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## Note

### Separation of homologous series on pre-adsorbed layers

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In a previous communication<sup>1</sup> it was shown that the pre-adsorption of organic vapours on silica gel layers determines the behaviour of organic compounds, all other experimental circumstances being constant. The present paper deals with the separation of homologues on such layers. This form of chromatography has the advantage of both the mobile and stationary phases being volatile liquids. Separated compounds eluted from such layers do not contain any non-volatile components from the chromatographic system and are therefore amenable to identification by infrared or mass spectrometry.

#### EXPERIMENTAL

The following series were tested: phenylazophenacyl esters of fatty acids, 2,4-dinitrophenylhydrazones of *n*-alkanals, 2,4 dinitrophenylhydrazones of *n*-alkenals, 2,4 dinitro-alkylanilides. These derivatives were prepared according to current methods<sup>2</sup>.

The following types of silica gel layers were used: silica gel type 60 (E. Merck), silica gel for column chromatography (sieved), silica gel D-O (Camag), silica gel IB-HP<sub>254</sub> (J. T. Baker). Layers were prepared using the Desaga equipment. Pre-adsorption was effected by placing a sheet of filter paper against the wall of the developing tank. The filter paper was thoroughly wetted with the liquid whose vapours were to be absorbed onto the silica gel layer. The spotted plate was allowed to equilibrate with the organic vapour for *ca.* 5 min. Subsequently, hexane was added carefully to the bottom of the tank, immediately followed by the development. In some cases multiple development was applied in which the plate was taken out of the tank after the first run and the mobile phase was allowed to evaporate for a few minutes. Then the plate was developed again in the same solvent; this procedure can be repeated *n* times. All  $R_F$  values were reduced to  ${}^1R_F$  values according to the formula

$${}^1R_F = 1 - \sqrt[n]{1 - {}^nR_F}$$

In order to obtain constant  $R_F$  values ( $R_F^c$ , ref. 3) a mixture of *p*-aminoazobenzene, dimethyl yellow and azobenzene was spotted on at least three different places on the plate. The correction equation of Van Wendel de Joode

$$\frac{1}{R_{Fi}} = \frac{a}{R_{Fj}} + b,$$

in which  $R_{Fi}$  stands for the  $R_F$  value of a compound on chromatogram  $i$  and  $R_{Fj}$  for the same compound on chromatogram  $j$ , was applied to all  $R_F$  values<sup>4</sup>. The  $R_F^0$  values for the reference compounds were

$$R_{F(p\text{-aminoazobenzene})}^0 = 0.041, R_{F(\text{dimethyl yellow})}^0 = 0.512, R_{F(\text{azobenzene})}^0 = 0.828.$$

## RESULTS AND DISCUSSION

In order to obtain sufficient separation, multiple development was found to be necessary in the systems silica gel–acetonitrile–hexane and silica gel–nitromethane–hexane. The number of developments could be reduced by preparing layers from mixtures of silica gel and Kieselguhr G (E. Merck). The experiments on mixed layers (Fig. 1) showed that the addition of Kieselguhr to the silica gel layer results in a set of

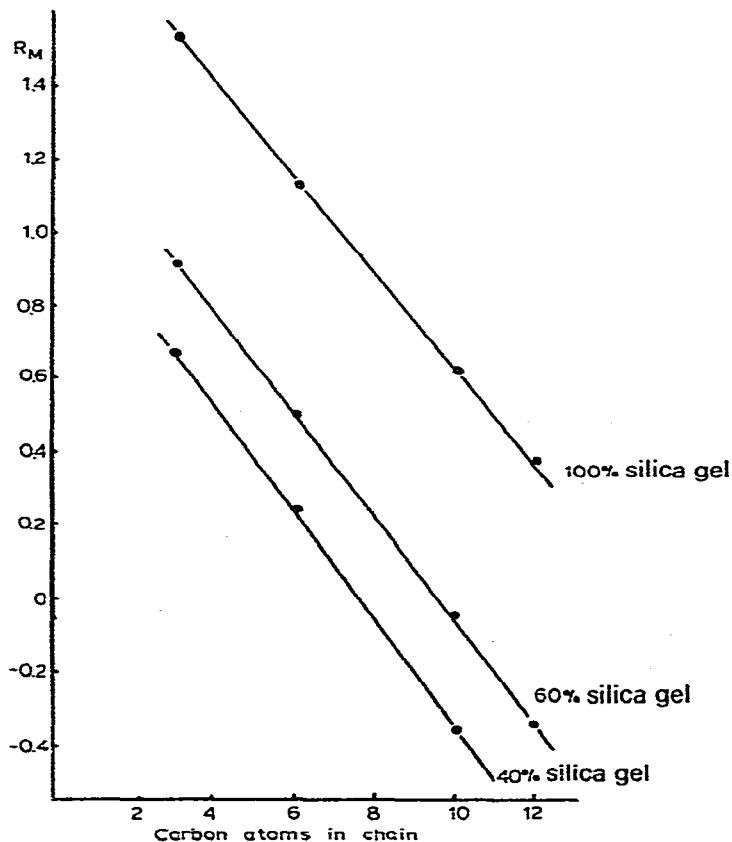


Fig. 1. Relation of  $R_F$  values and chain length of a homologous series on silica gel layers and on layers containing mixtures of silica gel with Kieselguhr G, test compounds 2,4-dinitrophenylhydrazones of  $n$ -alkanal. System: silica gel–acetonitrile–hexane.

parallel lines when  $R_{M}$  is plotted against chain length. In these experiments acetonitrile was used as the impregnating vapour and hexane as the solvent. Constant  $R_F$  values were obtained with Van Wendel de Joode's formula, irrespective of the amount of Kieselguhr in the layer. The results of some of these experiments are summarized in Table I.

TABLE I

$R_F$  AND  $R_F^c$  VALUES ON SILICA GEL LAYERS OF DIFFERENT ORIGIN AND WITH DIFFERENT AMOUNTS OF SILICA GEL

$n = 5$ .

Compound	$R_F \pm S.D.$	$R_F^c \pm S.D.$
Dimethyl yellow	$0.600 \pm 0.104$	$0.512 \pm 0.014$
propanal 2,4-dinitrophenylhydrazone	$0.133 \pm 0.044$	$0.073 \pm 0.005$
pentanal 2,4-dinitrophenylhydrazone	$0.208 \pm 0.071$	$0.154 \pm 0.011$
nonanal 2,4-dinitrophenylhydrazone	$0.490 \pm 0.120$	$0.405 \pm 0.024$

The figures show that the Van Wendel de Joode formula can be applied in this case. The  $R_F$  values collected during the last few years were obtained on silica gel layers of different origin (cf. Experimental section) having different ratios of silica gel: Kieselguhr and being usually run in multiple development. After calculating the  $^1R_F$  values, the Van Wendel de Joode equation was applied, using the  $R_F^0$  values of *p*-aminoazobenzene and azobenzene mentioned above. Some of the results obtained with the homologous series of *n*-alkanal 2,4-dinitrophenylhydrazones are given in Table II. Although these  $R_F$  values were obtained under widely different conditions, the standard deviations of the mean  $R_F^c$  values did not exceed 0.04  $R_F$ . The regression equations for a number of homologous series in the systems silica gel-acetonitrile-hexane and silica gel-nitromethane-hexane are summarized in Table III.

TABLE II

MEANS AND STANDARD DEVIATIONS OF CORRECTED  $R_F$  VALUES ( $R_F^c$ ) IN THE SILICA GEL-ACETONITRILE-HEXANE SYSTEM

Alkanal 2,4-dinitrophenylhydrazone	$R_F^c \pm S.D.$	$n^*$
Ethanal	$0.035 \pm 0.011$	10
Propanal	$0.073 \pm 0.006$	13
Butanal	$0.111 \pm 0.011$	13
Pentanal	$0.154 \pm 0.020$	12
Hexanal	$0.205 \pm 0.026$	14
Heptanal	$0.261 \pm 0.025$	7
Octanal	$0.331 \pm 0.033$	13
Nonanal	$0.405 \pm 0.040$	11
Decanal	$0.494 \pm 0.039$	13
Undecanal	$0.546 \pm 0.020$	7
Dodecanal	$0.620 \pm 0.037$	12

\* Mean of  $R_F$  values.

TABLE III  
REGRESSION EQUATIONS FOR SOME HOMOLOGOUS SERIES  
Systems: 1 = silica gel-acetonitrile-hexane; 2 = silica gel-nitromethane-hexane

Series	Type of silica gel (cf. Experimental section)			
	I	II	III	IV
Phenylazophenyl esters of <i>n</i> -fatty acids	1 $R_M = -0.156C + 1.39$	-	$R_M = -0.160C + 1.33$	-
	2 $R_M = -0.190C + 1.48$	$R_M = -0.191 + 1.49$	$R_M = -0.182 + 1.52$	-
2,4-Dinitrophenylhydrazones of <i>n</i> -alkanal	1 $R_M = -0.149C + 1.34$	$R_M = -0.150C + 1.44$	$R_M = -0.152C + 1.43$	$R_M = -0.148C + 1.31$
	2 $R_M = -0.167C + 1.71$	$R_M = -0.172C + 1.84$	-	-
2,4-Dinitroalkylamides	1 $R_M = -0.150C + 1.69$	-	-	$R_M = -0.150C + 1.51$
	2 -	-	-	-
2,4-Dinitrophenylhydrazones of <i>n</i> -alkenals	1 $R_M = -0.152C + 1.55$	$R_M = -0.150C + 1.51$	$R_M = -0.148C + 1.50$	-
	2 -	$R_M = -0.162C + 1.94$	$R_M = -0.160C + 1.95$	-

The homologous increment obtained under these conditions remains markedly constant, the mean value for the system containing acetonitrile being  $-0.151$  (S.D. =  $0.004$ ) and for the system containing nitromethane  $-0.175$  (S.D. =  $0.013$ ). The values of the first two members of each series were not incorporated in the calculations.

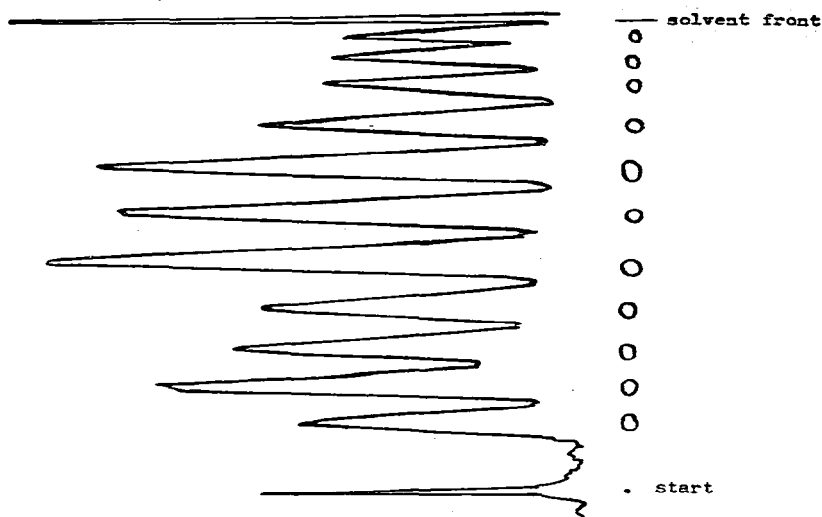


Fig. 2. Separation of a mixture of *n*-alkanal 2,4 dinitrophenylhydrazones. Layer: mixture of 50% silica gel and 50% Kieselguhr G pre-adsorbed with acetonitrile. Two developments in hexane. Distance from start to solvent front: 10 cm. Densitogram: reflection at 450 nm.

## CONCLUSION

Homologous series can be separated into their components on silica gel layers pre-adsorbed with either acetonitrile or nitromethane vapour. After elution of the components from the layers with a suitable solvent, the eluted fraction does not contain any non-volatiles from the system.

The corrected  $R_F$  values remain fairly constant, even under the widely different conditions applied. It is to be expected that more standardized conditions will result in an increased reproducibility of the corrected  $R_F$  values.

## REFERENCES

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