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THE R_F VALUE AS A CONSTANT IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A comparison has been made of the results of a number of experiments concerning R_F values in thin-layer chromatography randomly chosen from the literature. The study led to the conclusion that in thin-layer chromatography a constant R_F value can be obtained.

INTRODUCTION

There are two fields in which the reproducibility of R_F values might be of special interest, *viz.*, in the relationship between chemical structure and chromatographic behaviour and in systematic qualitative analysis.

In 1968, a symposium was held on "Reproducibility of R_F values in thin-layer and paper chromatography". Most of the contributions dealt with questions concerning the rigid standardization of operating conditions. Lederer[1], in his introduction to the symposium, disagreed with this concept because simplicity is in fact the great advantage of thin-layer and paper chromatography. He also wondered whether "when compiling chromatographic data one can combine R_F values by various authors into one table". During the same symposium, Hais[2] expressed the opinion that "no serious worker would consider it more than a dream to pool R_F values obtained in different laboratories".

Although it is still often questioned whether published R_F values can be reproduced, extensive inter-laboratory experiments have shown that these values can be made constant with the aid of a simple correction equation[3–5].

In this paper, the Galanos and Kapoulas equation[6] is compared with the equation recently developed by Van Wendel de Joode *et al.*[7]. The data used in the calculations have been taken from published tables.

CORRECTION EQUATIONS

One of the first attempts to arrive at a constant R_F value was the introduction of the relative R_F value, defined as[8]

$$R_X = \frac{R_F\text{ (compound)}}{R_F\text{ (reference compound)}} \tag{1}$$

The drawback of this procedure is that it is based on the supposition that the line representing the variations which cause the R_F value to deviate can be expressed by a straight line passing through the origin of the coordinate system, whereas this seldom occurs in practice.

Williams[9] formulated the following equation, containing factors that would correct R_F values for different experimental conditions:

$$R_{cp} = R_c + \alpha_p + \beta_p R_c + \varepsilon \tag{2}$$

where R_{cp} is the observed R_M of compound c on plate p, α_p and β_p are constants characterizing plate p, ε is the residual variation and R_c is the "ideal" R_M value of compound c in the system that one is trying to reproduce. This equation shows the additive character of the constants describing the variable conditions in practical chromatography and contains only extramolecular factors.

An important move towards the solution of the problem of obtaining constant R_F values was made by Galanos and Kapoulas[6]. They classified the variations caused by experimental conditions according to the mobility of compounds on chromatograms into the following groups:

(1) Variations that do not change the R_F values themselves, but the distances of the spots from the starting point. This type of variation is not contradictory to the postulate of a constant R_F value, *i.e.*

$$R_F^0 = R_F$$

where R_F^0 and R_F represent "tabular" and measured R_F values, respectively. The term "tabular" R_F values defines a set of R_F values, either given in the literature or determined in the lbaoratory, that are taken as models to be reproduced.

(2) Variations that change the R_F values but not the ratios of the distances of the spots from the starting point. Variations of this type can be expressed as follows:

$$R_F^0 = aR_F$$

(3) Variations that change both the individual R_F values and the ratios of the distances of the spots from the starting point.

Galanos and Kapoulas found the following equation to hold in practice:

$$R_F^0 = aR_F + b \tag{3}$$

One of the postulates on which the equation is based is that all R_F variations are additive properties, while the constants a and b are the sums of a number of individual constants $a^1, a^2, a^3, \ldots, a^n$ and $b^1, b^2, b^3, \ldots, b^n$, respectively. The constants a and b can be calculated by simple analytical geometry, provided that experimental R_F values are determined from authentic samples:

$$a = \frac{R_{FA}^0 - R_{FB}^0}{R_{FA} - R_{FB}}$$

$$b = R_{FA}^0 - aR_{FA}$$

where R_{FA}^0 and R_{FB}^0 are the tabular R_F values and R_{FA} and R_{FB} the measured R_F values of two reference compounds A and B. The basis of this equation is, in fact, very similar to that of eqn. 1. The equation of Galanos and Kapoulas has been tested in detail in inter-laboratory investigations[3–5].

Recently, Van Wendel de Joode *et al.*[7] derived an equation from thermodynamic principles:

$$\frac{1}{R_F^0} = \frac{a}{R_F} + b \tag{4}$$

The correction procedure is analogous to that of eqn. 3:

$$a = \left(\frac{1}{R_{FA}^0} - \frac{1}{R_{FB}^0}\right) \Big/ \left(\frac{1}{R_{FA}} - \frac{1}{R_{FB}}\right)$$

$$b = \frac{1}{R_{FA}^0} - \frac{a}{R_{FA}}$$

In this paper the results obtained with eqns. 3 and 4 are compared with the tabular R_F values.

RESULTS AND DISCUSSION

Geiss[10] published R_F values of some dyes on silica gels of different origin and under conditions of different relative humidity (63 and 79%). The R_F values obtained in the experiments were recalculated with the aid of eqns. 3 and 4 and by arbitrarily taking one set of R_F values obtained on silica gel G (Merck, Darmstadt, G.F.R.) as the R_F^0 values. The R_F^0 values are indicated as tabular and the calculated ones as corrected (R_F^c) values. The R_F^0 values of butter yellow and *p*-hydroxyazobenzene were applied as reference values for the calculation of the constants a and b in the equations. As these values were taken from the graph in the paper by Geiss[10], a slightly greater error might be expected than that occurring when tabular values are used. Table I shows the results [note: in the tables, (p.d.) stands for "per definition", R_{F3} for calculated R_F values from eqn. 3 and R_{F4} for those obtained when using eqn. 4].

TABLE I

R_F^0 VALUES, MEAN R_F VALUES AND STANDARD DEVIATIONS (s) FOR SOME DYES

Dye	$\overline{R_F}$*	s	R_{F3}^c	s	R_{F4}^c	s	R_F^0
Butter yellow	0.778	0.098	0.730	0(p.d.)	0.730	0(p.d.)	0.73
Impurity	0.692	0.143	0.649	0.057	0.639	0.031	0.63
Sudan red	0.508	0.184	0.465	0.097	0.437	0.047	0.40
Indophenol	0.377	0.175	0.332	0.077	0.301	0.031	0.29
Sudan black	0.265	0.169	0.221	0.065	0.196	0.023	0.19
p-Hydroxyazobenzene	0.185	0.119	0.13	0(p.d.)	0.13	0(p.d.)	0.13

* Mean R_F values taken from Geiss's paper[10].

The absolute differences between the tabular and the calculated R_F values are given in Table II.

TABLE II

ABSOLUTE DIFFERENCES BETWEEN TABULAR AND CALCULATED R_F VALUES

Dye	$R^0_F - R^c_{F3}$	$R^0_F - R^c_{F4}$
Butter yellow	0	0
Impurity	0.019	0.009
Sudan red	0.065	0.037
Indophenol	0.042	0.01
Sudan black	0.031	0.006
p-Hydroxyazobenzene	0	0

Tables I and II show that the application of eqn. 4 results in smaller deviations from the tabular values than that of eqn. 3, standard deviations for eqn. 4 are smaller than for eqn. 3, and R_F values can be reproduced to within 0.03 R_F unit, even under the widely different conditions of Geiss's[10] experiments.

Różyło[11] investigated the change in R_F values on layers of silica gel of different porosity, with mixtures of carbon tetrachloride and chloroform as eluents. The mean R_F values and the standard deviations are given in Table III.

TABLE III

MEANS AND STANDARD DEVIATIONS (*s*) OF ORIGINAL AND CALCULATED R_F VALUES OBTAINED ON SILICA GEL LAYERS OF DIFFERENT POROSITY

Solvent: 0.3 molar fraction $CHCl_3$ in CCl_4.

Compound	$\overline{R_F}$	*s*	$\overline{R^c_{F3}}$	*s*	$\overline{R^c_{F4}}$	*s*	R^0_F
1-Naphthol	0.373	0.200	0.347	0.036	0.341	0.015	0.36
2-Naphthol	0.25	0.221	0.250	0(p.d.)	0.250	0(p.d.)	0.25
Quinoline	0.230	0.104	0.275	0.047	0.262	0.043	0.29
8-Hydroxyquinoline	0.473	0.221	0.473	0.089	0.469	0.48	0.41
8-Methylquinoline	0.433	0.198	0.420	0.042	0.414	0.011	0.42
Carbazole	0.583	0.160	0.600	0.025	0.590	0.026	0.57
Fluorenone	0.620	0.089	0.620	0(p.d.)	0.620	0(p.d.)	0.62

The R_F values obtained on a silica gel of porosity 200 Å were arbitrarily used as the R^0_F values. The reference values $R^0_{F(\text{2-naphthol})} = 0.25$ and $R^0_{F(\text{fluorenone})} = 0.62$ were applied to calculate R^c_{F3} and R^c_{F4}. All original data show large deviations from the mean. Satisfactory results, however, are obtained with eqn. 4. The standard deviations of R^c_{F4} are smaller than those of R^c_{F3}. Table IV shows the absolute differences between the tabular and the calculated R_F values.

TABLE IV

ABSOLUTE DIFFERENCES BETWEEN TABULAR AND CALCULATED R_F VALUES

Compound	$R^0_F - R^c_{F3}$	$R^0_F - R^c_{F4}$
1-Naphthol	0.013	0.019
2-Naphthol	0(p.d.)	0(p.d.)
Quinoline	0.015	0.028
8-Hydroxyquinoline	0.063	0.059
8-Methylquinoline	0	0.006
Carbazole	0.030	0.020
Fluorenone	0(p.d.)	0(p.d.)

Again, in both instances a large increase in reproducibility was achieved. In this experiment both equations give the same tabular values, within certain limits.

Changes in R_F values caused by varying amounts of stationary phase in thin-layer partition chromatography can also be corrected by using eqn. 3 or 4. Graham *et al.*[12] prepared plates from cellulose slurries containing different amounts of formamide. In Table V some of the results they obtained on plates containing 0.5, 3.0 and 6.0 *M* formamide are recorded.

TABLE V

R_F VALUES FOR SOME PHENOLS ON CELLULOSE PLATES CONTAINING DIFFERENT AMOUNTS OF FORMAMIDE

Phenol	*Formamide concentration in slurry (M)*		
	0.5	*3.0*	*6.0*
2-Methylphenol	0.40	0.13	0.08
3,4-Dimethylphenol	0.38	0.125	0.06
2,6-Dimethylphenol	0.78	0.53	0.36
2,5-Dimethylphenol	0.63	0.265	0.14
2,3,4,6-Tetramethylphenol	0.92	0.80	0.72

When the values found on 3.0 *M* formamide are taken as the R^0_F values and $R^0_{F(\text{2-methylphenol})} = 0.13$ and $R^0_{F(\text{2,3,4,6-tetramethylphenol})} = 0.80$ as the reference values, the results given in Table VI are obtained.

TABLE VI

MEANS AND STANDARD DEVIATIONS (*s*) OF ORIGINAL AND CALCULATED R_F VALUES

Compound	$\overline{R_F}$*	*s*	$\overline{R^c_{F3}}$	*s*	$\overline{R^c_{F4}}$	*s*
2-Methylphenol	0.203	0.172	0.13	0(p.d.)	0.13	0(p.d.)
3,4-Dimethylphenol	0.188	0.196	0.113	0.011	0.115	0.01
2,6-Dimethylphenol	0.557	0.211	0.495	0.032	0.524	0.006
2,5-Dimethylphenol	0.345	0.255	0.296	0.031	0.255	0.034
2,3,4,6-Tetramethylphenol	0.813	0.103	0.800	0(p.d.)	0.800	0(p.d.)

* Calculated from Table V.

The deviations of the calculated R_F values (Table VI) from the tabular values are given in Table VII.

TABLE VII

ABSOLUTE DIFFERENCES BETWEEN TABULAR AND CALCULATED R_F VALUES

Compound	$R^0_F - R^c_{F3}$	$R^0_F - R^c_{F4}$
2-Methylphenol	0	0
3,4-Dimethylphenol	0.012	0.01
2,6-Dimethylphenol	0.035	0.006
2,5-Dimethylphenol	0.031	0.010
2,3,4,6-Tetramethylphenol	0	0

CONCLUSION

The problem of the inter-laboratory comparison of R_F values in TLC would seem to have been solved[3-5]. Compounds can be (tentatively) identified by a set of determined or already published R_F values. It is not necessary to run authentic samples along with the compounds to be identified. The R_F value can be handled as a constant, which is expecially useful when the compounds are difficult to obtain.

In this paper, both eqn. 3 and eqn. 4 have been shown to give reasonably reliable results, even on layers of different porosity, on layers containing different amounts of stationary phase or under conditions of different relative humidity. Eqn. 4 seems to give the smallest deviations.

In order to arrive at constant R_F values, the following precautions have to be taken: use solvents of known composition; run at least two easily obtainable reference compounds on every chromatogram, preferably with R_F values of about 0.1 and 0.9; run a "pilot" compound[*] as a control on every chromatogram, the R_F value of which should lie between *ca.* 0.3 and 0.8; the chromatogram should not be taken into consideration for the recording of R_F values if the R_F value of this compound differs by more than 0.03 R_F unit from its tabular value.

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[*] A pilot compound is used in order to detect irregularities in the thin-layer chromatogram that might influence the accuracy of the R_F measurement.