

CHROM. 5447

## Standardization of $R_F$ values in thin-layer partition chromatography

During the past two years various methods for standardizing the  $R_F$ -determination procedure in thin-layer chromatography (TLC) have been tested at the Central Institute for Nutrition and Food Research, TNO. The method of GALANOS AND KAPOULAS has been found to give consistently accurate  $R_F$  values, even when the determinations were carried out in different laboratories<sup>1</sup>. In the meanwhile, some results obtained in our Institute have been published<sup>2</sup>.

Our investigations on the use of polyvinyl acetate as a stationary phase in TLC<sup>3</sup> revealed that the standardization procedure of GALANOS AND KAPOULAS produced better results when the log of the  $R_F$  instead of the  $R_F$  was used. The corrected  $R_F$  values became largely independent of the quantity of stationary phase used in the preparation of the plates. This phenomenon was not only observed in the polyvinyl acetate system, but also in a number of other partition systems. The results of experiments carried out with various stationary phases are described in this paper.

### Experimental

*Preparation of plates for "normal" partition systems.* A weighed quantity of the stationary phase was dissolved in a mixture consisting of 40 ml of methanol and 20 ml of water. 25 g Kieselguhr G (Merck) was suspended in this solution by shaking it in an erlenmeyer flask. Plates were prepared, using standard Desaga equipment. The slit width was 0.25 mm throughout the tests. The plates were dried for 3 h at room temperature in contact with the laboratory atmosphere before being used. Hexane, saturated with the stationary phase, was used as solvent.

*Preparation of plates for the reversed phase system.* 2 g of powdered polyvinyl chloride were dissolved in 150 ml of methyl ethyl ketone. 25 g of Kieselguhr G (Merck) and a weighed quantity of paraffin oil were added to 60 ml of this solution.

The plates were allowed to dry overnight. The mobile phase consisted of a mixture of acetone-water (3:1) saturated with paraffin oil.

*Chromatographic technique.* Compounds were spotted 4 cm from the bottom edge of the plate in order to saturate the mobile phase with the stationary phase before the mobile phase had reached the line of application. A line was drawn through the layer 11.5 cm above the point of application. The plates were removed from the tank as soon as the solvent had reached this line. Normal tanks were used, lined with filter paper dipping in the solvent.

2,4-Dinitrophenylhydrazones of *n*-alkanals were used for determining the relation between  $R_F$  and the quantity of immobile phase on the plates.

### Results

*Dependence of  $R_F$  on the quantity of stationary phase.* Plates carrying the following quantities and types of stationary phases were studied.

- (a) 0.5, 2, 4 and 6 g of Carbowax 400 per 25 g of Kieselguhr G;
- (b) 1, 2, 3 and 5 g of 3,3'-oxydipropionitrile per 25 g of Kieselguhr G;
- (c) 1, 2, 4 and 6 g of 2-phenoxyethanol per 25 g of Kieselguhr G;
- (d) 0.25, 1.5, 4 and 6 g of paraffin oil per 25 g of Kieselguhr G.

Some of the results obtained are shown in Figs. 1 to 4 from which it is evident that, without exception, the  $\log R_F$  is linearly dependent on the quantity of stationary phase. This leads to the assumption that the correction equation of GALANOS AND KAPOULAS should be transformed as follows:

$$\log R_{F^c} = a \log R_F + b$$

$R_{F^c}$  = the corrected  $R_F$  value

$R_F$  = the  $R_F$  value found in the experiment.

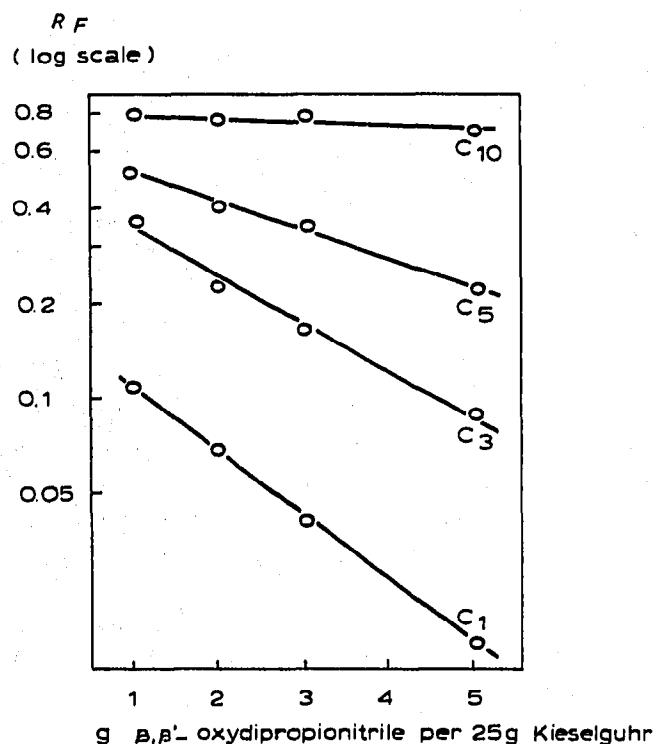


Fig. 1. Dependence of  $R_F$  on the quantity of stationary phase. Mobile phase, hexane; test compounds, 2,4-dinitrophenylhydrazones of  $n$ -alkanals.

TABLE I

$R_F^a$  VALUES OF SOME  $n$ -ALKANAL 2,4-DINITROPHENYLHYDRAZONES

Stationary phase, Carbowax 400; mobile phase, hexane.

2,4-Dinitrophenyl- hydrazone of	Grams of Carbowax per 25 g of Kieselguhr G			
	0.5	2	4	6
Ethanal	0.219	0.122	0.043	0.026
Propanal	0.374	0.218	0.104	0.070
Pentanal	0.547	0.365	0.265	0.174
Nonanal	0.826	0.730	0.625	0.608
Decanal	0.870	0.800	0.704	0.614
Dodecanal	0.904	0.860	0.817	0.765

<sup>a</sup> Means from three independent experiments.

TABLE II

$R_F^c$  VALUES CALCULATED FROM THE VALUES SHOWN IN TABLE I USING THE MODIFIED GALANOS AND KAPOULAS EQUATION

$R_F$  values on 2 g of Carbowax were taken as the standard values. Reference compounds, ethanal- and dodecanal-2,4-dinitrophenylhydrazone.

2,4-Dinitrophenyl- hydrazone of	Grams of Carbowax per 25 g Kieselguhr G				Mean $R_F$	Range	$s^a$
	0.5	2	4	6			
Propanal	0.26	0.22	0.22	0.19	0.22	0.07	0.033
Pentanal	0.43	0.37	0.41	0.43	0.41	0.06	0.029
Nonanal	0.76	0.73	0.72	0.71	0.73	0.05	0.024
Decanal	0.82	0.80	0.78	0.79	0.79	0.04	0.019

<sup>a</sup> Standard deviation calculated from the range.

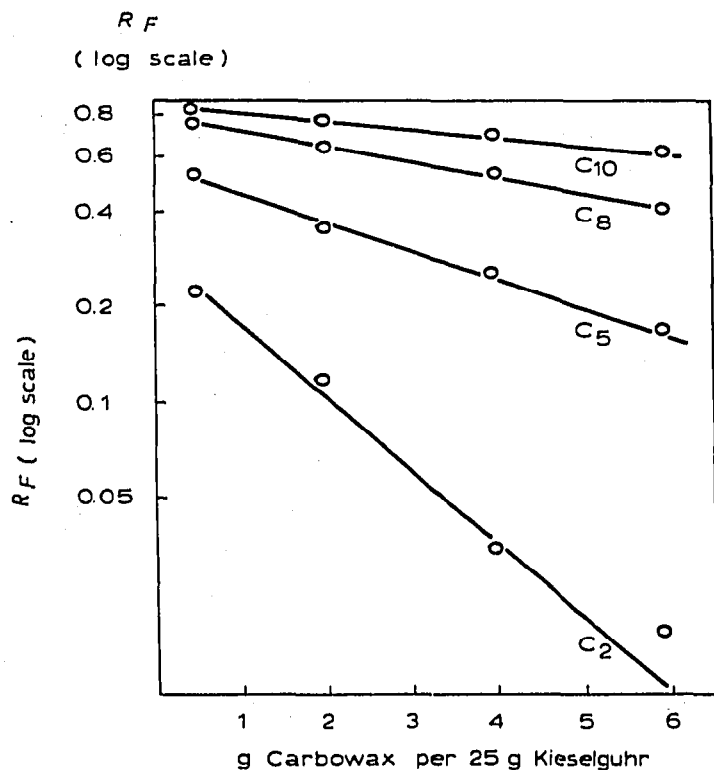


Fig. 2. Dependence of  $R_F$  on the quantity of stationary phase. Mobile phase, hexane; test compounds, 2,4-dinitrophenylhydrazones of *n*-alkanals.

The above formula has been used to correct a number of  $R_F$  values found on different quantities of stationary phase for one particular quantity. Tables I and II show the procedure followed.

Table I contains  $R_F$  values which were found on different quantities of Carbowax 400 in hexane as the mobile phase. From this table the values determined on 2 g of Carbowax 400 per 25 g of carrier material were adopted as the "standard" values. Subsequently, the values found on 0.5, 4 and 6 g of stationary phase were corrected, using the values of ethanal- and dodecanal-2,4-dinitrophenylhydrazone

as the reference  $R_F$  values in the modified GALANOS AND KAPOULAS equation. The results of these calculations are given in Table II, which shows that essentially constant  $R_F$  values are found in this way. As all the  $R_F$  values in the tables originate from one experimental observation, the reproducibility may be increased by taking mean values from different experiments.

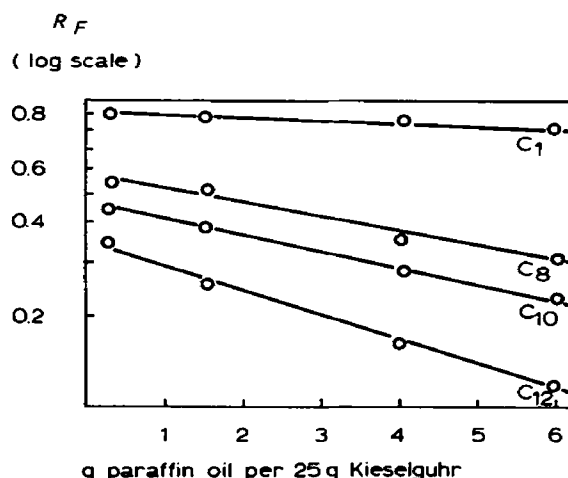
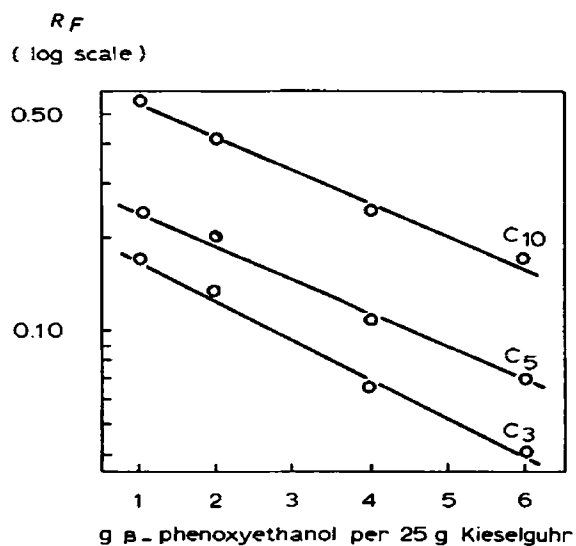


Fig. 3. Dependence of  $R_F$  on the quantity of stationary phase. Mobile phase, hexane; test compounds, 2,4-dinitrophenylhydrazones of  $n$ -alkanals.

Fig. 4. Dependence of  $R_F$  on the quantity of stationary phase. Mobile phase, acetone-water (3:1); test compounds, 2,4-dinitrophenylhydrazones of  $n$ -alkanals.

The results of the calculations of corrected  $R_F$  values in the other systems were comparable with those shown in Tables I and II.

In the 2-phenoxyethanol system it was found that, especially with greater quantities of stationary phase, multiple development must be used in order to obtain a satisfactory separation. By applying the formula

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

TABLE III

COMPARISON OF THE  ${}^1R_F^c$  VALUES ON DIFFERENT QUANTITIES OF 2-PHENOXYETHANOL AND AFTER A VARIOUS NUMBER OF DEVELOPMENTS

2,4-Dinitrophenyl- hydrazone of	Quantity of stationary phase (g/25 g Kieselguhr G)						
	5	5	2	2	2	6	6
Methanal	0.09	0.19	0.08	0.08	—	0.08	0.08
Pentanal	0.22	0.23	0.21	0.21	—	0.22	0.22
Undecanal	0.48	0.48	0.44	0.45	—	0.46	0.45
Pseudo-ionone	0.37	0.35	0.36	0.36	0.36	0.36	0.36
Number of developments	4	5	2	2	1	4	6

all the  $R_F$  values were calculated to give the values which should have been found after one development only, before being corrected by the modified equation. In this case constant  $R_F$  values were also obtained, as is shown in Table III.

#### *Conclusion*

Essentially constant  $R_F$  values can be expected in partition systems, provided that a linear relation can be found between  $R_F$  and variation in the quantity of the stationary phase. In the experiments described in this paper a linear relationship was found between the log  $R_F$  value and the quantity of stationary phase.

*Central Institute for Nutrition and Food Research TNO,  
Zeist (The Netherlands)*

J. H. DHONT  
J. C. DE BEAUVESER  
G. G. KUIJPERS

- 1 J. H. DHONT, C. VINKENBORG, H. COMPAAN, F. J. RITTER, R. P. LABADIE, A. VERWEY AND R. A. DE ZEEUW, *J. Chromatogr.*, 47 (1970) 376.
- 2 J. H. DHONT AND G. J. C. MULDER-DIJKMAN, *Analyst*, 94 (1969) 1090.
- 3 J. H. DHONT, G. J. C. MULDER-DIJKMAN, J. C. DE BEAUVESER AND G. G. KUIJPERS, *J. Chromatogr.*, 52 (1970) 429.

Received March 15th, 1971

*J. Chromatogr.*, 60 (1971) 265-269