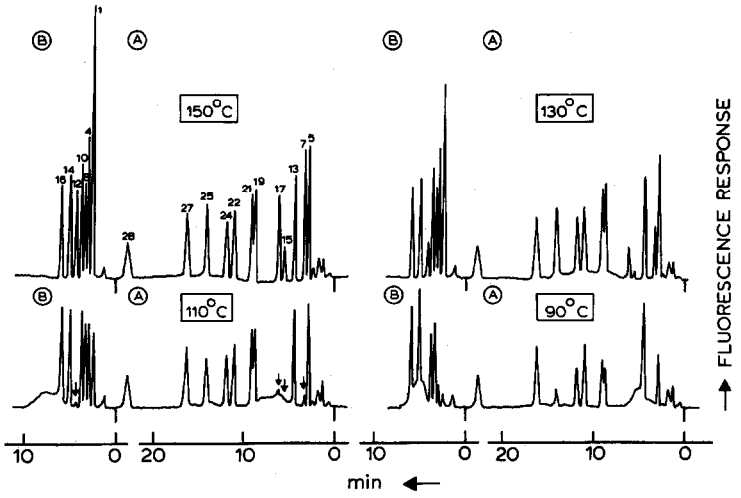

Fig. 2. Dependence of the catalytic hydrolysis effiency on reaction temperature for two standard mixtures of N-methylcarbamates (A and B), separated in the HPLC system Supersphere RP-8 with acetonitrile–water (35:65) at 0.8 ml min^{-1} and hydrolysed on MgO at 90, 110, 130 and 150°C. Injected amounts, 2 ng of each compound (except thiofanox, 20 ng). Numbers above the peaks correspond to those in Table III.

TABLE III

RELATIVE RETENTION TIMES (RRT) AND DETECTION LIMITS OF 22 N-METHYLCARBAMATES AND 10 OF THEIR METABOLITES IN THE REVERSED PHASE HPLC SYSTEM SUPERSPHERE RP-8 (5 μm) WITH ACETONITRILE–WATER (35:65)

Catalytic reactor packed with Aminex A-27 or magnesium oxide (operating temperature, 140°C). Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455 nm, respectively.

No.	*Compound*	*RRT*	*Detection limit (pg)*	*No.*	*Compound*	*RRT*	*Detection limit (pg)*
	Parent compounds:				*Metabolites:*		
5	Oxamyl	0.29	25	1	Aldicarb sulphoxide	0.24	25
7	Methomyl	0.34	25	2	Butocarboxim sulphoxide	0.25	25
9	Ethidimuron	0.39	50	3	Butoxycarboxim	0.28	25
11	Tranid	0.44	50	4	Aldicarb sulphone	0.29	25
13	Dioxacarb	0.46	50	6	Thiofanox sulphoxide	0.31	50
15	Butocarboxim	0.59	50	8	Methiocarb sulphoxide	0.34	50
17	Aldicarb	0.65	50	10	3-Hydroxycarbofuran	0.39	50
18	Cloethocarb	0.93	50	12	Thiofanox sulphone	0.45	50
19	Propoxur	0.94	50	14	Methiocarb sulphone	0.51	50
20	Bendiocarb	0.98	50	16	3-Ketocarbofuran	0.63	50
21	Carbofuran[a]	1.00	50				
22	Carbaryl	1.21	50				
23	Thiofanox	1.25	50				
24	Ethiofencarb	1.31	100				
25	Isoprocarb	1.55	100				
26	Landrin	1.59	100				
27	Carbanolate	1.83	100				
28	Methiocarb	2.58	100				
29	Promecarb	3.05	n.d.				
30	Bufencarb	>4	n.d.				
31	Mexacarbate	>4	n.d.				
32	Aminocarb	>4	n.d.				

[a] Absolute retention time of carbofuran = 10.5 min.

Analytical data

In Table III, relative retention times and detection limits of all the carbamates and their metabolites studied are reported. The retention times were obtained with an isocratic run, using acetonitrile–water (35:65) as the mobile phase at a flow-rate of 0.8 ml min^{-1}. Data for promecarb, bufencarb, mexacarbate and aminocarb are not given, as a different mobile phase would have been required to reduce their high retention. Detection limits, calculated for a signal-to-noise ratio of 3:1, are invariably in the 25–100 pg range for both catalytic solid phases. The only exception is thiofanox, which at a reaction temperature of 140°C displays a 10-fold higher detection limit on MgO compared with Aminex A-27. A further increase in the reactor temperature for MgO drastically improves this situation.

Repeatability studies were performed via repetitive injection of 5 ng of methomyl, carbofuran and methiocarb. For all three compounds a relative standard deviation of *ca.* 2% ($n = 10$) was obtained. No significant differences in repeatability between Aminex A-27 and MgO were observed.

Good linearity ($r = 0.995$) was observed on both solid phases over the practical

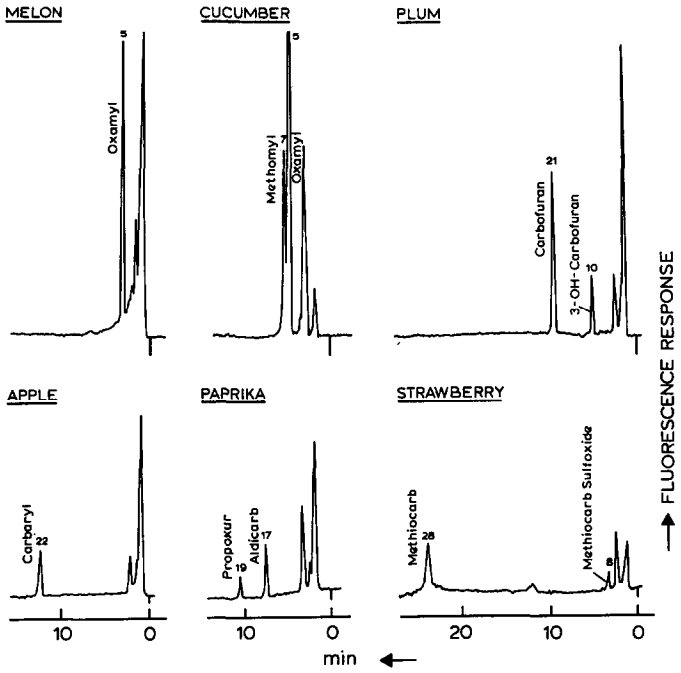

Fig. 3. Examples of HPLC of crop sample extracts containing real residues of various N-methylcarbamates. HPLC system as described under Experimental. Residue concentrations: (melon) 0.04 ppm oxamyl; (cucumber) 0.28 ppm oxamyl and 0.07 ppm methomyl; (plum) 0.02 ppm carbofuran and 0.01 ppm 3-hydroxycarbofuran; (apple) 0.05 ppm carbaryl; (paprika) 0.008 ppm aldicarb and 0.003 ppm propoxur; (strawberry) 0.02 ppm methiocarb and 0.005 ppm methiocarb sulphoxide.

working range of 0.1–10 ng injected amount of the test compounds methomyl, carbofuran and methiocarb. It should be stressed that good linearity was obtained despite the fact that no first-order kinetics were observed for catalytic hydrolysis. This finding is contrary to the expectations which were stated by Nondek *et al.*[5] in their study.

Crop sample analysis

The feasibility of the catalytic solid-phase hydrolysis was tested in crop sample analysis. An HPLC system including the Aminex A-27 reactor has been used routinely over the last 2 years with an annual sample throughput of *ca.* 1000. The catalytic reactor was in use for over 6 months before renewal of the phase was required. Hence no poisoning of the catalyst occurred provided that a guard column was installed in front of the analytical column and solid-phase extraction clean-up of samples was applied as a protective measurement. The catalysis may thus be assumed to be purely heterogeneous.

The carbamate pesticide system now includes the analysis of 22 N-methylcarbamates and ten of their major metabolites (mostly sulphoxides and sulphones of the oxime carbamates). In the optimized system a flow-rate of 0.8 ml min^{-1} of an acetonitrile–water (35:65) mobile phase and a reactor temperature of 140–150°C (for

Aminex A-27 or, alternatively, MgO) are being used. The major advantages of the use of the solid-phase hydrolysis system developed in this study, compared with the classical sodium hydroxide hydrolysis as used in crop sample analysis by Krause[2,3] and our group[4], are (1) the elimination of a reagent pump and a mixing tee and also reagent costs, (2) the possibility of using higher reaction temperatures with inherent higher hydrolysis efficiencies and thus lower detection limits (which is best illustrated in the case of thiofanox) and (3) reduced reaction band broadening resulting in optimum maintenance of the chromatographic resolution.

Compared with the work of Nondek *et al.*[5,6], the major improvements achieved are (1) the great extension of the range of application of the catalytic reaction to a total of 32 compounds, (2) a further reduction of reaction band broadening to the optimum value zero, (3) the inexpensive solid phase MgO (5–100 μm), compared with their inexpensive anion exchanger Amberlite CG 400 (70 μm), is easier to pack, has a much higher thermal stability and shows negligible retention for both reactant and products, (4) the suitability for application to even difficult crop samples and (5) practical problems with gradient elution due to shrinking and swelling of the Aminex A-27 are obviated with the rigid MgO.

In Fig. 3 chromatograms are shown of six routine crop samples that contained positive residues of various N-methylcarbamates. The clean chromatograms illustrate the selectivity and sensitivity of the method, which is due to the earlier developed improved clean-up method combined with the effective catalytic reaction detection system. Limits of determination are in the range 1–10 ppb when only 400 mg of the original sample is worked up. Owing to the difficult matrix, for citrus fruit products a 10-fold dilution of the original sample extract is recommended, which results in a 10-fold increase in the limits of determination. More results on reproducibility and quantitation, and also residue data from routine analysis, will be given in a subsequent paper.

Future work will be directed towards the extension of the application of solid-phase catalysis to other types of pesticides that might be hydrolysed, *e.g.*, phenylurea herbicides. Also, attention will be paid to the automation of the solid-phase extraction clean-up method, which is still the time-determining step in the whole method.

CONCLUSION

A postcolumn solid-phase reaction system, based on heterogeneous catalytic hydrolysis on a packed-bed reactor, has been optimized for the HPLC analysis of a complete group of 22 N-methylcarbamates and ten major metabolites. Complete hydrolysis was achieved at a reactor temperature of *ca.* 140°C on both the more expensive strong anion exchanger Aminex A-27 and on the inexpensive magnesium oxide. Magnesium oxide showed the additional advantages of lower reaction band broadening, even at lower than optimum temperatures, and the possibility of performing gradient elution analysis.

Reversed-phase HPLC combined with the solid-phase reactor and subsequent OPA derivatization of the resulting methylamine provides a selective and sensitive method for the routine determination of N-methylcarbamates in crop samples. The method has been in operation in our laboratory for more than 2 years, without any significant problems.

Limits of determination in the range 1–10 ppb can be obtained. Future research will be focused on the extension of the catalytic solid-phase reaction principle to other important classes of pesticides.

REFERENCES

1 I. S. Krull (Editor), *Reaction Detection in Liquid Chromatography*, Marcel Dekker, New York, 1986.
2 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 726.
3 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1986) 734.
4 A. de Kok, M. Hiemstra and C. P. Vreeker, *Chromatographia*, 24 (1987) 469.
5 L. Nondek, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 54 (1983) 1466.
6 L. Nondek, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.
7 L. Kun She, U. A. Th. Brinkman and R. W. Frei, *Anal. Lett.*, 17 (1984) 915.