UNC-WRRI-71-54

CHROMATOGRAPHIC CONCENTRATION OF PESTICIDES FROM A LARGE MASS OF WATER

By

Takeru Ito
Professor of Biology
Department of Biology
East Carolina University
Greenville, North Carolina 27834

The work upon which this publication is based was supported in part by funds provided by the Office of Water Resources Research, Department of the Interior, through the Water Resources Research Institute of the University of North Carolina as authorized under the Water Resources Research Act of 1964.

Project No. A-050-NC
Agreement No. 14-31-0001-3033, F. Y. 1970

August 1971

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author is indebted to the following manufacturers of pesticides for gifts of pesticide samples, to Zackie L. Tyndall for technical assistance, and to Water Resources Research Institute of University of North Carolina for partial financial support.

Allied Chemical Corporation, Morristown, N. J. 07960

Diamond Shamrock Chemical Co., Cleveland, Ohio 44115

Hercules Inc., Wilmington, Del. 19899

Rhodia Inc., Chipman Division, New Brunswick, N. J. 08903

Shell Chemical Co., Industrial Chemical Division, New York, N. Y. 10020

Stauffer Chemical Co., Agricultural Chemical Division, New York, N. Y. 10017

Union Carbide Corporation, New York, N. Y. 10017

Velsicol Chemical Corporation, Agricultural Product Division, Chicago, Ill. 60611

ABSTRACT

In order to test suitability of "anchored" stationary phases, or, chemically bonded stationary phases, for concentrating pesticides from a large amount of water, a number of such phases were synthesized. The method was as follows. To celite (39.7g) added was an alkyl or aromatic chlorosilane and n-hexane (500 ml). The mixture was shaken for 5 hours in a closed vessel and n-hexane was removed under reduced pressure. The powder so formed was shaken vigorously with passage of a continuous stream of warm air saturated with water to remove hydrogen chloride. The resulted damp material was dried completely in an oven at 110°C. The alkyl and aromatic chlorosanes applied for the above method were octadecyltrichlorosilane (12.29g), dichlorodiphenylsilane (8.02g), dichlorodimethylsilane (4.09g), trichlorophenylsilane (6.70g), dichloromethylphenylsilane (6.05g), trichloromethylsilane (6.38g) and trichloro-n-propylsilane (5.62g). The resultant "anchored" stationary phases were referred to OD, DP, DM, P, MP, M and nP respectively. The total organic content (carbon and hydrogen) of each phase ranged from 0.2 to 2.3%.

Duplicate columns (20 mm x 5 mm diameter) were packed with an "anchored" stationary phase synthesized above. To each column 0.1 ml n-hexane solution of a mixture of lindane (5.11 ppm), heptachlor (4.54 ppm), dieldrin (3.95 ppm), heptachlor epoxide (4.58 ppm) and pp'DDT (3.60 ppm) was applied. Each column was dried completely. One column was washed with 4 liters of deionized distilled water, and dried completely.

Another was unwashed. Each column was eluted with two portions of 5 ml n-hexane. THe hexane was evaporated to 0.1 ml before the eluent was subjected to gas-liquid chromatography. The "anchored" stationary phases examined were OD, MP, nP, P, DM, and Durapak. The recovery of the pesticides was not satisfactory. In addition considerable contamination of the columns was observed which was manifested by many large extra peaks in the gas-liquid chromatograms.

Since OD showed the best performance, however, the experiment with OD was repeated with some modifications. The column was washed with 50 ml n-hexane and dried completely before application of the mixture of pesticides. Elution with n-hexane was done with five portions of 5 ml n-hexane, and each eluent was evaporated to 0.1 ml before subjecting it to gas-liquid chromatography. The contamination observed previously was removed, but the recovery was unsatisfactory. It was concluded that the method studied was not satisfactory for concentrating pesticides from a large amount of water.

LIST OF TABLES

SUMMARY AND CONCLUSIONS

In analysis of pesticide residues in water the greatest difficulty at present is the lack of a good practical method to extract and concentrate the residues from water. This report is concerned with the attempt to improve presently available methods. The method studied here was partition chromatography with "anchored" stationary phases, that is, the stationary phases bonded chemically to a solid support.

In order to examine suitability of "anchored" stationary phases, synthesis of several such phases was attempted. The method of synthesis involved treatment of celite wiht an alkyl or aromatic chlorosilane. Seven such phases were synthesized.

A known amount of pesticides was applied to a column of each stationary phase synthesized, and the column was washed with four liters of water. The recovery of pesticides after such washing was studied. For all phases studied the recovery was not very satisfactory.

The method studied here is not satisfactory for concentrating pesticides from a large amount of water. However, the ideas behind the method seem good and sound, and further research should produce a practical method to analyze pesticides in a large mass of natural water.

RECOMMENDATIONS

Although the present study produced no satisfactory method of extracting and concentrating pesticides from a large mass of water, partition chromatography and countercurrent chromatography (Ito et al, 1970) appear to be the best method to be developed into a practical one. Further research in this direction should be pursued in order to develop a simple and accurate method to extract and concentrate pesticides from a natural water.

INTRODUCTION

Since the amount of pesticides in natural water is usually very small, to develop an accurate method to determine their amount in a large mass of water is still a formidable task. One common method is to treat the water with charcoal to adsorb pesticides, and desorb them with an appropriate solvent (Faust et al., 1966). However, as is almost always the case in adsorption chromatography (especially in the use of charcoal), adsorption of pesticides on charcoal and desorption from it is by no means complete (American Chemical Society, 1969). Another method is to extract a large volume (2-3 gallons) of the water with a small volume of an organic solvent. (500 ml). The solvent is evaporated, and concentrated solution is subjected to gas-liquid chromatography. The latter method suffers from the interference of the concentrated impurities in the solvent upon evaporation. Many problems involved in the analysis of pesticide residues were reviewed (Lisk, 1970).

This report is concerned with the attempt to develop a more adequate than the presently available methods of extracting and concentrating pesticides in a large mass of water. The method studied here was partition chromatography with "anchored" stationary phases (Stewart et al., 1968, Abel et al., 1966).

METHODS

Chemical Synthesis of "Anchored" Stationary Phases

Celite (Analytical Filter Aid) 200 g, was added to deionized distilled water to make 1.8 liters of a suspension. The suspension was shaken vigorously, and allowed to settle for exactly 10 minutes. The top 200 ml of the suspension was decanted off, and discarded. Deionized distilled water, 200 ml, was added to the remaining suspension. The suspension was shaken vigorously again, and allowed to settle for 10 min. The top 200 ml. was discarded. The procedure was repeated several times to obtain celite of large particle sizes.

Finally the suspension was filtered through a Buchner funnel. The celite was dried at approximately 150° for 100 hours. To this dry selected Celite (39.7g) was added n-octadecyltrichlorosilane (12.29 g) and about 500 ml of n-hexane. The resulting mixture was stirred with a magnetic stirrer for several hours. The white powdery product was filtered on a Buchner funnel, and was dried under a reduced pressure.

The product was next stirred with a magnetic stirrer in a three neck one liter flask through which a continuous stream of warm air saturated with water was passed for about 12 hours until no further hydrogen chloride could be detected in the exit stream. The solid product was filtered on a Buchner funnel, washed with a large volume of n-hexane, and dried at 110° overnight.

The preceding synthesis procedure was repeated with other chlorosilanes. The amount used is given below:

dichlorodiphenylsilane	8.02 g
dichlorodimethylsilane	4.09 g
trichlorophenylsilane	6.70 g
dichloromethylphenylsilane	6.05 g
trichloromethylsilane	6.38 g
trichloro-n-propylsilane	5.62 g.

The products thus formed from each of the silanes are referred to octadecylsilyl-celite (OD), diphenylsilyl-celite (DP), dimethylsilyl-celite (DM), phenylsilyl-celite (P), methylphenylsilyl-celite (MP), methylsilyl-celite (M), and n-propylsilyl-celite (nP), depending on the silane used as a reactant. The abbreviations are in parenthesis.

Recovery of Pesticides from the "Anchored" Stationary Phases

Duplicate Hagihara's columns (Hagihara et al., 1960) were packed with approximately 75 mg of a stationary phase prepared above. To each column 0.1 ml n-hexane solution containing the following pesticides to wet about one third of the packed column.

Lindane	5.11 mg/l
Heptachlor	4.54 mg/l
Dieldrin	3.95 mg/l
Heptachlor epoxide	4.58 mg/l
pp'DDT	3.60 mg/l

All pesticides are obtained from Beckman except heptachlor epoxide (98.6% pure) which is a gift from Velsicol Chemical Corporation. The columns were dried completely in an oven at 100°C for about 30 minutes.

One column was washed with 4 liters of deionized distilled water. Another unwashed. The former column was dried in an oven at 100-150° overnight. Each column was eluted with two portions of 5 ml n-hexane.

The two portions eluted from each column were combined and evaporated to 0.1 ml under nitrogen gas stream, before analysis (5 microliters) by gas-liquid chromatography. The "anchored" stationary phases examined were OD, MP, nP, P, DM, and Durapak, the last of which was a product of Waters Associates, Inc. In some experiments modified was the elution procedure, which is described in Results and Discussion.

Gas-liquid chromatography was carried out by a Fisher-Victoreen Gas Chromatograph with an electron capture detector. The column was six feet stainless steel packed with 3% SE-30 on 40-50 mesh Anakrom Abs. Temperature of the oven, the injection port, the detector was 190°, 170° and 320° respectively. Carrier gas flow rate was 50 ml/minute. The detector voltage was set for maximum sensitivity.

RESULTS AND DISCUSSION

Chemical Synthesis of "Anchored" Stationary Phases

The results of carbon and hydrogen analyses, done by Galbraith Laboratories, Inc. (Knoxville, Tennessee 37921) are given in Table 1.

Table 1

Per Cent C and H in the "Anchored" Stationary Phases

Phases	%C	%H
OD	2.00	0.29
DP	1.38	0.17
DM	0.11	0.10
P	1.05	0.23
MP	0.35	0.06
nP	0.28	0.13
M	Not analyzed	

The organic content (C and H) of the "anchored" stationary phase synthesized from n-hexadecyltrichlorosilane by Abel et al. was 14%. The organic content of the phases synthesized in the present study appeared to be very low even in the case of OD which is similar to the phase of Abel et al. THe discrepancy may be due to the slightly modified synthetic procedure employed in the present study. The differences in the organic content seen in Table 1 between the phases suggest that the incorporation of the hydrocarbon moieties into celite depends on the kinds of silanes employed and on the kinds of hydrocarbon substituents in the silanes.

Recovery of Pesticides from the "Anchored" Stationary Phases

The results of the recovery experiments are given in Table 2 (See Appendices). As can be seen, the recovery from various phases before the water-wash was low, and that after the water wash was even lower in every case except OD. Durapak after the water wash lost over 90% of pesticides. The loss of the pesticides was probably due to the hydrolysis of Durapak. None of the stationary phases performed satisfactorily. Retention of pesticides on the phases during the water wash appeared to be related to the organic content of each phase.

The best performance was shown by OD. The recovery from OD after the water wash indicated that there was a considerable contamination which may have come from either the stationary phases or the glasswares and the plastic wares (Bevenue et al., 1971) which were contact with four liters of water.

Since OD seemed to show the best performance, the preceding experiment with OD was repeated with some modifications. These modifications were as follows. The packed columns were washed with 50 ml n-hexane and dried completely before application of the mixture of pesticides. Elution with n-hexane was done with five portions of 5 ml n-hexane, and each eluent was evaporated to 0.1 ml before subjecting to gas-liquid chromatography. The results were summarized in Table 3. (See Appendices). The recovery after the second elution was mostly less than one per cent. The elution from the unwashed columns was not complete. Lindane, heptachlor epoxide and dieldrin were almost entirely washed away with the large amount of water. In every eluent a large peak named i appeared consistently. The nature of the peak was not determined. It is probably related to the silanes employed in the synthesis of the "anchored" stationary phases. Most other contamination observed in the previous experiments disappeared. The disappearance was probably due to the prewash of the columns with a large amount of n-hexane and the avoidance of use of plastic wares during the water wash.

REFERENCES

1. Abel, E. W., Pollard, F. H., Uden, P. C., and Nickless, G., "A New Gas-Liquid Chromatographic Phase," J. Chromatog., 22, 23 (1966).

2. American Chemical Society, "Cleaning Our Environment--The Chemical Basis for Action,"--A Report by the Subcommittee on Environmental Improvement, Committee on Chemistry and Public Affairs, Washington, D. C., 1969, p. 193-244.

3. Bevenue, A., Kelley, T. W., and Hylin, J. W., "Problems in Water Analysis for Pesticide Residues," J. Chromatogr. 54, 71 (1971).

4. Faust, S. D., Suffet, I. H., "Recovery, Separation, and Identification of Organic Pesticides from Natural and Potable Waters," Residue Rev., 15, 44 (1966).

5. Hagihara, B., and Lardy, H. A., "A Method for the Separation of Orthophosphate from Other Phosphate Compounds," J. Biol. Chem., 235, 889 (1960).

6. Ito, Y., and Bowman, R. L., "Countercurrent Chromatography: Liquid-Liquid Partition Chromatography Witnout Solid Support," Science, 167, 281 (1970).

7. Lisk, D. J., "The Analysis of Pesticide Residues: New Problems and Methods," Science, 170, 589-593 (1970).

8. Stewart, H. N. M., and Perry, S. G., "A New Approach to Liquid Partition Chromatography," J. Chromatogr. 37, 97 (1968).

GLOSSARY

Adsorption - A type of adhesion which takes place at the surface of a solid or a liquid in contact with another medium, resulting in an accumulation of molecules from that medium in the immediate vicinity of the surface.

Adsorption Chromatography - A type of chromatography in which separation of substances is achieved by differential migration of solutes resulting from differences in affinity of the solutes between an adsorbing stationary phase (usually solid) and a mobile phase.

"Anchored" Stationary Phase - Stationary Phase which is chemically (covalently) bonded to an inert solid support.

Celite - Diatomite (also called diatomaceous earth, diatomaceous silica, or kieselguhr) which is composed of the skeletons of single-celled algae, or diatoms.

Chromatography - An analytical technique for resolution of solutes, in which separation is made by differential migration of the solutes in a medium and the migration is caused by flow of solvent. All chromatographic processes have two basic parts--A mobile phase and a stationary (or immobile) phase. When the mobile phase containing the solutes moves through the stationary phase, the solutes migrate according to their differential affinity to these phases. The stationary phase may be either a liquid, an active solid or a liquid coated over an active solid. The mobile phase may be a liquid or a gas.

Countercurrent Chromatography - Liquid-liquid partition chromatography (in which the mobile and the stationary phases are immiscible liquids) without a solid support. (Ito et al., 1970).

Desorption - The reverse of adsorption, or the release of a substance which has been held in concentrated form upon a surface.

DM - Dimethylsilyl-celite, an "anchored" stationary phase in which dimethylsilyl group is chemically bonded to celite.

DP - Diphenylsilyl-celite, an "anchored" stationary phase in which diphenylsilyl group is chemically bonded to celite.

Durapak - an "anchored" stationary phase, a commercial product (Waters Associates).

Gas-Liquid Chromatography - A type of partition chromatography in which a mobile phase and a stationary phase are a gas and a liquid coated over (or chemically bonded to in the case of "anchored" stationary phase) an inert solid support respectively.

M - Methylsilyl-celite, an "anchored" stationary phase in which methylsilyl group is chemically bonded to celite.

MP - Methylphenylsilyl-celite, an "anchored" stationary phase in which methylphenylsilyl group is chemically bonded to celite.

nP - n-propylsilyl-celite, an "anchored" stationary phase in which n-propylsilyl group is chemically bonded to celite.

OD - Octadecylsilyl-celite, an "anchored" stationary phase in which octadecylsilyl group is chemically bonded to celite.

P - Phenylsilyl-celite, an "anchored" stationary phase in which phenylsilyl group is chemically bonded to celite.

Partition Chromatography - A type of chromatography in which separation of substances is achieved by differential migration of solutes resulting from differences in distribution between two immiscible solvents, which are the stationary phase and the mobile phase.

APPENDICES

Table 2. Recovery of Pesticides from the Columns Packed with "Anchored" Stationary Phases

Pesticides or Peak Names	Relative Retention Time	a Standard	%Eluted from Columns by n-Hexane: Stationary Phases: OD u	OD w	MP u	MP w	nP u	nP w	Durapak u	Durapak w	P u	P w	DM u
Lindane	0.140	100	87.8	114.3	44.8	5.8	44.9	21.8	73.1	1.3	69.6	41.0	5.2
Heptachlor	0.246	100	71.3	90.8	28.2	2.2	11.2	trace	64.9	2.4	50.0	3.0	trace
Heptachlor Epoxide	0.390	100	97.5	102.8	95.3	58.8	86.1	55.1	88.6	2.8	98.0	64.8	80.1
Dieldrin	0.587	100	100.0	148.0	103.6	24.6	83.3	58.7	101.9	2.7	105.3	31.4	47.1
pp'DDT	1.00	100	98.5		117.1	65.1	99.5		98.8	9.8	111.0	67.1	102.5
e	1.17		85.0	722[b]	13.4	65.7	15.2	608[b]	93.2	4.2	13.4	113.9	49.3
f												11.3	15.1
g				58.8									
h				109.0									
i	2.52		76.2	826[b]	95.8	68.7	79.2	750[b]	123	44.6	101	62.6	84.8
j				102.7									
k				373									

The columns were either unwashed or washed with 4 liters of water and dried before elution by n-hexane. All % of extra peaks designated by the alphabets are % equivalents of DDT. Washing the DM column with 4 liters of water would have taken several days, and not completed. Abbreviations are given in METHODS and GLOSSARY parts of the text.

[a]The n-hexane solution of the pesticide mixture was directly analyzed by gas-liquid chromatography.

[b]These numbers indicate % equivalents (to DDT) of more than the designated peaks.

[u]The columns were not washed with water.

[w]The columns were washed with 4 liters of water.

Table 3. Recovery of Pesticides from the Columns Packed with OD

Pesticides or Peak Names	Relative Retention Time	Standard[a]	% Eluted from the Columns of OD by n-Hexane									
			Unwashed with water					Washed with 4 l water				
			Elution					Elution				
			1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Lindane	0.140	100	67.1	1.5	1.44	0.92	0.73	0.35	0.45	0.70	0.66	0.53
Heptachlor	0.246	100	49.1	0.32	0.21	0.09	0.09	24.2	0.30	0.17	0.11	0.10
Heptachlor Epoxide	0.390	100	58.3	0.84	0.45	0.10	0.05	1.54	0.06	0.00	0.06	0.99
Dieldrin	0.587	100	46.5	0.72	0.61	0.08	0.00	6.68	0.15	0.08	0.06	1.27
pp'DDT	1.00	100	30.1	1.12	0.42	0.49	0.38	39.8	0.69	0.58	0.61	2.64
e	1.17			1.17							6.00	
i	2.52		7.85	15.7	17.2	23.7	25.6	13.5	12.8	21.0	24.2	17.8

The columns were either unwashed or washed with 4 liters of water and dried before elution by five portions of 5 ml n-hexane. All % of extra peaks designated by alphabets are % equivalent of DDT.
[a]The n-hexane solution of the pesticide mixture was directly analyzed by gas-liquid chromatography.