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POLYVINYL ACETATE AS A STATIONARY PHASE IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The use of polyvinyl acetate as a stationary phase in thin-layer chromatography has been investigated using the derivatives of some organic compounds as the test substances. The phase gives very rigid layers and can be used for the separation of derivatives of homologous series as well as for the separation of derivatives of other types of organic compounds.

INTRODUCTION

In a study concerning the use of polyamide for thin-layer separations COPPIUS PEEREBOOM used polyvinyl acetate as a binder¹. During our investigations on the behaviour of 2,4-dinitrophenylhydrazones on thin layers of polyamide containing polyvinyl acetate, the R_F values of the 2,4-dinitrophenylhydrazones were found to depend to a large extent on the amount of polyvinylacetate used as binder for the polyamide layers. Presumably the polyvinyl acetate itself took part in the distribution of the compounds between the mobile and the immobile phases. It was then observed on substituting kieselguhr for the polyamide that the polyvinyl acetate itself acted as a convenient stationary phase for the separation of organic derivatives.

Some of the potentialities of this immobile phase for this purpose were then investigated.

EXPERIMENTAL

Preparation of plates

Measured amounts of polyvinyl acetate (Mowilith 50, Hoechst, G.F.R.) were dissolved in a mixture of 25 ml of methanol and 15 ml of ethyl acetate. The volume was adjusted to 60 ml with methanol and 25 g of Kieselguhr G (Merck) were suspended in the solution by shaking in an erlenmeyer flask. Chromatoplates were prepared using the standard Desaga equipment. The equipment should be cleaned immediately after use by rinsing with methanol until all polyvinyl acetate solution has been removed.

The plates were used after 3 h of drying at room temperature in the atmosphere of the laboratory.

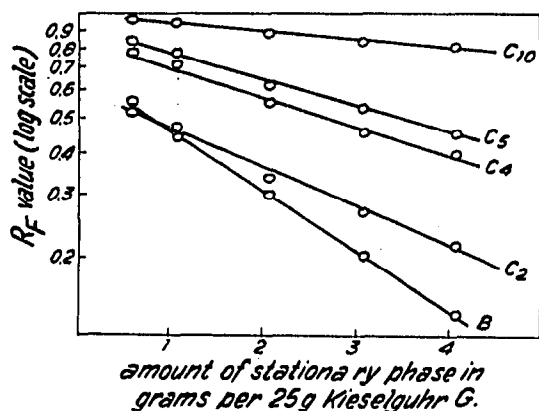


Fig. 1. Dependence of the R_F values of carbonyl 2,4-dinitrophenylhydrazones on the amount of immobile phase (polyvinyl acetate).

RESULTS AND DISCUSSION

Dependence of R_F and R_F^c values on the amount of polyvinyl acetate

A series of plates was prepared containing 0.5 to 4 g of polyvinyl acetate to 25 g of Kieselguhr G. A number of 2,4-dinitrophenylhydrazones were spotted at the bottom edge of the plates. The solvent consisting of a mixture of hexane-di-*n*-butyl ether-methanol-ethyl acetate (85:5:5:5) was allowed to rise to 11.5 cm above the point of application.

The results of these experiments are presented in Fig. 1 and it is seen that the log R_F values are linear with respect to the amount of stationary phase. It was shown earlier that perfect agreement between R_F values measured on different chromatograms can be obtained by using the standardization procedure²⁻⁵ of GALANOS AND KAPOULAS. The relation found in Fig. 1 suggests that this correction procedure would apply to our data on different amounts of immobile phase by taking the log of the R_F values instead of the R_F values as such. The GALANOS AND KAPOULAS equation thus takes the form:

$$\log R_F^c = a \log R_F + b$$

The R_F values shown in Fig. 1 were then corrected using the R_F values found on 3 g polyvinyl acetate as the standard values. The results are shown in Table I.

TABLE I

R_F^c VALUES ON PLATES CONTAINING DIFFERENT AMOUNTS OF IMMOBILE PHASE, AFTER CORRECTION ACCORDING TO THE MODIFIED EQUATION OF GALANOS AND KAPOULAS
Solvent system B.

| 2,4-Dinitrophenyl- hydrazone of | Amount of immobile phase per 25 g of Kieselguhr G (g) | | | | |
|------------------------------------|--|------|------|------|------|
| | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 |
| Butanal | 0.56 | 0.50 | 0.49 | 0.46 | 0.46 |
| Pentanal | 0.62 | 0.56 | 0.56 | 0.54 | 0.52 |
| Benzaldehyde | 0.29 | 0.23 | 0.24 | 0.20 | 0.25 |

The R_F values of the 2,4-dinitrophenylhydrazones of ethanal and decanal were used as the reference compounds on all chromatograms, and it was from these values that those of butanal, pentanal and benzaldehyde were computed. As is shown in Table I, the values are nearly constant with the exception of those obtained on 0.5 g immobile phase which are significantly higher. It is clear that using the modified GALANOS AND KAPOULAS correction procedure, constant R_F values are obtainable even if there are slight variations in the amount of immobile phase present on the plates.

Behaviour of some organic derivatives on polyvinyl acetate

Two chromatographic systems were studied. One series of plates contained 2 g of polyvinyl acetate per 25 g of Kieselguhr G (M_2 plates), the other type contained 3 g of polyvinyl acetate for the same amount of support material (M_3 plates).

The solvent systems used were: System A/ M_2 plates: hexane-di-*n*-butyl ether-methanol-ethyl acetate (65:10:10:15). System B/ M_3 plates: hexane-di-*n*-butyl ether-methanol-ethyl acetate (85:5:5:5). Point of application: 1.5 cm from the bottom edge of the plate; front: 11.5 cm above the point of application.

2,4-Dinitrophenylhydrazones (2,4-DNPH's) and 2-diphenylacetyl-1,3-indanedione hydrazones (DAIH's). 2,4-DNPH's were prepared in the normal way. The DAIH's were prepared as follows: 3–5 mg of 2-diphenylacetyl-1,3-indanedione hydrazone was dissolved in 0.5 ml chloroform. To this solution about 3 mg of the carbonyl compound and a very small drop of concentrated hydrochloric acid were

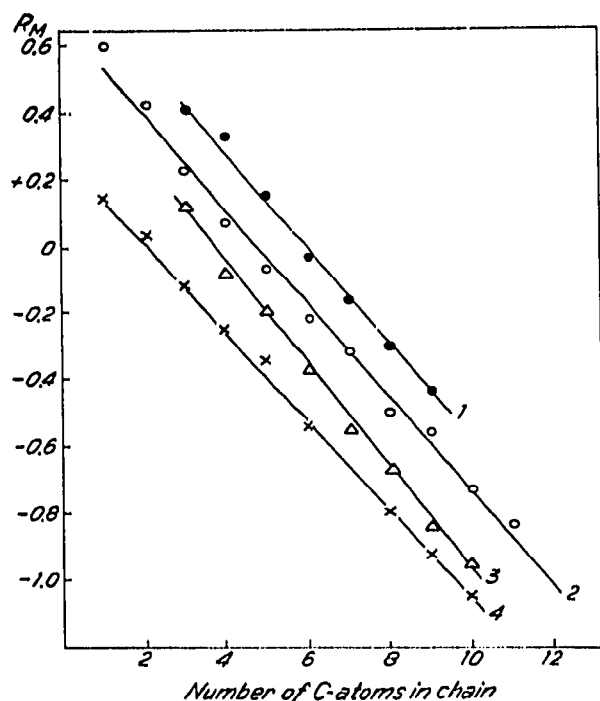


Fig. 2. Relation between R_M value and number of C atoms in chain for: (1) alk-2-enal 2,4-dinitrophenylhydrazones; (2) *n*-alkanals 2,4-dinitrophenylhydrazones; (3) 2-alkanones 2,4-dinitrophenylhydrazones; (4) *n*-alkanol 3,5-dinitrobenzoates in system B.

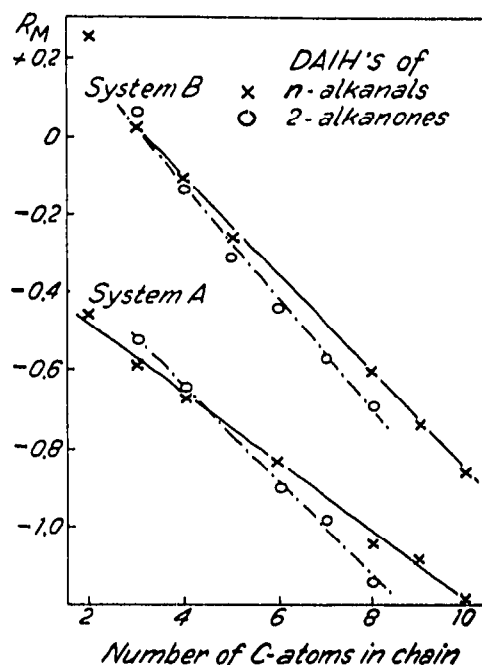


Fig. 3. Relation between the number of carbon atoms in the chain and the R_M value for aliphatic 2-diphenylacetyl indanedione hydrazones.

TABLE II

REGRESSION LINE EQUATION RELATING R_M VALUE AND CARBON NUMBER FOR SOME CARBONYL DERIVATIVES

| | System | Equation |
|-------------------------------------|--------|---------------------------|
| DAIH's of <i>n</i> -alkanals | A | $R_M = -0.0850 C - 0.307$ |
| | B | $R_M = -0.1250 C + 0.392$ |
| DAIH's of 2-alkanones | A | $R_M = -0.1239 C - 0.148$ |
| | B | $R_M = -0.1488 C + 0.474$ |
| 2,4-DNPH's of <i>n</i> -alkanals | B | $R_M = -0.1394 C + 0.668$ |
| 2,4-DNPH's of 2-alkanones | B | $R_M = -0.1631 C + 0.681$ |
| 2,4-DNPH's of <i>n</i> -alk-2-enals | B | $R_M = -0.1454 C + 0.870$ |

added. After standing overnight the solution was evaporated on a boiling water bath and the residue was taken up in 0.2–0.5 ml of chloroform. 1–2 μ l of this solution was spotted on the plate. The derivatives have an intense yellow color and on the plates they exhibit a strong fluorescence in filtered UV light (363 nm)^{6,7}. R_M values were calculated for each homologous series and the relation between R_M and the chain length for the particular series tested. The results are shown in Figs. 2 and 3. With the exception of some of the first members of the series a linear relation was found. The lines of best fit were calculated using least square methods and the results are shown in Table II.

System B was the most suitable for the separation of homologous series of aliphatic derivatives. The R_F values of the 2,4-DNPH's and the DAIH's of the same

TABLE III

 R_F VALUES OF SOME 2,4-DNPH'S AND DAIH'S FROM AROMATIC CARBONYL COMPOUNDS

| Compound | 2,4-DNPH's | | DAIH's | |
|-------------------------------|------------|------|--------|------|
| | A | B | A | B |
| Furfural | 0.52 | 0.13 | — | — |
| 5-Methylfurfural | 0.63 | 0.28 | — | — |
| | 0.59 | 0.18 | — | — |
| 5-Hydroxymethylfurfural | 0.70 | 0.41 | — | — |
| | 0.13 | 0.10 | — | — |
| Vanillin | 0.34 | — | — | — |
| | 0.35 | 0.10 | — | 0.12 |
| Isovanillin | 0.43 | 0.11 | 0.39 | — |
| Ethylvanillin | 0.43 | 0.10 | 0.35 | — |
| Benzaldehyde | 0.65 | 0.20 | — | 0.26 |
| <i>p</i> -Hydroxybenzaldehyde | 0.37 | 0.10 | — | 0.03 |
| <i>o</i> -Methoxybenzaldehyde | 0.61 | 0.21 | — | 0.26 |
| Phenylacetaldehyde | 0.67 | 0.27 | 0.64 | — |
| Phenylpropionaldehyde | 0.67 | 0.29 | — | 0.34 |
| Protocatechualdehyde | 0.21 | 0.10 | — | — |
| Gentisic aldehyde | 0.23 | — | — | — |
| Anisaldehyde | 0.55 | 0.15 | 0.53 | — |
| Cyclamenaldehyde | 0.82 | 0.63 | 0.84 | 0.64 |
| Cinnamaldehyde | 0.62 | 0.20 | 0.61 | — |
| <i>p</i> -Methoxyacetophenone | 0.62 | 0.23 | — | 0.25 |
| Benzophenone | — | 0.49 | 0.70 | — |

carbonyl compound are not very different from each other. However the DAIH's have a much lower detection limit than the 2,4-DNPH's owing to their strong fluorescent colors which can be observed by inspection under filtered UV light. Table III contains the R_F values of a number of aromatic type derivatives and it is seen that system A in combination with system B separated a number of aromatic type derivatives.

3,5-Dinitrobenzoates. The 3,5-dinitrobenzoates of a number of aliphatic alcohols and phenols were prepared by reacting the individual compounds with 3,5-dinitrobenzoylchloride⁸. The regression line equation for the homologous series of aliphatic alcohols in relation to carbon number and R_M was found to be

$$R_M = -0.1312 C + 0.279 \text{ for system B.}$$

The line of best fit together with the experimental points are shown in Fig. 2.

The regression line equation for system A was not calculated because of the very high R_F values found in this system.

The R_F values of some 3,5-dinitrobenzoates of phenols are shown in Table IV.

TABLE IV

 R_F VALUES OF SOME 3,5-DINITROBENZOATES

| 3,5-Dinitrobenzoate of | R_F | |
|------------------------|----------|----------|
| | System A | System B |
| 2,5-Dimethylphenol | 0.76 | 0.50 |
| 1-Naphthol | 0.61 | 0.23 |
| Thymol | 0.82 | 0.39 |
| <i>m</i> -Cresol | 0.70 | 0.40 |
| Phenol | 0.65 | 0.29 |
| Geraniol | 0.88 | 0.85 |
| Benzyl alcohol | 0.67 | 0.35 |

Derivatives of amines. Amines were reacted with 2,4-dinitrochlorobenzene or 2,4-dinitrofluorobenzene to form the corresponding 2,4-dinitroanilides⁸.

The regression lines relating carbon number and R_M value for the derivatives of homologous primary aliphatic amines were found to be:

$$\text{for system A: } R_M = -0.1195 C - 0.049$$

$$\text{for system B: } R_M = -0.1456 C + 0.800.$$

The first member of the series for system A, and the first two members for system B were excluded from the calculations. The resulting lines of best fit are shown in Fig. 4. A number of derivatives of other types of amines were separated in the two systems. The R_F values found are shown in Table V.

The homologues belonging to the series described in this paper are best separated from each other in system B. By lowering the amount of stationary phase and at the same time raising the polarity of the solvent a number of aromatic type derivatives can be separated, especially when present in a single mixture with aliphatics.

The running time of a chromatogram was 20–25 min which is usual in thin-layer chromatography. The reproducibility of R_F values was studied during the time the

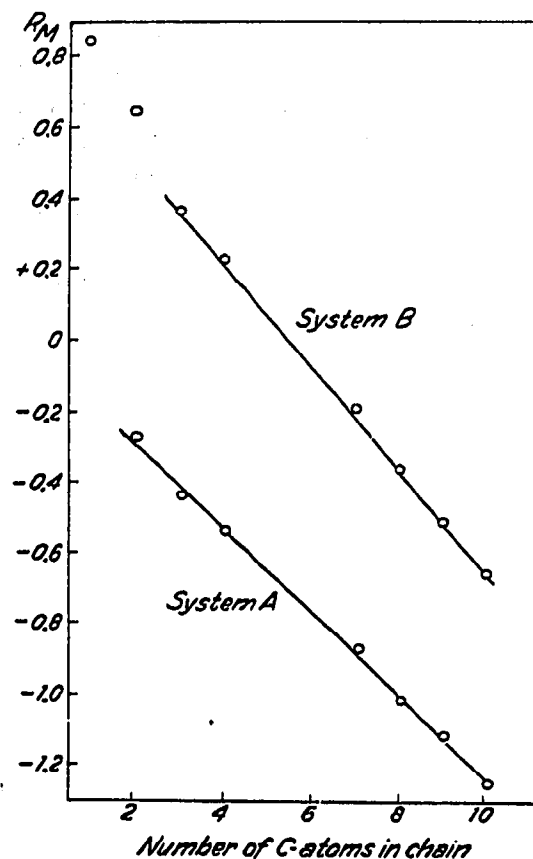


Fig. 4. Relation between carbon number and R_M value for 2,4-dinitroalkylanilides.

experiments were performed (about 18 months) using the 2,4-DNPH of pentanal in system B as a check and was found to be 0.122 R_F units.

However, using the correction procedure of GALANOS AND KAPOULAS the reproducibility was raised to 0.031 R_F units using the 2,4-DNPH's of ethanal and of decanal as reference compounds.

In conclusion, polyvinyl acetate seems to be a very useful addition to the range of thin-layer stationary phases. It gives very rigid layers which can be stored indefinitely. Separations can be obtained in a short time and R_F values are highly reproducible.

TABLE V

2,4-DINITROANILIDES OF SOME NON-ALIPHATIC TYPE AMINES

| 2,4-Dinitroanilide of | R_F | |
|-------------------------|----------|----------|
| | System A | System B |
| α -Naphthylamine | 0.70 | 0.36 |
| Benzidine | 0.26 | 0.02 |
| β -Phenethylamine | 0.65 | 0.22 |
| Cyclohexylamine | 0.82 | 0.53 |
| Aniline | 0.66 | 0.28 |
| <i>p</i> -Nitroaniline | 0.50 | 0.06 |

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