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A MATHEMATICAL TREATMENT OF THE VARIATION OF THE R_F VALUE CAUSED BY THE PRESENCE OF ANOTHER COMPONENT

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SUMMARY

The R_F value of a component (A) of a mixture is influenced by the presence of another component (B) if the latter alters the distribution coefficient, α_A. The results obtained show that ΔR_M (the difference between the R_M values in the presence and absence of the other component) is directly proportional to the amount of the other component and can be given by the equation:

$$\Delta R_M = K.m_B$$

where K is a constant and m_B is the amount of component B.

This rule can be used to obtain real $R_{F(0)}$ values, by determining $R_{M(t)}$ values for component A in the presence of various amounts of component B and then extrapolating to zero.

The presence of other components in a mixture may have a great effect on the reproducibility of R_F values for various substances. It is, however, impossible to anticipate this influence. The mathematical treatment of this problem is interesting and it can help in chromatographic work since by mathematical extrapolation it is possible to obtain the correct R_F value.

PRINCIPLE

According to the equation of MARTIN AND SYNGE[1], the R_F value is a function of the distribution coefficient α ($\alpha = C_S/C_M$) and the ratio of the quantity of the cross sectional areas occupied by the mobile and stationary phases (A_M/A_S). In the ideal case the following relation holds:

$$\frac{1}{R_F} = \frac{A_M}{A_S} \cdot \alpha + 1 \tag{1}$$

The purpose of this work was to study the change in the R_F value caused by the

presence of another component. The functional dependence of ΔR_F may be given by the equation:

$$\Delta R_F = F\left[\Delta\alpha,\ \Delta\left(\frac{A_M}{A_S}\right),\ \Delta d_s\right] \tag{2}$$

where

$\Delta\alpha$ = the change in α,
$\Delta(A_M/A_S)$ = the change in the ratio A_M/A_S,
d_s = the variation of the layer thickness.
Supposing that $\Delta(A_M/A_S) \rightarrow 0$ and $\Delta d_s \rightarrow 0$, eqn. (2) becomes:

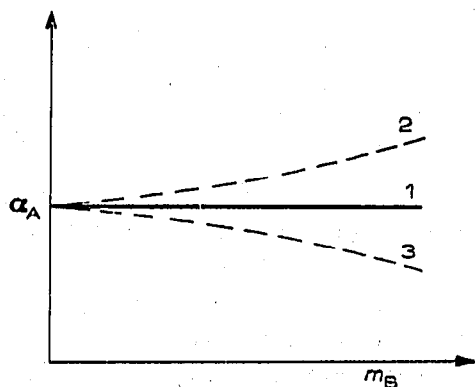$$\Delta R_{F(A)} = f(\Delta\alpha_A) \tag{3}$$

Fig. 1. The influence of component B on α_A. (1) Component B does not effect α_A; (2) component B causes an increase in α_A; (3) component B causes a decrease in α_A.
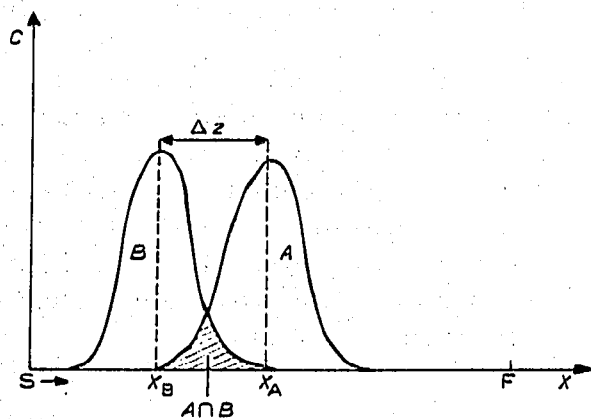

Fig. 2. Diagram showing the ratio of the amount of component B that influences component A $(A\cap B/A)$.

The distribution coefficient α_A for a substance A depends on the chromatographic system. The presence of another component, B, changes the properties of the system and thus can also change α_A, as is shown in Fig. 1. Analogously component A can change α_B.

At the start the influence of component B on α_A is greatest, but as the chromato-

gram develops this influence dimishes because of the increasing distance between the components.

Theoretically, four cases are possible, *viz.*:

(1) If component B (low R_F value) influence component A (high R_F value) so that α_A increases, the time of overlapping of the components during the chromatographic process is prolonged.
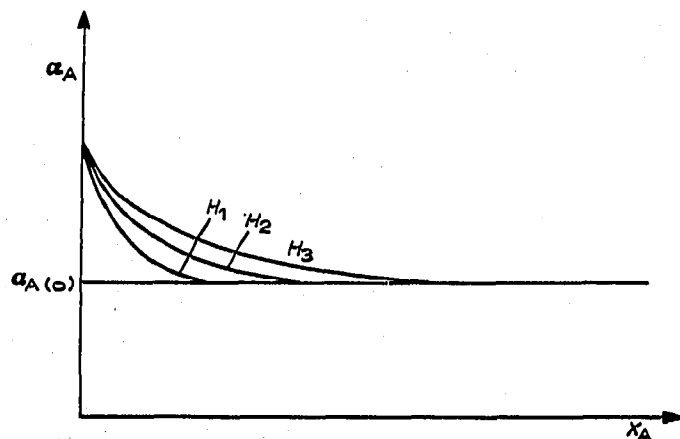

Fig. 3. The change in α_A during the chromatographic process for three HETP values ($H_1 < H_2 < H_3$).

(2) If component B decreases α_A, the time of overlapping is shortened.

(3) If component A increases α_B, the time of overlapping is shortened.

(4) If component A decreases α_B, the time of overlapping is prolonged.

In cases 1 and 4, we have the phenomenon of "spot approaching" and in cases 2 and 3, the phenomenon of "spot repulsion".

In Fig. 2, the area $A \cap B$ represents the amount of component B that influences component A, and *vice versa*. With a decrease in the ratio $(A \cap B)/A$ or $(A \cap B)/B$, this influence becomes smaller.

The sharpness of the spot is directly proportional to the number of theoretical plates, or inversely proportional to the HETP value for each component. When the number of theoretical plates increases, for the same distance between the peaks for components A and B, the ratio $(A \cap B)/A$ diminishes, because the peaks are narrower.

Fig. 3 shows the change in α_A during the chromatographic process, when the ratio of the amounts of components A and B is constant, for three HETP values: $\text{HETP}_1 < \text{HETP}_2 < \text{HETP}_3$. The curvature of the lines decreases with the increase in HETP value, and the differential form can be given by the expression:

$$\left(\frac{d^2\alpha}{dx^2}\right)_{H_1} > \left(\frac{d^2\alpha}{dx^2}\right)_{H_2} > \left(\frac{d^2\alpha}{dx^2}\right)_{H_3} \tag{4}$$

The real R_F value is a function of $\bar{\alpha}$, which is obtained from the following equation:

$$\bar{\alpha} = \frac{\int_s^{x_A} f(\alpha)\,dx}{x_A} \tag{5}$$

Substitution of the expression for $\bar{\alpha}$ in the equation of MARTIN AND SYNGE gives:

$$\varDelta R_F = \frac{A_M}{A_S}\left[\alpha_A - \frac{\int_s^{x_A} f(\alpha_A)\mathrm{d}x}{x_A}\right] R_F \cdot R_F' \tag{6}$$

In mathematical calculations it is better to convert the R_F values into R_M values, because the relation for the latter values is linear:

$$\varDelta R_M = \log|\alpha| - \log|\bar{a}| \tag{7}$$

or

$$a = \bar{a} \cdot e^{-\varDelta R_M} \cdot {}^{2,3} \tag{8}$$

From the diagram in Fig. 3 it can be concluded that the difference between $\bar{a}$ and α diminishes when the plate number increases. By extrapolation it is possible to obtain α:

$$a = \lim_{\varDelta R_M \to 0} \bar{a} \tag{9}$$

As is shown in Fig. 4, and in accordance with eqn. (8), the approximate relation between $\varDelta R_{M(A)}$ and the amount of component B, M_B, can be given by eqn. (10), in which K is a constant:

$$\varDelta R_{M(A)} = K m_B \tag{10}$$

or

$$R_{M(0)(A)} - R_{M(A)} = K m_B$$

which is valid for all four cases.

From eqn. (10) it is evident that if $R_{M(t)(A)}$ values for various values of $m_{t(B)}$ are empirically determined, the $R_{M(0)}$ value can be obtained by means of statistical methods (either graphical or mathematical):

$$R_{M(0)} = \frac{(\varSigma R_{M(t)}) \cdot (\varSigma m_t{}^2) + (\varSigma R_{M(t)} \cdot m_t) \cdot (\varSigma m_t)}{N(\varSigma m_t{}^2) - (\varSigma m_t)^2} \tag{11}$$

where N is the number of measurements,

$R_{M(0)}$ is converted into the standard $R_{F(0)}$ value, by means of a conversion table or the equation $R_M = \log[1/R_F - 1]$.

TABLE I

FIRST PLATE

Amounts in μg.

	I	II	III	IV	V	VI
Component A	10	10	10	10	10	10
Component B	2	5	8	10	15	20

TABLE II

SECOND PLATE

Amounts in μg.

	I	II	III	IV	V	VI
Component A	2	5	8	10	15	20
Component B	10	10	10	10	10	10

EXPERIMENTAL

The experiments were performed according to the usual method[2]. On the plates (20 × 20 cm) the adsorbent (Silica Gel H; thickness, 0.25 mm) was activated at 120° for 20 min. The chromatograms were developed with toluene. The substances investigated were: component B—Sudan Red III (R_F 0.220); component A—Fettrot (R_F 0.403). The mixture was applied in the ratios given in Tables I and II.

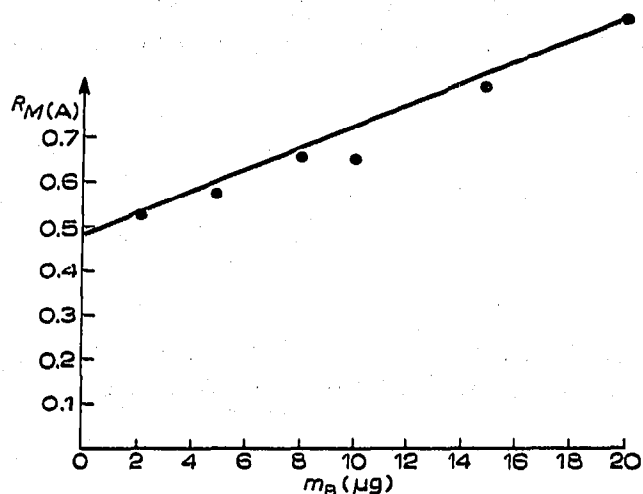The R_M values obtained are given in Fig. 4, where it can be seen that the change

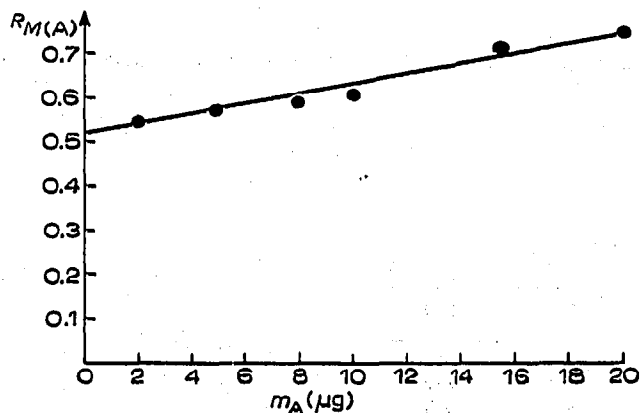Fig. 4. Change in $R_{M(A)}$ *versus* amount of component B.

Fig. 5. Change in $R_{M(A)}$ *versus* amount of component A.

in $R_{M(A)}$ is proportional to the amount of component B, thus establishing eqn. (10).

Fig. 5 shows that when the amount of component B is kept constant, the change in the R_M value is proportional to the amount of component A, because the ratio $(A \cap B)/A$ increases with the increase in the amount of component A.
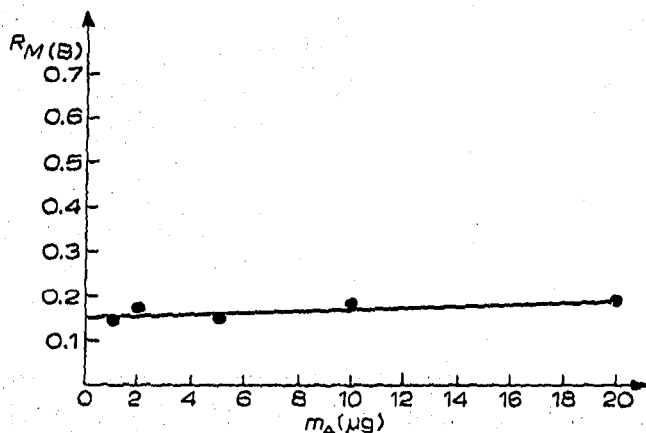

Fig. 6. Diagram showing that the presence of component A does not influence $R_{M(B)}$.
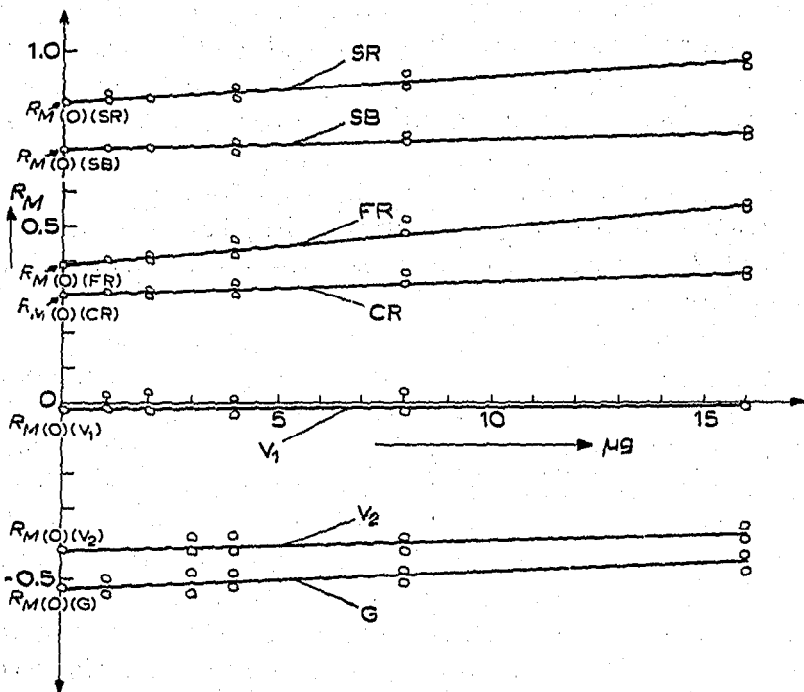

Fig. 7. Extrapolation method for calculation of the $R_{M(0)}$ values of seven components of a mixture. For abbreviations, see Table III.

The presence of component A does not affect the distribution coefficient, α_B, as is shown in Fig. 6. The effect of component A on component B was described previously as case (3).

The R_M values obtained are given in Fig. 7. The corresponding $R_{M(0)}$ values have been converted into $R_{F(0)}$ values, which are given in Table III.

TABLE III

THIRD PLATE

Amounts in μg.

Substances	I	II	III	IV	V	$hR_{F(0)}$
Sudan Red III (SR)	16	8	4	2	1	12.4
Sudan Blue (SB)	16	8	4	2	1	16.0
Fettrot (FR)	16	8	4	2	1	29.0
Ceres Red (CR)	16	8	4	2	1	33.3
Sudan Violet (V$_1$)	16	8	4	2	1	51.2
Sudan Violet (V$_2$)	16	8	4	2	1	72.6
Fettreingelb (G)	16	8	4	2	1	77.2

DISCUSSION AND CONCLUSION

The working conditions should be constant throughout, *e.g.* the areas of the applied spots, the saturation of the chamber and the uniformity of the layer. It is important to keep the area of the applied spot constant, which is possible only by always applying the same volume of solution. The saturation of the chamber should also be complete in order to avoid edge effects.

The above arguments are only valid when the zone width is so small that the distance substance A has to go to traverse substance B is negligible. Obviously, if the zone is wider (*e.g.* in preparative work), not only the R_F value but also the shape of the spot will change because the tail of zone A will remain in contact with zone B much longer than the front.

This method can be adopted not only to obtain the "real" R_F value, but also to standardize the R_F value of compounds which change on increasing the amount of compound. In this case the standard R_F value can be given as $R_{F(0)}$ (when the amount of the compound tends toward zero).

REFERENCES

1 A. J. P. MARTIN AND R. L. M. SYNGE, *Biochem. J.*, 35 (1941) 1358.
2 E. STAHL, *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin, 1962.