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THE APPLICATION OF GEL CHROMATOGRAPHY TO THE SEPARATION OF PESTICIDES

PART I. ORGANOPHOSPHORUS PESTICIDES*

J. H. RUZICKA, J. THOMSON, B. B. WHEALS AND N. F. WOOD
Laboratory of the Government Chemist, Ministry of Technology, Cornwall House, Stamford Street, London, S.E. 1 (Great Britain)
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SUMMARY

This paper reports the use of gel chromatography as a clean-up and separatory technique in the identification of organophosphorus pesticides. Differences in separation on gels swollen in acetone and ethanol are investigated.

INTRODUCTION

Gel chromatography or gel filtration[1,2] has been widely used in the field of protein chemistry[3] but has not, to any extent, been applied to the separation of pesticides. The possibility of separating compounds on the basis of molecular size attracted our attention to this technique as it seemed potentially useful as a clean-up method for separating organophosphorus pesticides (mol.wt. *ca.* 200–350) from common vegetable co-extractives such as chlorophyll (mol.wt. 906) and carotene (mol.wt. 536). This paper describes the separation of organophosphorus pesticides on Sephadex LH-20, a modified dextran gel of lipophilic character.

EXPERIMENTAL

Sephadex LH-20 was allowed to swell for approximately 24 h in the solvents: acetone, tetrahydrofuran or ethanol. The swollen gel slurries were poured into glass chromatographic columns of 2 cm diameter to give a packed bed volume of approximately 75 ml. One millilitre of a mixture of pesticides containing 20 μg of each compound was placed on the top of each column in the corresponding solvent, and eluted at a rate of 1.0 ml/min. Each mixture contained up to five pesticides and always included parathion as an internal standard. Fractions of the eluted solutions were collected and examined for the presence of the component pesticides using gas chromatography[4]. Certain oxidation products which could not be detected on our

gas chromatographic columns were examined by carrying out a colorimetric phosphorus determination of each fraction.

To evaluate the clean-up potential of these columns several cabbage extracts fortified with pesticides were passed down them. The extracts contained about 60 mg of material, mainly natural pigments, resulting from the extraction of 100 g of cabbage with acetone. Each extract was added to the column dissolved in 1 ml of acetone or

TABLE I

THE SEPARATION OF ORGANO PHOSPHORUS PESTICIDES ON SEPHADEX LH-20

Gel swollen and eluted with acetone			*Gel swollen and eluted with ethanol*	
Pesticide in sequence of elution	*Mol.wt.*	*Elution volume relative to parathion (= 100)*	*Pesticide in sequence of elution*	*Elution volume relative to parathion (= 100)*
Aspon	378	87	Aspon	66
Ethion	384	92	Dimefox	71
Sulfotep	322	93	Phosphamidon	72
Malathion	330	95	Tepp	73
Mecarbam	329	96	Phorate-O-analogue	74
Diazinon	304	98	Sulfotep	75
Demeton-S	258	99	Malathion-O-analogue	76
Pyrimithate	333	99	Bidrin	77
Disulfoton	274	100	Diazinon	77
Parathion	291	100 (Elution vol. 43.5 ml)	Chlorfenvinphos	77
			Demeton-S	79
Fenitrothion	277	101	Mevinphos	79
Phorate	260	103	Thionazin-O-analogue	79
Demeton-O-methyl	230	103	Demeton-S-methyl-sulphoxide*	79
Phorate sulphone	309	104	Trichlorphon	80
Malathion-O-analogue	314	104	Pyrimithate	81
Chlorthion	283	107	Ethion	82
Fenthion	278	108	Phorate sulphoxide	82
Thionazin	248	108	Dichlorvos	83
Thiometon	248	108	Mecarbam	85
Parathion-methyl	249	109	Parathion-O-analogue	86
Fenchlorphos	321	109	Demeton-S-methyl	86
Parathion-O-analogue	275	112	Malathion	86
Chlorfenvinphos	359	114	Disulfoton	87
Bromophos	366	115	Phorate	89
Mevinphos	224	116	Thionazin	89
Phosphamidon	264	117	Demeton-O-methyl	93
Phorate-O-analogue	244	118	Dimethoate	95
Trichlorphon	257	121	Phorate sulphone	98
Dichlorvos	221	122	Demeton-S-methyl-sulphone*	99
Morphothion	285	124	Parathion	100 (Elution vol. 75.5 ml)
Demeton-S-methyl-sulphone*	262	125		
Demeton-S-methyl	230	130	Thiometon	100
Thionazin-O-analogue	232	133	Bromophos	100
Tepp	290	134	Fenchlorphos	101
Bidrin	237	141	Morphothion	106
Phorate sulphoxide	293	147	Fenthion	112
Dimethoate	229	148	Parathion-methyl	114
Dimefox	154	150	Fenitrothion	114
Demeton-S-methyl-sulphoxide*	246	200	Chlorthion	118

* These pesticides were determined by a total phosphorus method.

ethanol containing a mixture of pesticides, each at 10 μg. Fractions were collected and examined as above, and the quantity of cabbage coextractive emerging at various stages of the elution was determined by evaporating the portion to dryness to obtain a dry weight of residue.

RESULTS

The pesticide elution sequence, molecular weights and elution volumes relative to parathion on the gel swollen in acetone and ethanol are shown in Table I. The elution sequences in acetone and tetrahydrofuran were identical and the data for the latter solvent have been omitted.

The elution curves for all the compounds, with the exception of trichlorphon, were symmetrical, and recovery from the columns was apparently quantitative, although recovery experiments were only carried out with a few of the pesticides. No significant change in the elution volume of parathion occurred over a period of several weeks on a given column and its band-spread was found to be independent of sample concentration over the range investigated (10–1000 μg/ml), but dependent on flow rate. Fig. 1 shows the separation of parathion and its analogues on the gel swollen in acetone and in ethanol. The higher loading of the oxygen analogue was necessitated by the decreased sensitivity of the gas chromatographic method to this compound[4].

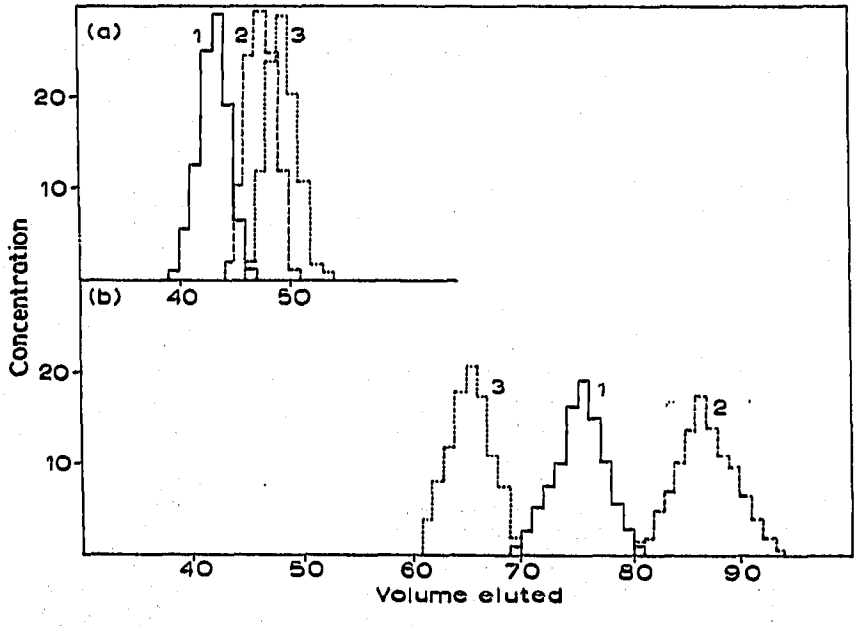


Fig. 1. Separations on a gel swollen in acetone (a) or ethanol (b), elution rate 1 ml/min. 1 = Parathion; 2 = parathion-methyl; 3 = parathion-O-analogue.

The elution volumes of the pesticides added to the cabbage extracts were identical with those obtained using pure pesticides, and did not change despite repeated use of the columns. Table II shows the percentage of cabbage co-extractive material eluting in the various portions of the eluate.

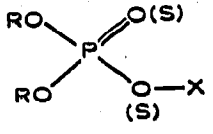
TABLE II

THE SEPARATION OF A CABBAGE EXTRACT ON SEPHADEX LH-20

Gel swollen and eluted with acetone		*Gel swollen and eluted with ethanol*	
Portion of eluate	*% of total coextractive (average of five experiments)*	*Portion of eluate*	*% of total coextractive (average of five experiments)*
0– 35 ml	18	0– 50 ml	24
35– 75 ml	57	50– 85 ml	62
75–100 ml	25	85–150 ml	14

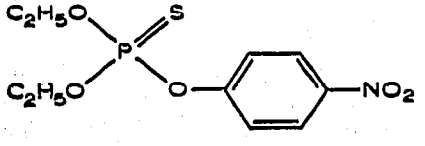
DISCUSSION

Gel chromatography is primarily a means of separating molecules on the basis of molecular size, but departures from "ideal" behaviour can occur; JANSON[5] has recently reviewed this topic. Departures from "ideality" manifest themselves in two ways, compounds either eluting before their "ideal" position as a result of exclusion from the gel or eluting later due to adsorption or electrostatic retardation. In the results shown in Table I the separations on the gel swollen with acetone (also tetrahydrofuran) correspond more closely to "ideality" than is the case with the gel swollen with ethanol. The organophosphorus pesticides are a closely related group of compounds, mainly esters of phosphoric or phosphorothioic acids, having the following general structure:

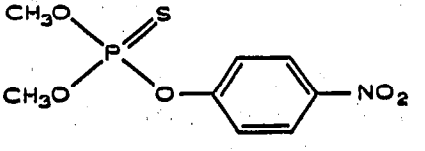


where: R = an alkyl group and X = an organic radical.

Variation in the size of the alkyl group produces the anticipated effect on both columns, *i.e.* the molecule containing the larger alkyl group elutes first. Thus parathion elutes before parathion-methyl (see Fig. 1).



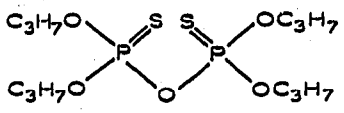
Parathion
R_{va} = 100
R_{ve} = 100



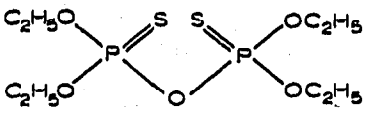
Parathion – methyl
R_{va} = 109
R_{ve} = 114

where R_{va} and R_{ve} are the relative elution volumes in acetone and ethanol, respectively.

Analogous behaviour is shown by the pairs of compounds demeton-S–demeton-S-methyl and disulfoton–thiometon. The sulphur-containing pyrophosphate analogues aspon and sulfotep show similar separation.

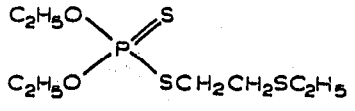


Aspon
R_{VA} = 87
R_{VE} = 66

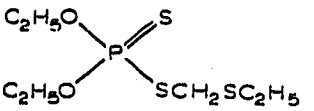


Sulfotep
R_{VA} = 93
R_{VE} = 75

Increasing the chain length by $-CH_2-$ in the group X apparently results in a less significant change in molecular dimensions with little separation occurring. Disulfoton and phorate were the only compounds available to us on which this effect could be studied.

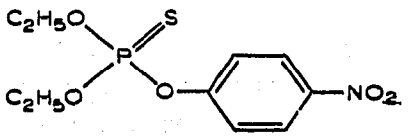


Disulfoton
R_{VA} = 100
R_{VE} = 87

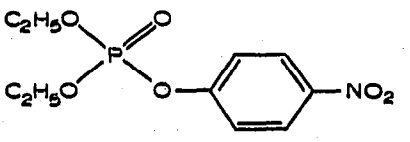


Phorate
R_{VA} = 103
R_{VE} = 89

Many pesticides are dependent for their biological activity on conversion of P=S to P=O after application. It would be anticipated that the separation of a mixture of such compounds would result in the larger molecule containing the P=S group eluting first. On the acetone-swollen gel the behaviour is as anticipated but in ethanol the behaviour is anomalous. This may be due to an exclusion process in which the more electrophilic oxygen atom results in a molecule sufficiently polarized to be repelled by the gel in a polar solvent such as ethanol, or is perhaps due to an increase in overall molecular size as a result of solvation induced by hydrogen bonding. Parathion and its oxygen analogue are typical examples (see Fig. 1).

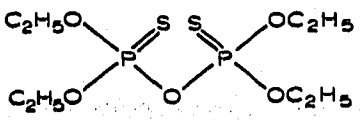


Parathion
R_{VA} = 100
R_{VE} = 100

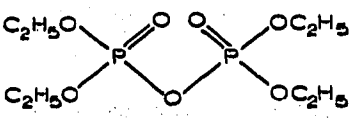


Parathion-O-analogue
R_{VA} = 112
R_{VE} = 86

The compounds malathion, phorate, thionazin and thiometon and their oxygen analogues show the same elution pattern as above. The pyrophosphates tepp and sulfotep behave similarly.

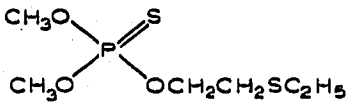


Sulfotep
R_{VA} = 93
R_{VE} = 75

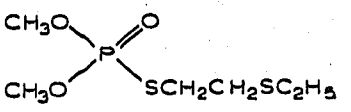


Tepp
R_{VA} = 134
R_{VE} = 73

Thiono-thiolo isomerisation of certain pesticides can occur, *e.g.* demeton-O-methyl isomerising to demeton-S-methyl, which results in replacement of P=S by P=O. The elution pattern is as above.



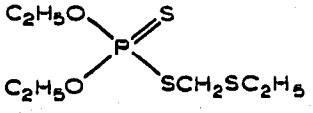
Demeton-O-methyl
$R_{VG} = 103$
$R_{Ve} = 93$



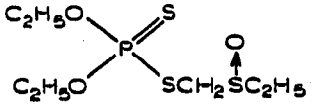
Demeton-S-methyl
$R_{VG} = 130$
$R_{Ve} = 86$

Variations in the structure of the side chain X lead to the great diversity of organophosphorus pesticides. The nature of the side chain also plays an important part in determining the elution characteristics of the compound. In the pesticides studied some noticeable correlations emerge. For example those pesticides containing aromatic side chains, *e.g.* parathion, parathion-methyl, bromophos, chlorthion, fenchlorphos, fenitrothion and fenthion, all elute much later in ethanol than pesticides which lack such aromaticity. This retardation of aromatic molecules by gels swollen in ethanol has been noticed by other workers[6]. Substituents on the aromatic ring do not lead to changes resulting in pronounced separations.

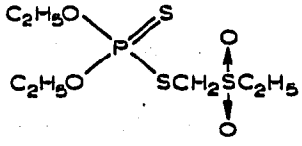
Several pesticides contain side-chains in which an oxidisable sulphur atom is present. Oxidation of such compounds to form the sulphoxide and sulphone is important as such oxidation occurs under natural conditions[7], and the oxidation products are frequently of greater biological activity than the parent compounds. The elution pattern for the phorate oxidation products is shown below; the oxidation products of demeton-S-methyl show a similar inversion of elution order in the two



Phorate
$R_{VG} = 103$
$R_{Ve} = 89$



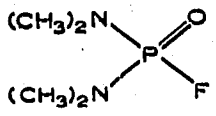
Phorate sulphoxide
$R_{VG} = 147$
$R_{Ve} = 82$



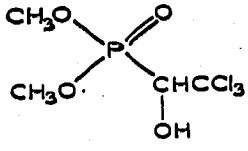
Phorate sulphone
$R_{VG} = 104$
$R_{Ve} = 98$

solvents. The reversal of the elution order is most marked and, if typical, may well provide a means of characterisation which usually requires infra-red spectroscopy[8]. We have used the separations attained on the Sephadex gel to isolate pure samples of the components of oxidised pesticides[4].

Two pesticides which are atypical in structure are dimefox and trichlorphon. The former compound displays a dramatic change in its elution position when the solvent is changed, and the latter was the only pesticide examined which showed marked tailing of its elution peak in all solvents.



Dimefox



Trichlorphon

The results in Table II indicate that Sephadex LH-20 is of little use as a clean-up technique, with much of the pigment material eluting in the range over which pesticides are found to emerge. The constant nature of the elution volume of a particular pesticide on the Sephadex gel might make a separation on this material a useful adjunct to the identification of organophosphorus pesticides at residue levels now based on thin-layer or gas chromatography.

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