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# Ion Exchange Resin Impregnated Paper Chromatography of Some Typical Chlorinated Pesticides

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# ION EXCHANGE RESIN IMPREGNATED PAPER CHROMATOGRAPHY OF SOME TYPICAL CHLORINATED PESTICIDES

by

Stephen F. Nagy III UC 1967

Senior Thesis Submitted

in Partial Fulfillment

of the Requirements of Graduation

DEPARTMENT OF CHEMISTRY

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This Thesis

Submitted by

Stephen F. Nagy

Stephen F. Nagy

to the

Department of Chemistry of Union College

in partial fulfillment of the requirements of the degree of

Bachelor of Science with a Major in Chemistry

is approved by

Robert W Schaefer

## ACKNOWLEDGMENT

The author would like to express his appreciation to Professor Robert W. Schaefer of the Union College Chemistry Department for his guidance in research and helpful remarks concerning the construction of this paper.

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# HISTORICAL BACKGROUND

# I

## HISTORICAL BACKGROUND

Many methods employing paper chromatography for the determination of chlorinated pesticides appear in the literature (1, 2). No publications have been found on the application of ion exchange resin impregnated paper chromatography to chlorinated pesticides determination.

Ion exchange resin impregnated papers are high quality alpha-cellulose papers uniformly loaded with 45-50% resin by weight (3). In ion exchange resin impregnated paper chromatography the resin serves as the stationary phase over which a mobile phase (developing solvent) is drawn, as in paper chromatography, during developing.

If in the process of developing the rate of partition of a solute (spotted onto a chromatogram) between the resin and the developing solvent is less than the rate at which the mobile phase traverses a chromatogram, solute retention occurs. The ratio of the distance

a solute moves on a chromatogram during developing to the distance over which the developing solvent front travels is an indication of retention, and is termed an $R_f$ value. Differences in $R_f$ values of various solutes in a given chromatographic system are an indication of the solutes' separability in that system.

The purpose of this project was to examine the effect the ionic immobile phase of various ion exchange resin-impregnated papers has on the separability of some typical chlorinated pesticides; the ultimate goal was devising an ion exchange resin-impregnated paper chromatographic system which would provide better chlorinated pesticide resolution than conventional paper chromatographic methods. Specific aims of the project were:

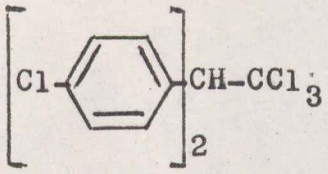
(1) obtaining $R_f$ values for the pesticides used in different resin paper chromatographic systems for separability comparisons,

(2) devising methods of establishing the whereabouts of pesticides on developed chromatograms to obtain the distances pesticides move in developing for calculating $R_f$ values,

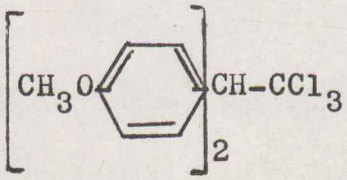
(3) developing a chromatogram elution technique for quantitative detection in the UV.

DDT, Methoxychlor, and 24D were the chlorinated pesticides that were chosen for ion exchange resin-impregnated paper chromatography (see Figure 1). DDT and Methoxychlor were selected because of their similar structures for comparative purposes.

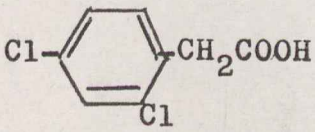
Zweig (1) tried Dowex cationic resin in the column chromatography of acidic 24D. He reports a low 24D recovery. The same worker postulates that the low recovery was probably due to the irreversible reaction of 24D with the resin. 24D was chosen for the project to test Zweig's findings in ion exchange resin-impregnated paper systems.

DDT 

1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane

Methoxychlor 

2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane

24D 

2,4-dichlorophenoxyacetic acid

Figure 1

# II

# DESCRIPTION OF APPARATUS

# II

## DESCRIPTION OF APPARATUS

An ordinary chromatography chamber with a glass tree was used (Will catalogue #8421). Weighing bottles were secured to the glass tree with the aid of rubber bands for descending chromatograms; the glass tree would also accommodate stainless steel hooks for ascending chromatograms. Other important apparatus included an all glass atomizer for spraying chromogenic agents onto developed chromatograms, a heat lamp to facilitate spotting, and a portable UV light source.

The following resin papers had been obtained from the H. Reeve Angel and Co. of Clifton, New Jersey:

(1) Amberlite IRA-400 resin anion exchanger, strong base (SB-2) type with the resin in the $Cl^-$ form

(2) Amberlite IR-120 resin cation exchanger, strong acid (SA-2) type with the resin in the $NA^+$ form

(3) Amberlite IR-4B resin anion exchanger, weak base (WB-2) type with the resin in the $OH^-$ form .

(4) Amberlite IRC-50 resin cation exchanger, weak acid (WA-2) type with the resin in the $H^+$ form.

DDT (77% PP' isomer, control #1807) and 24D (control #8213) were obtained from the Nutritional Biochemicals Corporation of Cleveland, Ohio. Methoxychlor (technical, 88% PP' isomer) was provided by the Biochemicals Division of the DuPont Co. at Wilmington, Delaware.

UV spectra were obtained on the Perkin Elmer 202 visible and ultraviolet spectrophotometer.

# III

# EXPERIMENTAL RESULTS AND CONCLUSIONS

# III

## EXPERIMENTAL RESULTS AND CONCLUSIONS

Resin paper sheets were cut into 2 x 25cm strips, and an origin was marked off on each strip by means of a graphite pencil. Then strips were shaken twice with technical chloroform in a 500cc separatory funnel to eliminate greases, oils, and other substances that might have contaminated the resin paper during the course of handling. After the chloroform had evaporated, strips were washed with distilled water and dried under a heat lamp.

Strips were carefully spotted with the appropriate substances under a heat lamp with the aid of lambda pipettes in order to keep the area of spots to a minimum. 1mg/ml technical chloroform solutions of each pesticide were used throughout the project for spotting.

Descending chromatograms were developed by freely hanging them from mobile solvent containing weighing bottles, which were secured to the top of the glass tree,

until the front traveled about 15cm (a limiting distance set by the chromatographic chamber's size); ascending chromatograms were developed by suspending them from stainless steel hooks on the glass tree into the developing solvent pool on the bottom of the chamber. The developing solvent pool was maintained also for descending chromatography so as to provide a developing solvent saturated atmosphere in the chromatography chamber--a piece of cardboard against the inner side of the chamber provided additional surface for evaporation.

Most of the project work was concerned with devising a reliable technique for detecting the whereabouts of the spotted pesticides on developed chromatograms.

Silver Nitrate Detection Method

A chromogenic agent of 1.7g silver nitrate in 3% (by volume) aqueous acetone (200ml) and 10 drops of hydrogen peroxide was prepared.

Approximately $3cm^2$ portions of each resin paper were spotted with 60λ (1mg/ml chloroform solution) of each

pesticide. The squares were then sprayed with the chromogenic agent to test the agent's effectiveness with the different pesticides on the various resin papers before chromatography. The following observations were made:

Table I

| Resin Paper | Observation DDT | Methoxychlor | 24D |
|---|---|---|---|
| SB | + | + | - |
| SA | 0 | 0 | 0 |
| WB | + | + | - |
| WA | 0 | 0 | 0 |

In Table I, "+" indicates a positive result, or the presence of a distinct brown reduced silver spot on a resin paper corresponding to the area of spotting after standing under a heat lamp for a few minutes. "-" denotes an indefinite result, or the absence of a distinct brown spot over the spotted area after a few minutes of heat lamp treatment, but the presence of a uniformly brown resin paper square after prolonged heat lamp treatment. "0" means a negative result, that is, no color change was observed even

after prolonged heat lamp treatment.

The fact that only a relatively light brown spot appeared after spraying 10λ DDT on WB demonstrated the suitableness of 60λ as a spotting amount from the point of view of resolution.

The following observations were made after developed chromatograms were sprayed with the chromogenic agent:

(1) A descending chromatogram of 60λ DDT on SB with aqueous acetone (75% acetone by volume) as the developing solvent gave a positive result with about a $1cm^2$ spot. An $R_f$ value of 0.94 was obtained; a 0.94 $R_f$ value was calculated for a similar ascending chromatogram. The same chromatographic systems with 60λ Methoxychlor gave poor positive results, since a large smeary reduced silver area near the front was seen--no $R_f$ values were obtained; 24D showed indefinite results. In the instance of each pesticide the front traveled 14.5cm in 50 minutes.

(2) Descending--60λ--SA--aqueous acetone systems

gave negative results for all pesticides; front traversed 14.5cm in 55 minutes.

(3) Descending--60λ--DDT--WB--aqueous acetone chromatograms afforded the following $R_f$ values:

Table II

| $R_f$ |
|---|
| 0.94 |
| 0.92 |
| 0.94 |
| 0.93 average |

The reduced silver spots were about $2cm^2$ each in area. The same system with Methoxychlor showed more elongated spots than with DDT, and $R_f$ values of 0.90 and 0.92 were calculated; with 24D indefinite results were obtained. In this group the front traveled 15cm in 60 minutes.

(4) Descending--60λ--WA--aqueous acetone

chromatograms showed indefinite results with each pesticide. Fronts traversed 14.5cm in 120 minutes.

(5) Descending--60λ--WB--aqueous acetone chromatograms gave results, for both DDT and Methoxychlor, that were identical to those of (3) with the exception that the front traversed 14.5cm in 45 minutes. 60λ Methoxydhlor on SB with water as mobile phase gave a very smeary spot. Neither DDT nor Methoxychlor yielded positive results on WB with dioxane as developing solvent.

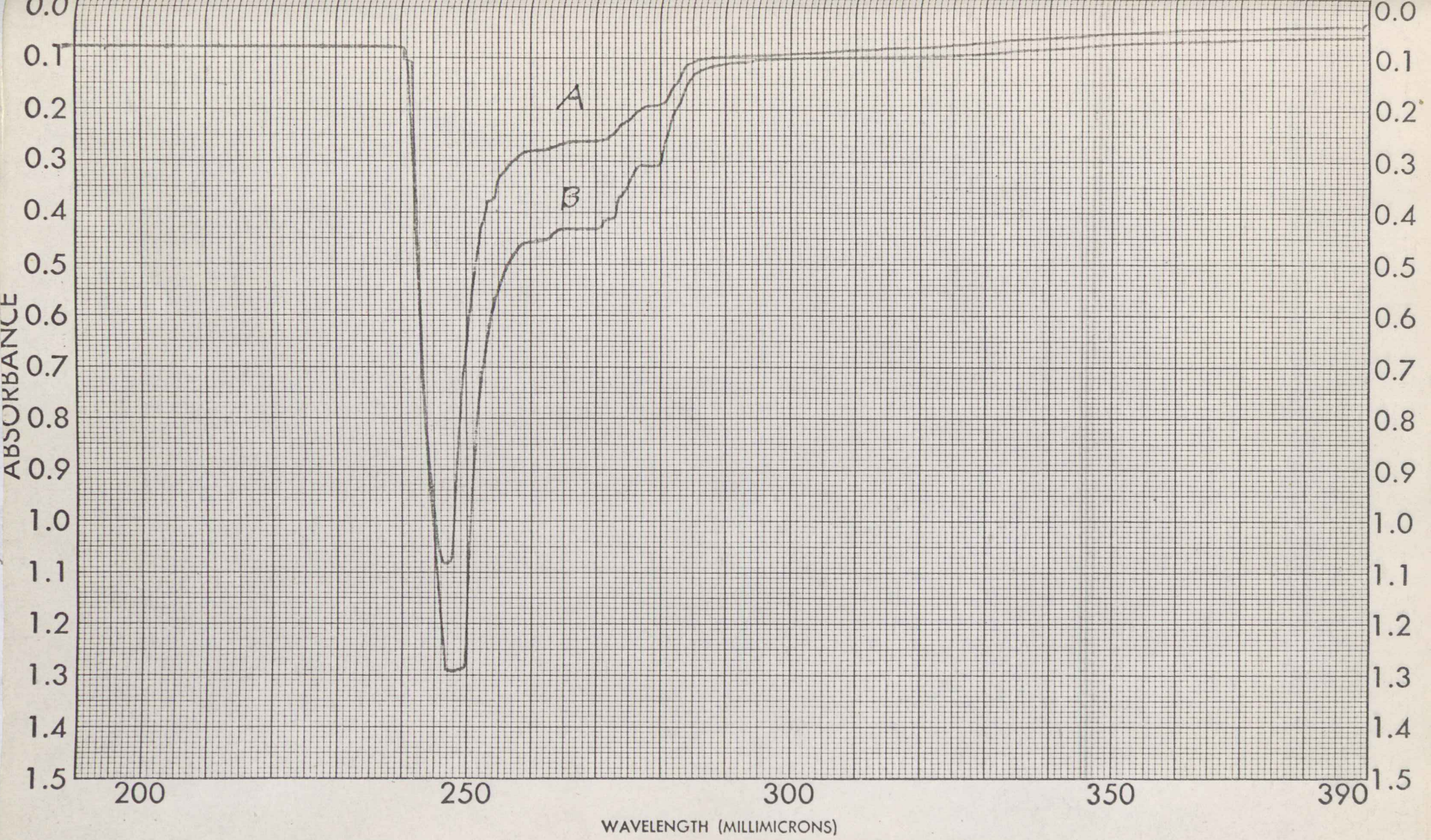
The above data indicate that silver nitrate affords positive results only with DDT and Methoxychlor, only on anionic papers (SB, WB) and only with relatively polar developing solvents (aqueous acetone, water). These observations and the fact that both DDT and Methoxychlor possess an alkyl chloride group intimate the possibility of chloride ion liberation by basic hydrolysis and the subsequent reaction of the chromogenic agent with free chloride near the front (high $R_f$ values) instead of the

bulk of the pesticide (which may be somewhere else). In short, it is postulated that the $R_f$ values that have been cited above may, in fact, be "pseudo-$R_f$ values"; a more definitive detection method is needed.

Elution-UV Detection Method

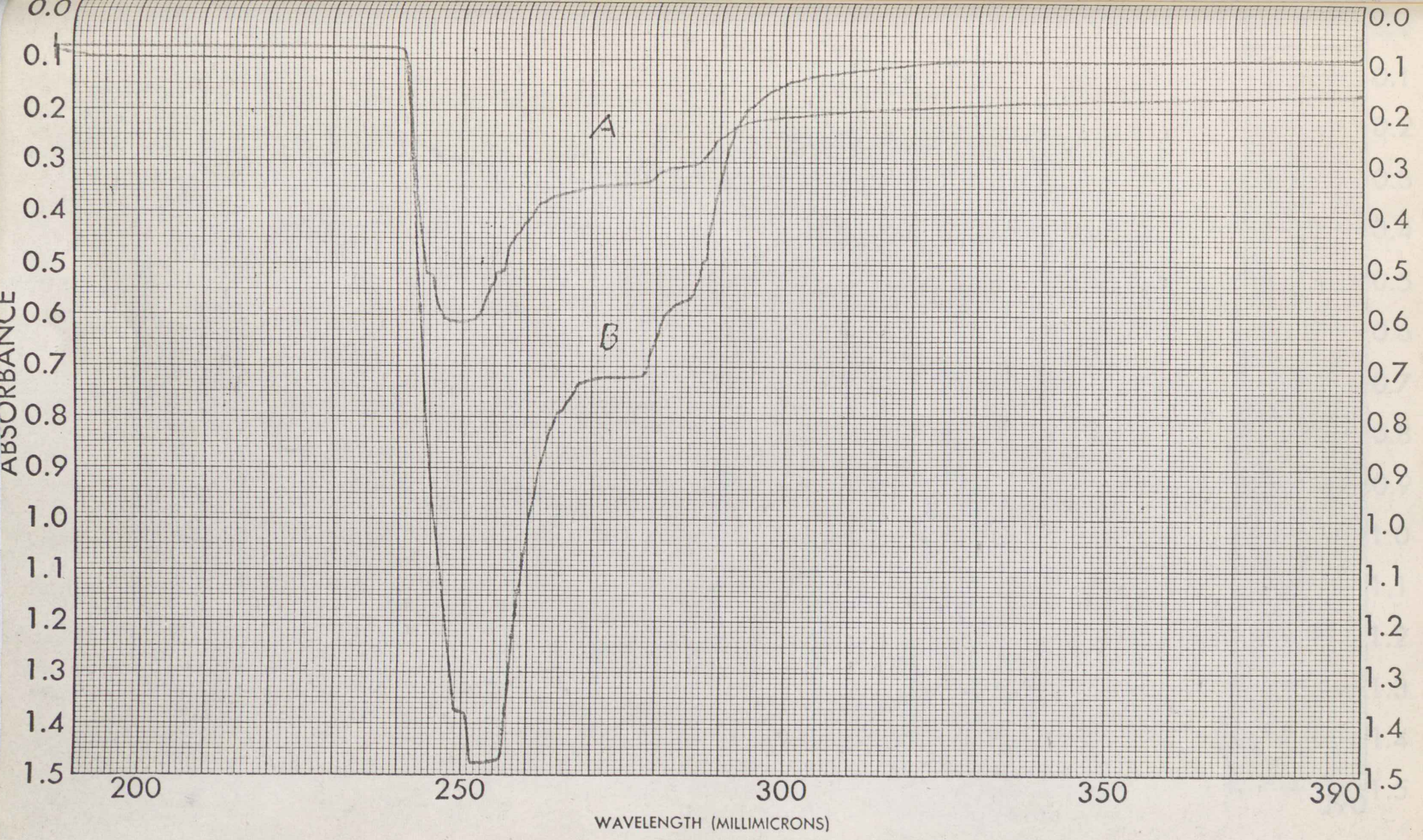
The position of spotted substances on developed chromatograms may be established quantitatively from UV spectra of the properly concentrated and blanked chloroform elutes of segments of developed chromatograms.

On the basis of the UV spectrum of each pesticide (see Figures 2, 3, and 4), optimum concentration ranges were determined for quantitative (or qualitative) work in the UV.




| | | | |
|---|---|---|---|
| SAMPLE D D T | CURVE NO. Figure 2. | SCAN SPEED 5 | OPERATOR Nagy |
| | CONC A. 0.3 mg/ml, B. 0.6 mg/ml | SLIT 2 5 | DATE |
| ORIGIN | CELL PATH 1cm | REMARKS | |
| SOLVENT C H Cl3 | REFERENCE | | |

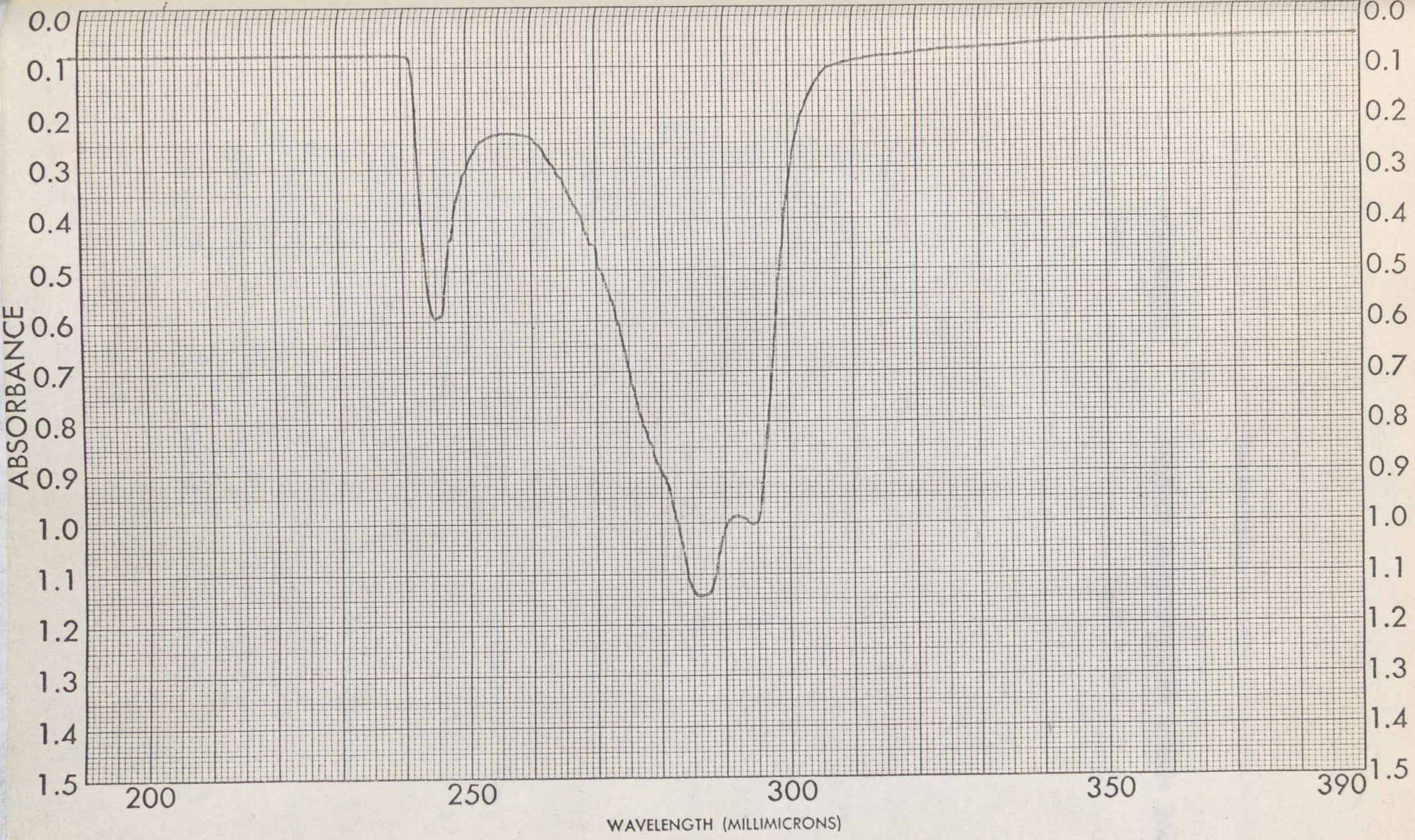
17




| | | | |
|---|---|---|---|
| SAMPLE Methoxychlor | CURVE NO. Figure 3 | SCAN SPEED S | OPERATOR Nagy |
| | CONC. A. 0.02 mg/ml, B. 0.1 mg/ml | SLIT 2.5 | DATE |
| ORIGIN | CELL PATH 1cm | REMARKS | |
| SOLVENT $CHCl_3$ | REFERENCE | | |

PART NO. 202-1511

PERKIN-ELMER




| | | | |
|---|---|---|---|
| SAMPLE 24D | CURVE NO. Figure 4 | SCAN SPEED S | OPERATOR Nagy |
| | CONC. 0.1 mg/ml | SLIT 2.5 | DATE |
| ORIGIN | CELL PATH 1 cm | REMARKS | |
| SOLVENT $CHCl_3$ | REFERENCE | | |

PERKIN-ELMER

Table III

| Pesticide | Optimum Concentration Range, mg/ml |
|---|---|
| DDT | 0.05 - 0.7 |
| Methoxychlor | 0.05 - 0.4 |
| 24D | 0.01 - 0.1 |

A 2 x 25cm strip of WB was shaken twice in a 500cc separatory funnel with 30ml chloroform. After each shaking the chloroform appeared to contain progressively less suspended material and became less yellow, the color of WB paper. A UV spectrum was obtained for a third 30ml portion after another shaking. The spectrum indicated strong absorption from 265 millimicrons to the end of the viewing range (390mu); a very similar spectrum was obtained for a 2 x 25cm SB strip with the same procedure. These findings obviated the necessity of blanking out whatever extracted out from the resin paper strips before a spotted substance could be viewed in the UV.

After additional UV spectra made it apparent that simply filtering off suspended material does not remove resin paper interference with the UV spectrum of an eluted pesticide, the following blanking procedure was devised:

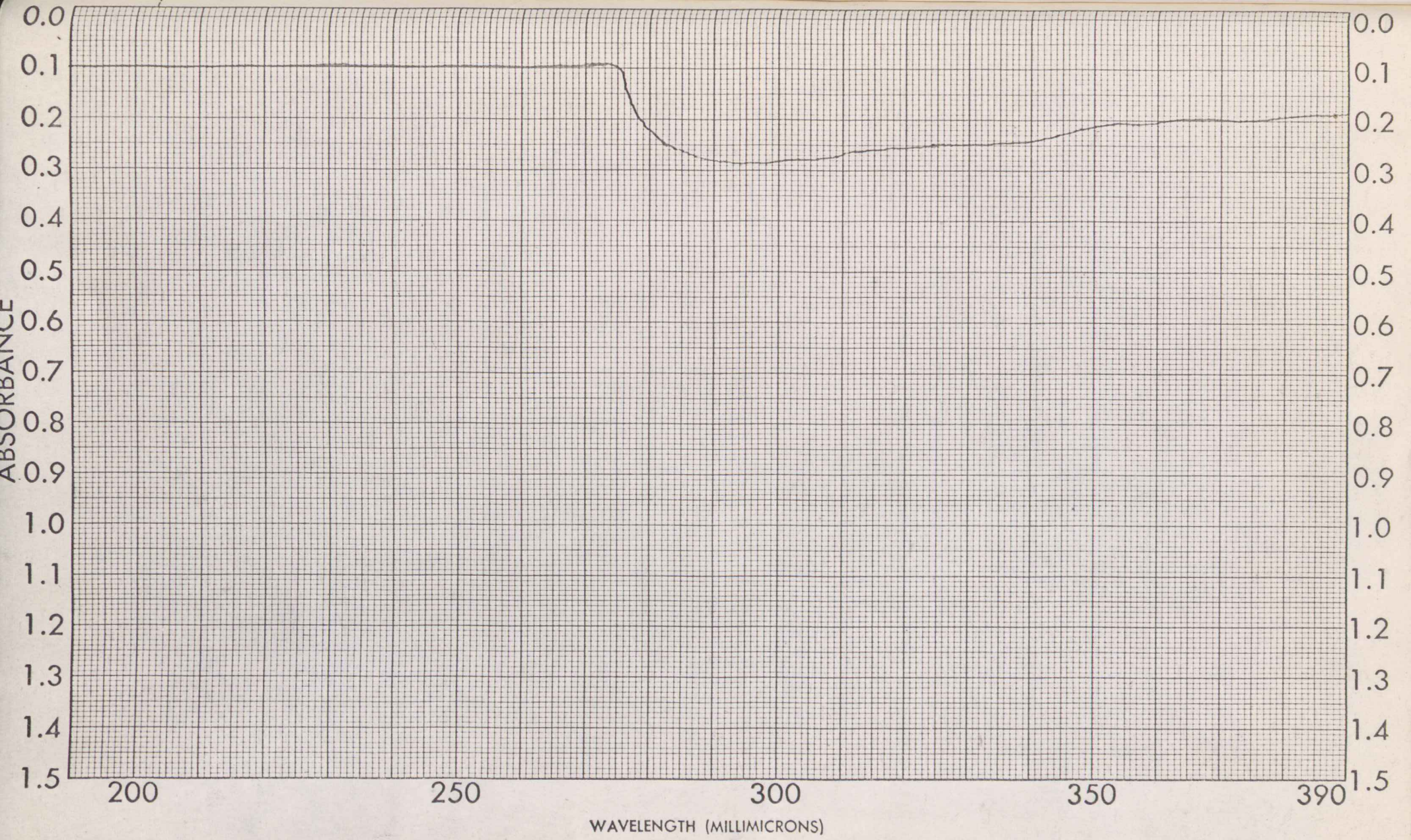
Shake a resin paper strip four times with 50ml chloroform; then shake twice with the same amount of distilled water; dry, and spot; develop the chromatogram, and elute desired segments by shaking twice with 20ml chloroform taking care to add aliquots. Finally, concentrate the resulting solution via evaporation until a practical UV viewing concentration is reached. Prepare a blank solution by exactly duplicating the history of a spotted strip on an identical strip with the exception of spotting.

With knowledge of the optimum detection concentration ranges of DDT, Methoxychlor, and 24D in the UV and the convenience consideration that about three milliliters of solution are needed in order to use ordinary 1cm (3ml) spectrophotometric cells, it was concluded that 200λ of 1mg/ml substance should be spotted, then eluted, and concentrated to 3ml. The assumption was made that strips would not be overloaded at 200λ of sample, and that the elute would contain the original spotted sample (i.e., the absorption coefficient of a spotted substance would not change in chromatography).

Figures 5 and 6 were obtained with the aforementioned blanking procedure. In the instance of Figure 5, a whole

chromatogram was eluted, concentrated to 3ml, and viewed. Figure 6 was obtained for the elute of the "front" half of a chromatogram, also concentrated to 3ml. Both curves show the spotted pesticide in a reacted form--compare Figure 5 with Figure 2 and Figure 6 with Figure 4: The curve of Figure 5 is possibly the spectrum of hydrolized DDT. (In Figure 5, absorption begins at 275mu as compared to 265 mu for a blank against chloroform curve.) In Figure 6, evidence is seen that may parallel Zweig's postulates (see p. 4); the same curve demonstrates the need for blanking.

Because the procedure promised to be too time consuming, no attempt was made to establish $R_f$ values by a method of progressive segments elution. However, developed, descending 60λ DDT and Methoxychlor WB-aqueous acetone chromatograms were irradiated with UV light to see if the pesticides on these chromatograms absorbed and/or fluoresced under UV light, thus becoming visually perceivable. UV light of two different wavelengths was tried with negative results.

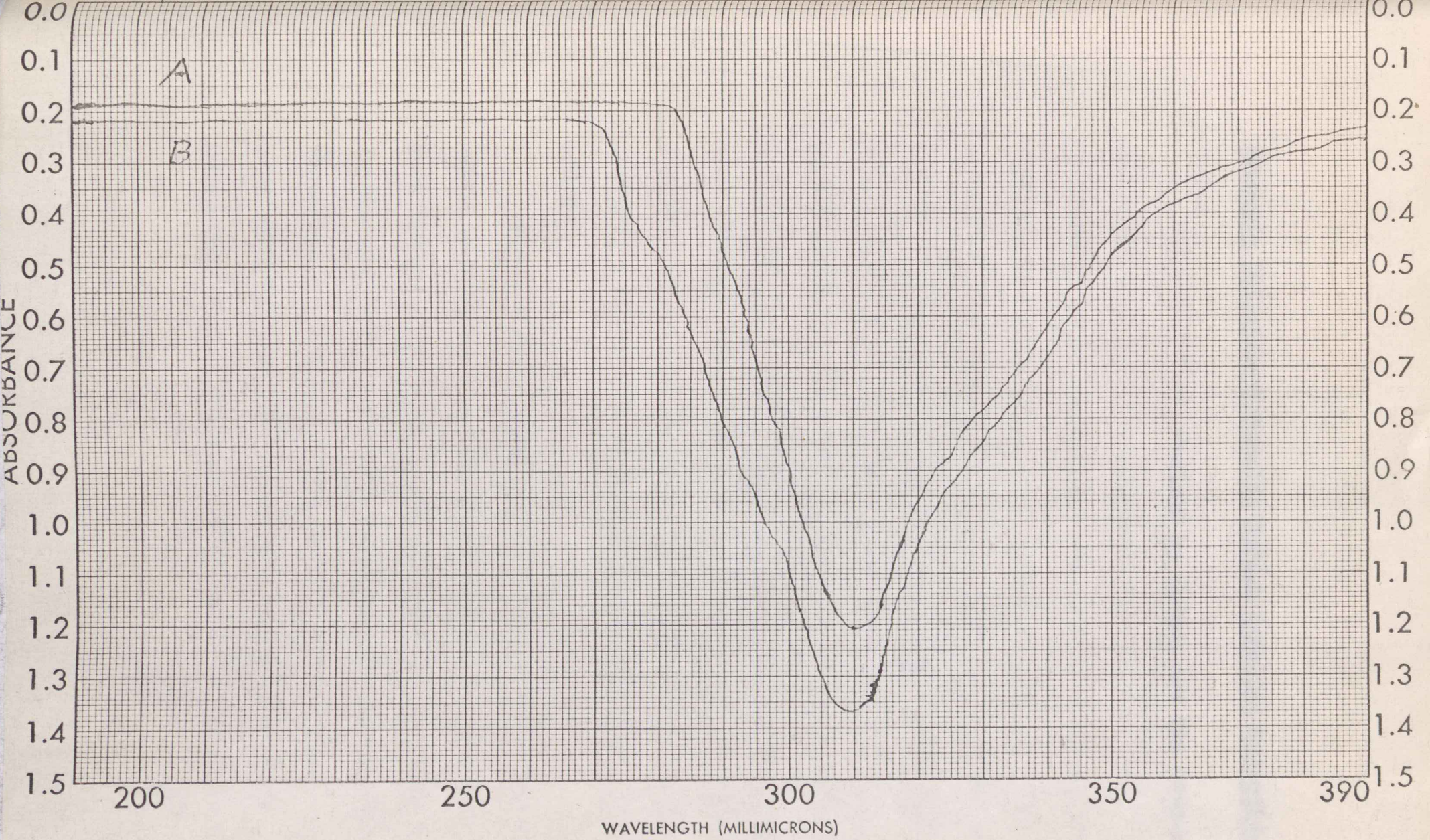



| | | | |
|---|---|---|---|
| SAMPLE Desc.- 200λ DDT - WB- $H_2O$ - Acetone elute → 3ml | CURVE NO. Figure 5 | SCAN SPEED F | OPERATOR Nagy |
| ORIGIN | CONC. | SLIT 2.5 | DATE |
| SOLVENT $CHCl_3$ | CELL PATH 1 cm | REMARKS run vs. WB blank | |
| | REFERENCE | | |



PART NO. 202-1511 ®

PERKIN-ELMER




| | | | |
|---|---|---|---|
| SAMPLE Desc.-60λ 240-WB-$H_2O$-Acetone, "front" half | CURVE NO. Figure 6 | SCAN SPEED F | OPERATOR Nagy |
| ORIGIN elute → 3 ml | CONC. | SLIT 25 | DATE |
| SOLVENT $CHCl_3$ | CELL PATH 1 cm | REMARKS A. run vs WB. blank | |
| | REFERENCE | B. run vs. $CHCl_3$ | |



PART NO. 202-1511 ®

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Mercuric Chloride--Ethanolic Sodium Ethoxide Detection Method

This procedure, devised by Connor and Van Campen (4), was tried with DDT and Methoxychlor. Reagents were prepared by dissolving 10g of mercuric chloride in 100ml absolute ethanol, and dissolving 1g of sodium in another 100ml portion of absolute ethanol. 1ml of NaOEt solution plus 2 drops of 1mg/ml solutions of DDT and Methoxychlor plus 5 drops of mercuric chloride solution yielded a white precipitate with DDT and Methoxydhlor. According to Connor and Van Campen a white ppt. indicates a positive test; the same workers also remark that an orange ppt. is inconclusive--an orange ppt. forms when aliquots of sodium ethoxide solution and mercuric chloride solution alone are mixed.

Several attempts were made to apply this test to 60λ spots of DDT and Methoxychlor on WB and SB. However, in each instance--even when reagents were added to the spots in the successful test tube test proportions--an orange ppt. formed on the papers, giving inconclusive results. In view of these observations it was concluded that the resin paper apparently interferes with the test. Consequently

the test was judged impractical for resin paper detection work.

Anhydrous Aluminum Chloride Detection Method

Work on devising a reliable way of establishing the whereabouts of a spotted pesticide on a developed chromatogram was continued with the effort to adapt to the chromatographic environment the Knotz (5) method of detecting trace amounts of DDT. This test, based upon the color-producing internal Friedel-Crafts reaction of DDT with anhydrous $AlCl_3$, is also applicable to Methoxychlor which has a similar structure to DDT. A "pinch" of $AlCl_3$ powder added to 0.2ml of 1mg/ml DDT and Methoxychlor in chloroform gave, as is predicted by Knotz, a brown ppt. with DDT and a violet ppt. with Methoxychlor, constituting a positive test.

The Handbook of Chemistry and Physics indicates that $AlCl_3$ is soluble in $CCl_4$, absolute ethanol, and chloroform; $AlCl_3$ was found to be soluble also in chlorobenzene and nitrobenzene. Portions of 0.1g/5ml solutions and of saturated solutions of $AlCl_3$ in each of these solvents were added to both pure solid DDT and Methoxychlor and to 0.2ml aliquots of 1mg/ml solutions of DDT and Methoxychlor.

Positive results were found only in the instance of $AlCl_3$ in nitrobenzene added to pure solid Methoxychlor. When saturated $AlCl_3$ in nitrobenzene was applied to dry Methoxychlor spots (200λ 1mg/ml per ~ $1cm^2$ spot) on WB and SB papers negative results were obtained. Thus, it was concluded that applying $AlCl_3$ in solution to developed chromatograms is apparently not feasible.

However, when dry 200λ 1mg/ml per ~ $1cm^2$ spots of DDT and Methoxychlor on WB paper were "wetted" with a drop of chloroform and $AlCl_3$ powder was applied (the powder was firmly pressed onto a wetted area with a porcelain spatula) positive results were observed with no apparent resin paper interference ($AlCl_3$ powder, added to WB extract in chloroform, showed no immediate reaction, although the suspension turned brown with a brown ppt. in two days' time). This adaptation of the Knotz method was tried on developed chromatograms of DDT and Methoxychlor. Chromatograms were wetted and powdered progressively from origin to front.

The following chromatograms showed good resolution with aluminum chloride--about $1cm^2$ spot could be seen easily:

Table IV

| System | $R_f$ |
|---|---|
| Descending--200λ--DDT--WB--aqueous acetone | 0.52 |
| Descending--300λ--DDT--WB--aqueous acetone | 0.76 |
| Descending-- 60λ--DDT--SB--aqueous acetone | 0.29 |
| Descending--200λ--Methoxychlor--WB--aqueous acetone | 0.44 |

Initially several DDT and Methoxychlor--WB--aqueous acetone chromatograms yielded a zero $R_f$ value when spotting was performed very near the heat lamp (about 5cm away)--previously all spotting had been done at about 30cm. A round area with a brownish outline was clearly visible on the origin after spotting, and after developing the very same area showed a chromogenic reaction product with aluminum chloride, thus affording a zero $R_f$ value. When spotting was again carried out at 30cm from the heat lamp, the above-indicated non-zero $R_f$ values were obtained. It is possible that at 5cm the spotted pesticide was "baked" onto the resin paper and would not move with developing.

300λ--Methoxychlor--WB--aqueous acetone chromatograms showed poor resolution with the chromogenic agent--smeared

out violet areas were seen at about the halfway point on each strip. Poor resolution might have been the result of overloading a chromatogram at 300λ of Methoxychlor, since good results were obtained at 200λ. These findings are qualitative evidence that Methoxychlor shows about 50% retention in a WB--aqueous acetone system.

In Table IV the difference between the two WB--DDT $R_f$ values might be due to the difference in spotting amount.

Diphenylamine--Zinc Chloride Detection Method

An attempt was made to adapt Katz's (6) qualitative test for DDT and Methoxychlor on ordinary paper chromatograms to resin paper chromatography. The chromogenic agent was prepared by dissolving 0.5g of diphenylamine and 0.5g of anhydrous zinc chloride in 100ml acetone. According to Katz the whereabouts of DDT and Methoxychlor on developed paper chromatograms is indicated by the appearance of pink-red and blue-black spots respectively on chromatograms after spraying with the chromogenic agent and heating at 160°C.

The Katz test showed positive results with DDT and Methoxychlor on pieces of both WB and SB. A brown DDT

spot appeared on WB.

The following $R_f$ values were obtained:

Table V

| System | $R_f$ |
|---|---|
| Descending--100λ--DDT--WB--aqueous acetone | 0.63 |
| Descending--100λ--Methoxychlor--WB--aqueous acetone | 0.38 |
| Descending--100λ--Methoxychlor--SB--aqueous acetone | 0.49 |
| Descending--100λ--Methoxychlor--WB--dioxane | 0.0 |
| Descending--100λ--Methoxychlor--SB--dioxane | 0.0 |

This detection method is probably much more practical than the Aluminum Chloride Detection Method from the technician's point of view.

# IV

# SUMMARY

# IV

## SUMMARY

To a limited extent the goals of the project have been realized. An ion exchange resin-impregnated paper chromatogram elution-blanking technique with chloroform has been devised for UV work; $R_f$ values have been established for some chlorinated pesticide ion exchange resin-impregnated paper chromatographic systems.

$R_f$ values obtained with the Silver Nitrate Detection Method seem unreliable for reasons already stated. However, $R_f$ values obtained with the Anhydrous Aluminum Chloride and Diphenylamine-Zinc Chloride Methods are reliable at least as a general indication of retention, since the chromogenic agent of both methods forms a colored product with the bulk of the pesticide. From the latter $R_f$ values (see Tables IV and V) it is evident that Methoxychlor shows greater retention than DDT in a descending--WB--aqueous acetone system; there is about a 0.2 $R_f$ value difference. While retention is greater for

both DDT and Methoxychlor on WB than on WB with aqueous acetone as developing solvent, the same 0.2 $R_f$ value difference remains as on WB. The fact that with dioxane as the mobile phase zero $R_f$ values were obtained for Methoxychlor on WB and SB papers with the Diphenylamine-Zinc Chloride Detection Method may be an indication of a requirement of using polar solvents in order to obtain non-zero $R_f$ values on WB and SB papers.

For further work the Diphenylamine-Zinc Chloride Detection Method may be used on DDT and Methoxychlor--aqueous acetone systems on all resin papers at a constant spotting amount (e.g., 100λ) and under otherwise highly controlled conditions, in order to obtain consistent and statistically verified $R_f$ values. Then these $R_f$ values may be compared for spread with the paper chromatography $R_f$ values for chlorinated pesticides, reported by L.C. Mitchell in J. Assoc. Offic. Agr. Chemist, 41, 781, 1958. (This publication was not available to the author at the time of this writing.) From a comparison of $R_f$ value spreads the chromatographic system most suited for the separation of DDT and Methoxychlor could be ascertained.

# V

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# V

## BIBLIOGRAPHY

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