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INVESTIGATIONS ON LOSS OF CHLOROTHALONIL, DICHLOFLUANID, TOLYLFLUANID AND VINCLOZOLIN BY COLUMN CHROMATOGRAPHIC CLEAN-UP ON SILVER-LOADED ALUMINA IN A GAS CHROMATOGRAPHIC MULTIRESIDUE PROCEDURE

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SUMMARY

The loss of the fungicides chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin during column chromatography on alumina was found to be dependent on temperature, contact time, pH, deactivation by water and loadability of the column material. Silver nitrate added to the column material increased the loss of chlorothalonil, probably by the formation of charge transfer complexes between chlorothalonil and silver ions. The addition of substrate reduced the loss of compounds.

On the basis of the experimental results an alumina clean-up procedure is proposed for multiresidue determination of chlorinated pesticides in fruit and vegetables. By rapid elution with 30% diethyl ether–light petroleum (b.p. 50–60°C) at 0°C from a chilled microcolumn consisting of 1 g of neutral alumina standardized with 7% water and loaded with 1% silver nitrate, recoveries better than 90% were obtained for the compounds tested.

INTRODUCTION

The monitoring of pesticide residues often is performed by multiresidue methods that permit analysis of a large number of compound and commodity combinations. However, all methods are limited in application mainly by the lack of specificity and sensitivity of the detection system or by insufficient recovery. Recently, the field of application of the FDA multiresidue procedure was extended by the recording of data for chemicals known to be only partially recovered, if at all[1].

A multiresidue procedure used in the Danish National Food Institute for the determination of chlorinated insecticides in fruit and vegetables failed to yield reproducible recovery of the fungicides chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin. The method includes acetone extraction, addition of water and dichloromethane, and liquid–liquid partitioning, followed by column chromatography on silver-loaded alumina with 30% diethyl ether–light petroleum as solvent. The final

determination is performed by gas chromatography (GC) with an electron capture detector (ECD). The loading of chromatographic materials with silver nitrate (silver ions) is widely used in separation techniques of column chromatography, thin-layer chromatography, and GC[2], and has also been applied to high-performance liquid chromatography[3]. Silver-loaded alumina was introduced in 1972 for the clean-up of pesticides in crops containing interfering compounds such as carotenes and organic sulphides, which are found in, *e.g.*, onions, cabbage and carrots[4].

Preliminary experiments have shown that the loss of compounds occurred during the alumina clean-up, and at first the silver loading was thought to be an important cause. However, further experiments revealed that a number of factors were responsible. The causes of change and loss of substance during chromatographic separation, particularly on alumina, have been investigated and reviewed by Hesse[5]. The most important effects reported were acid or basic reaction, oxidation by impurities of heavy metal oxides, and autooxidation as well as catalytic decomposition by the column material. In this laboratory, the loss of compounds was investigated by variation of chromatographic elution time, temperature, pH, water deactivation, loadability and addition of silver nitrate to the column material. A successful general procedure was elaborated by chilling the chromatographic system and increasing the elution rate.

During the investigations a test tube procedure was developed, which was suitable for the simulation of the reactions of test compounds and alumina on chromatographic columns. The test tube procedure worked with better reproducibility than the original micro column procedure, and the latter was therefore mainly used for experiments involving clean-up of extracts.

EXPERIMENTAL

Materials

Acetone, dichloromethane and 2,2,4-trimethylpentane were analytical reagent grade. Acetone was further distilled, light petroleum (b.p. 50–60°C) was freshly distilled before use. Liquid paraffin oil for spectroscopy was used in a 1% (w/v) solution in light petroleum as a keeper during evaporation to dryness. Deionized water (pH 7) was used. Reagents were reagent grade. Alumina of varying activity and acidity in the mesh range 70–300 (BDH, Poole, Great Britain; Merck, Darmstadt, G.F.R.; Woelm, Eschwege, G.F.R.) was treated at 500°C for 4 h followed by cooling in a desiccator before standardization with water or a water solution of silver nitrate.

Chlorothalonil (2,4,5,6-tetrachloro-1,3-dicyanobenzene, m.p. 248.5–251°C), dichlofluanid (N-[(dichlorofluoromethyl)thio]-N′,N′-dimethyl-N-phenylsulphamide, m.p. 105°C) and tolylfluanid (N-[(dichlorofluoromethyl)thio]-N′,N′-dimethyl-N-tolylsulphamide, m.p. 92.5–95°C) were recrystallized from methanol. Vinclozolin (3-(3,5-dichlorophenyl)-ethenyl-5-methyl-1,3-oxazolidin-2,4-dione, m.p. 107.5–109°C (Bayer, Leverkusen, G.F.R.)) was used without further purification. The structures are given in Fig. 1.

Test tube method

10-ml test tubes, 13 mm I.D. and provided with glass stoppers, were wrapped

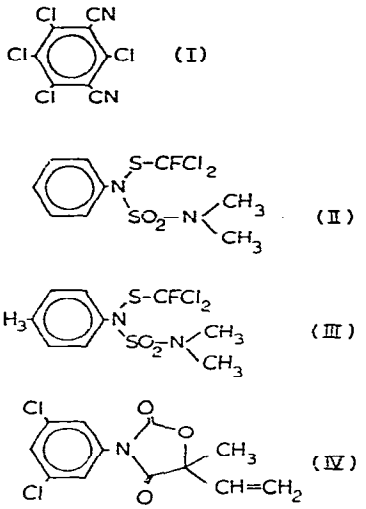

Fig. 1. Structures of chlorothalonil (I), dichlofluanid (II), tolylfluanid (III) and vinclozolin (IV).

in aluminium foil to exclude light. 1.00-ml standard pesticide solutions in 2,2,4-trimethylpentane were placed in closed test tubes in a thermostatted water bath. 1.70 g of standardized alumina was placed in closed test tubes in the same water bath. After temperature equilibration, alumina was added to each tube containing standard solutions resulting in complete wetting of the alumina. The time of contact between alumina and pesticide solution was measured. Each tube was removed from the water bath immediately. 4 ml of diethyl ether were added and the tube shaken vigorously for 120 sec. A 2-ml portion of the solution was transferred to a clean tube, and 2 ml of light petroleum were added. The solutions were dried with anhydrous sodium sulphate and analyzed by GC–ECD. Aldrin was used as internal standard for vinclozolin, and *p,p'*-DDE for chlorothalonil, dichlofluanid and tolylfluanid. It was shown that neither aldrin nor *p,p'*-DDE was degradated or absorbed under the experimental conditions.

Micro column method

Alternatively, the experiments were conducted by use of micro chromatography columns of pyrex glass, 6 mm I.D. and provided with a 15-ml reservoir and screw thread head for quick connection to nitrogen pressure.

On a small cotton plug 1.00 g of standardized alumina was placed and, without the column tip being touched, a rubber cap was placed on the bottom of the column. The prepared column was placed in a thermostatted water bath. The pesticide standard solution was placed in the same water bath. The rubber cap was removed and 1.00 ml of pesticide solution in 2,2,4-trimethylpentane was added. A light pressure was applied until the solvent level reached the alumina. The cap was replaced and the column wrapped in aluminium foil and placed in the water bath. After the treatment the column was removed from the water bath. The cap was removed immediately, and the column tip wiped, followed by rapid elution with 10 ml of 30% diethyl ether–light petroleum previously stored in the water bath. A light

pressure was applied to the upper end of the column. The eluate was collected and the volume adjusted to 10 ml with light petroleum. The solution was analyzed by GC–ECD.

Alumina pH

The pH of standardized alumina was determined in a slurry of 1.00 g of alumina in 10 ml of deionized carbon dioxide-free water (pH 7) by use of a Radiometer pH Meter 25 equipped with a glass electrode.

Gas chromatography

A Packard 427 gas chromatograph was fitted with a 6 ft × 2 mm I.D. glass column packed with 5% OV-17 on 80–100 mesh Chromosorb WHP. A constant-current ^{63}Ni ECD (15 mCi) was used. The flow-rates were: column flow 25 ml/min, and by-pass to detector 15 ml/min, both oxygen-free nitrogen. The injection heater was kept at 225°C and the detector at 275°C. The column temperature was isothermal at 200°C for all determinations. The ECD current was 0.92 nA and the output attenuation 32.

Extracts from fruit and vegetables

The effect of extracts was investigated by clean-up with micro chromatographic columns. Strawberries or apples (100 g) were extracted with acetone (200 ml) and filtered by suction, and the filter cake was rinsed with acetone (150 ml). To an aliquot of the extract (125 ml) water (250 ml) and saturated sodium chloride solution (10 ml) were added, followed by repeated extraction with dichloromethane (2 × 50 ml). The extract was dried with anhydrous sodium sulphate, and 5 ml of keeper (paraffin oil) was added, followed by evaporation just to dryness. After addition of 5–10 ml of light petroleum the evaporation to dryness was repeated and the volume adjusted with light petroleum to a concentration corresponding to 2.00 g of fresh sample per ml. This procedure was in accordance with the extraction and clean-up steps in a multiple residue procedure used by the official laboratories in Denmark for the control of residues of chlorinated pesticides in fruit and vegetables[6].

RESULTS AND DISCUSSION

In most experiments it was found that the rate of disappearance by contact with alumina of chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin showed good agreement with a first order kinetic model

$$\ln G_t = -k\,t + \ln G_0$$

where G_t is the recovery of compound after the time t (sec), k the first-order rate constant (sec^{-1}), and G_0 the recovery at the time 0 defined by the intercept ln G_0. In some experiments G_0 was found to be less than 100%, indicating a very rapid reaction or formation of complexes of a limited and well-defined amount of substance present in the experiments. In the following discussion G_0 is denoted as the intercept. The results conform to chlorothalonil[7], captan, captafol, and folpet[8] hydrolysis in water following first-order models at constant pH and temperature.

In Fig. 2 the micro column procedure and the test tube procedure are compared by the loss of vinclozolin on the same standardized alumina and found to be equivalent. A prewash of the micro column with 10 ml of 30% diethyl ether–light petroleum caused a loss of vinclozolin by removing water and increasing the alumina activity. There was no difference between dry and water-saturated 30% diethyl ether–light petroleum as solvent in the micro column test procedure.

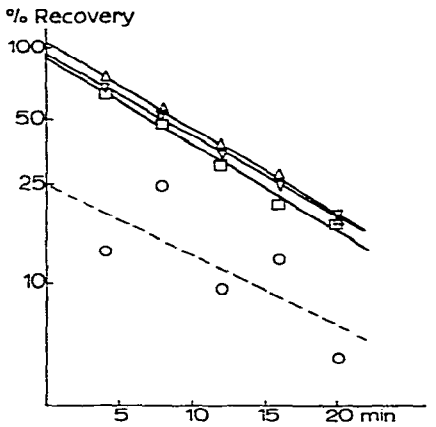

Fig. 2. Comparison of micro column procedure (△) and test tube procedure (□) by the loss of 0.2 μg of vinclozolin at 0° as a function of time on 1.7 g of neutral alumina standardized with 7% water and 1% silver nitrate. The contact was performed in 2,2,4-trimethylpentane, followed by elution with 30% diethyl ether–light petroleum. ▽, Micro column eluted with water saturated solvent; ○, micro column prewashed with solvent.

Fig. 3 shows the solvent effect on the loss of vinclozolin by contact with standardized alumina: the test tube procedure was carried out with solutions of the compound in either 2,2,4-trimethylpentane or in 30% diethyl ether–light petroleum. The increase of the rate of loss by use of a non-polar and weak solvent may be explained according to Snyder[9] as a result of competition of solvent and solute for adsorption sites in the column material. The proton acceptor, diethyl ether, is held more strongly on the most active adsorption sites than light petroleum, resulting in the adsorption of pesticide test compounds mainly on the weakened remainder of the adsorbent surface. A similar effect was observed by comparison of a micro column prewashed with 10 ml of 30% diethyl ether–light petroleum and a column with the same prewash followed by an additional prewash with 10 ml of light petroleum, resulting in removal of diethyl ether and consequently an increased rate of loss of vinclozolin on the column.

The ratio of solvent and standardized alumina did not affect the loss of vinclozolin in a range corresponding to 25–100% of complete wetting of the alumina as shown in Table I.

The ratio of amounts of pesticides and standardized alumina did not affect the rate constant k significantly in the ranges 0.2–20 μg chlorothalonil/g alumina, 0.4–40 μg dichlofluanid/g alumina, 0.4–40 μg tolylfluanid/g alumina, and 0.12–12 μg vinclozolin/g alumina. The results for vinclozolin are shown in Table II.

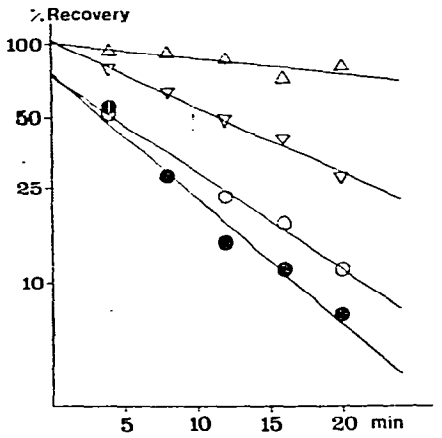

Fig. 3. Solvent effect on the loss of 0.2 μg of vinclozolin as a function of time at 0° C on 1.7 g of neutral alumina standardized with 7% water and 1% silver nitrate. △, Test tube procedure, reaction in 30% diethyl ether–light petroleum; ▽, test tube procedure, reaction in 2,2,4-trimethylpentane; ○, micro column procedure, prewashed with 30% diethyl ether–light petroleum; ●, micro column procedure prewashed with 30% diethyl ether–light petroleum followed by additional wash with light petroleum

TABLE I

RATE CONSTANTS FOR THE LOSS OF VINCLOZOLIN

0.2 μg of vinclozolin dissolved in 2,2,4-trimethylpentane in contact with alumina at 0° C, standardized with 7% water and 1% silver nitrate, by variation of the amount of solvent. 95% confidence limits.

Solvent (ml)	*Standardized alumina (g)*	*Wetting (%)*	$k \times 10^4$ *(sec^{-1})*
0.25	1.7	25	1.9 ± 0.5
0.50	1.7	50	2.3 ± 0.2
0.75	1.7	75	2.3 ± 0.5
1.00	1.7	100	2.7 ± 0.7

TABLE II

RATE CONSTANTS AND INTERCEPTS OF THE FIRST-ORDER MODEL FOR VARIOUS CONCENTRATIONS OF VINCLOZOLIN

Vinclozolin in 1 ml 2,2,4-trimethylpentane added to 1.7 g of alumina at 0° C standardized with 7% water and 1% silver nitrate. 95% confidence limits.

Vinclozolin (μg/ml)	*Intercept (%)*	$k \times 10^3$ *(sec^{-1})*
0.2	84 (66–106)	1.4 ± 0.2
2.0	90 (78–103)	1.4 ± 0.1
20	97 (76–124)	1.2 ± 0.2

The rate constants were found to be dependent on the temperature following the simplified Arrhenius expression

$$k = A \cdot e^{-E_a/RT}$$

where E_a = the apparent activation energy, $R = 8.314$ J/°C mol, T = temperature, (°K) and A = constant (frequency factor).

Fig. 4 gives the rate constants k as function of the reciprocal temperature ($°K^{-1}$) for the compounds chlorothalonil (0.2 μg), dichlofluanid (0.4 μg), tolylfluanid (0.4 μg) and vinclozolin (0.2 μg). Consequently the loss of all the test compounds during chromatography on alumina is markedly reduced by lowering the column temperature and increasing the elution rate.

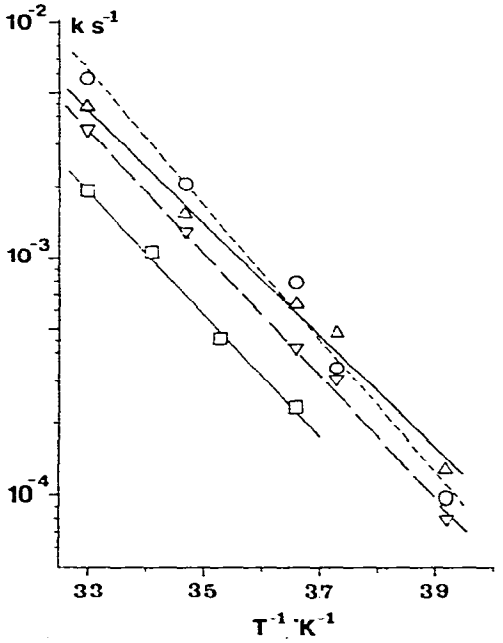

Fig. 4. Rate constants, k (sec^{-1}), of chlorothalonil (○, --------) dichlofluanid (△, ———), tolylfluanid (▽, -·-·-·-), and vinclozolin (□, ———) as a function of reciprocal temperature ($°K^{-1}$). Micro column procedure with solvent prewash was used for chlorothalonil, dichlofluanid and tolylfluanid, and test tube procedure was used for vinclozolin.

The apparent activation energy E_a may be a composite quantity including the energy of activation, the heat of absorption of the compound and possible reaction products, and the heat of solution. From Fig. 4 the apparent activation energies were calculated: chlorothalonil 54 kJ/mol, dichlofluanid 46 kJ/mol, tolylfluanid 50 kJ/mol and vinclozolin 50 kJ/mol.

The influence on the loss of vinclozolin in 2,2,4-trimethylpentane solution by the contact with either acid, neutral or basic alumina was tested at 0°C by use of the test tube method. Alumina from different sources was standardized with 7% water and 1% silver nitrate or with 7% water. The rate constant k as function of pH and the source of the alumina are shown in Fig. 5. Some scattering of the points was probably caused by origin and batch variations. The first-order k values seemed to increase with increasing pH values above 6.5. This effect may be interpreted as

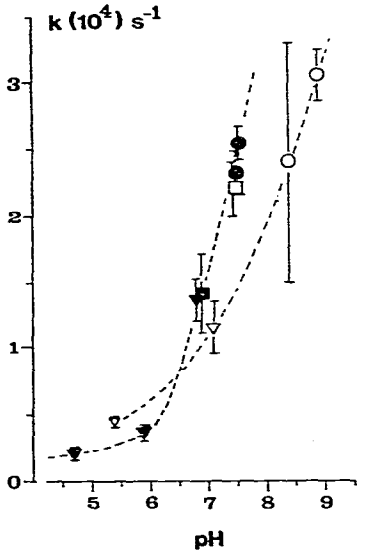

Fig. 5. Effect of pH on the rate constant of the loss of 0.2 μg of vinclozolin at 0 °C on different aluminas standardized with 7% water (open symbols) or 7% water and 1% silver nitrate (closed symbols). ▽, Woelm; □, Merck; ○, BDH. 95% confidence limits of single points.

second-order reactions of vinclozolin and hydroxide similar to water hydrolysis of captan as function of pH[8] given by

$$-\frac{\mathrm{d[vinclozolin]}}{\mathrm{d}t} = k[\text{vinclozolin}] + k_{OH}[\text{vinclozolin}]\,[\text{hydroxide}]$$

In Fig. 5 a tendency towards increasing k values was observed following the addition of silver nitrate to the alumina. Although dichlofluanid and tolylfluanid were not included in these detailed pH studies, a similar dependence on pH was found. This was also found for chlorothalonil, but only in the absence of silver ions. Consequently, neutral or acid alumina should be chosen for the chromatographic clean-up of chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin.

The influence of water and silver nitrate added to the alumina on the loss of vinclozolin was tested by use of the test tube method at 0°C. 0.2 μg of vinclozolin dissolved in 1.00 ml of 2,2,4-trimethylpentane was added to 1.70 g of neutral alumina (Woelm W-200) standardized with 2, 4, 8, or 16% of water with or without the addition of 17 mg of silver nitrate. As shown in Fig. 6, the k values decreased and the intercept value increased as the amount of water added was increased. The blocking of active centres by water adsorption is a general way to deactivate alumina. It seems to be necessary to have three or more complete layers of water molecules for complete homogenization of the alumina surface[10]. The adsorbed water molecules, on the strong centres of adsorption, lead to a strong deformation in the electronic density of other molecules attaching the water molecules[10]. With a specific area of *ca.* 200 m²/g, and a surface area occupied by one molecule of water on the alumina of *ca.* 30 Å², a triple monolayer of water corresponds to *ca.* 6% (w/w) water[11].

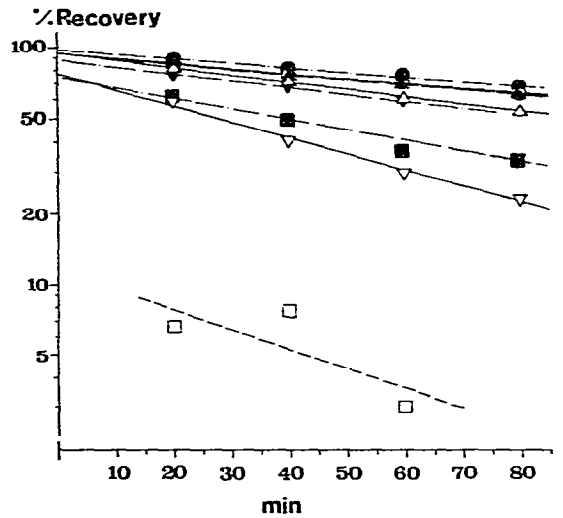

Fig. 6. The effect of water deactivation of alumina on the loss of 0.2 μg of vinclozolin at 0° C as a function of time. ○, 16%; △, 8%; ▽, 4%; □; 2% water. The addition of 1% silver nitrate is marked by closed symbols. Test tube procedure.

The addition of silver nitrate to the alumina at any experimental water standardization reduced the k values and increased the intercept value. However, a slight silver nitrate reagent acidity caused a lowering of the pH by *ca.* one pH unit in the range of pH used in this experiment. As shown in Fig. 5, the addition of silver nitrate increased the k values at fixed water percentage and pH value. The effect of the pH change caused by addition of silver nitrate seemed to dominate the silver ion effect, resulting in a total effect of decreasing k values by silver nitrate loading of alumina. Similar effects were found for dichlofluanid and tolylfluanid.

Silver ions, acting as electron acceptors, and certain classes of organic compounds, functioning as electron donors, can form π-electron charge transfer complexes, which are normally very unstable and exist in equilibrium with the free components. Generally, the formation of such complexes is very rapid, and the heats of interaction are small[2]. In this investigation the increase of loss of compounds by silver loading of the column material may be explained by increased reaction rates of active alumina centres and charge transfer complexes, in comparison with the reaction of active centres and free test compounds. The reactions between silver ions and dichlofluanid, tolylfluanid, or vinclozolin were weak, and the reactions were depressed by the addition of water to the column material. The dicyano compound chlorothalonil was strongly bound by silver ions, and the reaction was promoted by the addition of water. However, the influence of temperature and time on the rate of loss of chlorothalonil on silver-loaded alumina followed the same pattern as for the other compounds with a slightly higher apparent activation energy, as shown in Fig. 4. Consequently, alumina should be deactivated with a minimum of 6% water for the chromatographic clean-up of the compounds tested. The silver nitrate loading of the column material has only a minor effect on the loss of dichlofluanid, tolylfluanid and vinclozolin, but special care should be taken

to minimize the reaction between chlorothalonil and silver ions.

The influence of extracts on the loss of compounds was tested with substrates prepared as described in the section *Extracts from fruit and vegetables*. The clean-up involved could not be simulated by the test tube method, so all experiments were carried out using the micro column procedure with its inherent uncertainty. The following amounts of extracts were left after the extraction steps determined by drying and weighing, and referred to the original amount of sample: melon 0.6 mg/g, strawberries 3.5–4.4 mg/g, gooseberries 3.6 mg/g, red currants 1.5 mg/g, and apples 2.7 mg/g. By following the micro column method for clean-up only 10–30% weight of extracts was removed, but this step was found to be necessary to omit interferences and to achieve reproducibility in the final GC–ECD determination.

In the experiments the columns were loaded at 24°C with extracts corresponding to 2 g of crop and spiked with 0.2 μg of chlorothalonil, 0.4 μg of dichlofluanid, and 0.4 μg of tolylfluanid. Initial experiments showed that double the amount of substrate, corresponding to 4 g of crop, resulted in a marked decrease of reproducibility and of recovery. All columns were standardized with 7% of water and 1% of silver nitrate, and they were rinsed with 30% diethyl ether in light petroleum before use.

Fig. 7 shows the recovery of dichlofluanid and tolylfluanid as a function of contact time with alumina. Obviously a more complicated model is followed than the first-order model. Reference columns without substrate (closed symbols) showed complete loss of both compounds after 20 min. First-order models are indicated by dotted lines in the figure. The fate of chlorothalonil on alumina loaded with substrate is shown in Fig. 7. The loss was complete after 15 min, probably owing to the addition of silver nitrate, with only a slight difference between columns with or without substrate.

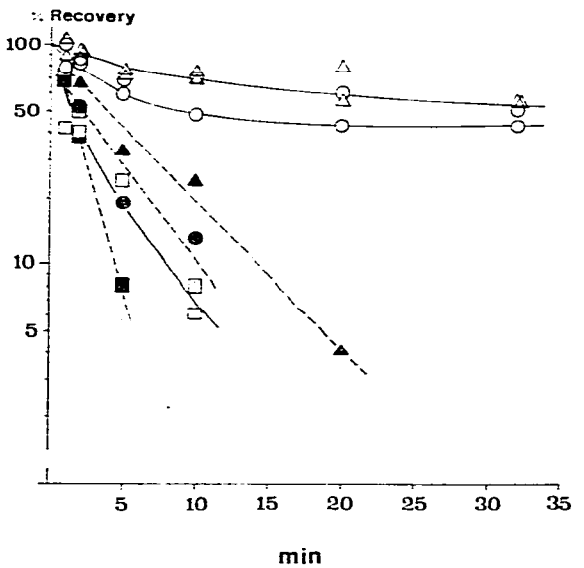

Fig. 7. The effect of substrate loading of micro columns of alumina at 24 °C on the loss of 0.2 μg of chlorothalonil (□), 0.4 μg of dichlofluanid (○), and 0.4 μg of tolylfluanid (△) as a function of contact time. Reference columns without substrate loading are marked by closed symbols and dotted lines.

The effect of strawberry substrate was found to be similar to that of apple substrate on the compounds chlorothalonil, dichlofluanid, and tolylfluanid. Consequently, it is important to test the performance of pesticide residue methods by standards taken through the method both alone and in the presence of each new substrate.

CONCLUSIONS

Chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin are lost by the chromatography on alumina following a first-order model. The loss of compounds was reduced by increasing water deactivation of the column material. The results indicated second-order reactions with hydroxide on basic alumina. A general deactivation was observed by the loading of crop extracts on the column material. Silver nitrate loading of the alumina caused a slight increase of the rate of loss of dichlofluanid, tolylfluanid and vinclozolin, but the loss of chlorothalonil was markedly increased, apparently by the formation of strong charge transfer complexes with silver ions. Some batch to batch variation was observed, presumably caused by the influence of impurities of heavy metal oxides[5], or by differing centres of adsorption, with respect to energy and the manner of interactions with the samples. In some cases, a limited portion was lost by very rapid and irreversible reactions with the alumina. In this study the isolation and identification of possible hydrolysis or degradation products were not included. However, the reactions were dependent on time and temperature, following a simplified Arrhenius expression.

On basis of the results a clean-up procedure was set up including a wide range of electron capturing compounds. Neutral alumina standardized with 7% water and 1% silver nitrate was chosen as column material. The use of basic alumina increased the rate of loss markedly, and acid alumina might reduce recovery of some compounds, e.g. pentachloroaniline. The solvent 30% diethyl ether–light petroleum eluted a wide range of components. On top of the column material were placed 4 g of anhydrous sulphate to keep 1 ml of sample solution prior to the start of elution. The column should not be prewashed to remove impurities causing GC interference. The prewash with solvent was found to increase the activity of the alumina by removal of water. However, if clean reagents and glassware are used the prewash is unnecessary. To reduce the degree of reaction of alumina and labile compounds the solvent was cooled to 0°, and the elution was performed rapidly (10–20 ml/min) by applying a light pressure of nitrogen at the top of the column. By this procedure the recovery of chlorothalonil, dichlofluanid, tolylfluanid and vinclozolin was better than 90%. The method was found to be very time-saving and versatile for routine purposes. At present the procedure is being tested for a variety of non-polar and medium-polar halogenated compounds as a general routine screening multiresidue procedure.

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