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 $\Delta R_{M(\text{stand})}$ AS A RELIABLE VALUE FOR IDENTIFICATION PURPOSES
IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

This report experimentally confirmed the possibility of calculating the R_M value of a substance on different layers if the following data are known: R_M value of the reference substance on the standard layer; $\Delta R_{M(\text{stand. subst.})}$ of a substance on the standard layer and $\Delta R_{M(\text{stand. layer})}$ for the standard substance.

For some carbohydrates, characteristic deviations from calculated R_M values appeared on different layers.

INTRODUCTION

The R_F value is used for the characterisation of a substance by the position of its spot in a given chromatographic system. According to MARTIN AND SYNGE'S¹ equation

$$\alpha = \frac{A_M}{A_S} \left(\frac{1}{R_F} - 1 \right) \quad (1)$$

the R_F value depends on two factors: the partition coefficient, α , and the A_M/A_S value. It is possible to reproduce the partition coefficient satisfactorily by strict adherence to the given working conditions. On the other hand, it is almost impossible (in TLC) to control the A_M/A_S factor (the ratio of the cross-sectional areas of the mobile and stationary phase). This factor depends on the dimensions of the adsorbent particles, *i.e.* on the packing density of the adsorbent in the layer.

On the basis of the above considerations good reproducibility of R_F values in TLC cannot be expected, and thus the possibility of the identification of different substances by means of tabulated R_F values is appreciably diminished.

As a result of this some authors tried to introduce the $R_{(\text{stand})}$ value (the ratio between the R_F value of a substance A and the R_F value of a reference substance), but the introduction of such an expression could not eliminate the effects of variations in the A_M/A_S ratio on the $R_{(\text{stand})}$ values under experimental conditions. BATE-SMITH AND WESTALL² proposed a function which eliminates this A_M/A_S factor.

$$R_M = \log \left(\frac{1}{R_F} - 1 \right) \quad (2)$$

For two substances A and B differing by a group X (*i.e.* B = AX), it was established by MARTIN that:

$$\log \alpha_B - \log \alpha_A = \Delta \log \alpha_X = K_X \quad (3)$$

where K_X is a constant characteristic for group X in the respective chromatographic system. Substituting in eqn. 1, MARTIN's equation is transformed into the following:

$$K_X = \left[\log \frac{A_M}{A_S} + \log \left(\frac{1}{R_{FB}} - 1 \right) \right] - \left[\log \frac{A_M}{A_S} + \log \left(\frac{1}{R_{FA}} - 1 \right) \right] \quad (4)$$

or in terms of R_M

$$K_X = R_{MB} - R_{MA} = \Delta R_{MX} \quad (5)$$

Other authors³⁻⁵ later established that characteristic ΔR_M values apply not only to different substituents on the same molecular skeleton but also to the changes of temperature, pH, solvent composition, etc. The present report is an attempt to investigate the possibility of expressing the dependence of the ΔR_M value on the kind of adsorbent used.

EXPERIMENTAL

Plates

The following pre-coated (ready for use) plates were tested: Silica Gel F₂₅₄, Alumina F₂₅₄, and Cellulose F, all manufactured by Merck; ChromAR Sheet 500 (Mallinckrodt); and Eastman-Kodak Chromagram Sheets of Cellulose F, silica gel, alumina, Alumina F, polycarbonate and polyamide.

Samples

Samples tested are D-glucose, D-fructose, L-sorbose, D-xylose and sucrose. Samples were dissolved in methanol (1% w/v).

Chromatography

Application of the samples on the starting line was performed by an Autoliner (Desaga). Pressure, 0.8 kp/cm²; speed, 10; gouttex-control, 7; band-width, 0.3-0.6 mm.

Ascending chromatography was performed in a saturated chromatographic chamber for preparative purposes (Desaga) at room temperature.

Solvent mixture was ethyl acetate-acetic acid-water (3:2:3)⁶. The developing mixture was allowed to ascend 10 cm.

Visualisation was done with (a) phenol-sulphuric acid reagent⁷ or (b) 1,2-dihydroxynaphthalene-phosphoric acid reagent⁸.

RESULTS AND DISCUSSION

The R_M values and corresponding ΔR_M values were calculated from the measured R_F values. D-Glucose and cellulose pre-coated plates (Merck) were chosen as reference standards. The results for the various substances on the different layers are given in Tables I-III.

TABLE I

 R_M VALUES OF SOME CARBOHYDRATES ON DIFFERENT LAYERS USING THE SAME SOLVENT MIXTURE⁰

| Layer | Glucose | Fructose | Sorbose | Xylose | Sucrose |
|-------------------------|---------|----------|---------|--------|---------|
| Cellulose (Merck) | +0.027 | -0.014 | +0.003 | -0.074 | +0.052 |
| Silica gel (Merck) | -0.248 | -0.284 | -0.267 | -0.345 | -0.220 |
| Alumina (Merck) | -0.087 | -0.129 | -0.113 | -0.188 | -0.063 |
| Cellulose (Eastman) | -0.448 | -0.487 | -0.472 | -0.550 | -0.421 |
| Silica gel (Eastman) | -0.310 | -0.355 | -0.336 | -0.415 | -0.286 |

TABLE II

 ΔR_M (stand. glucose) VALUES OF SOME CARBOHYDRATES ON DIFFERENT LAYERS USING THE SAME SOLVENT MIXTURE⁰

| Layer | Glucose (standard) | Fructose | Sorbose | Xylose | Sucrose |
|-------------------------|-----------------------|----------|---------|--------|---------|
| Cellulose (Merck) | 0.0 | -0.041 | -0.024 | -0.101 | +0.025 |
| Silica gel (Merck) | 0.0 | -0.036 | -0.019 | -0.097 | +0.028 |
| Alumina (Merck) | 0.0 | -0.042 | -0.026 | -0.101 | +0.024 |
| Cellulose (Eastman) | 0.0 | -0.039 | -0.024 | -0.102 | +0.027 |
| Silica gel (Eastman) | 0.0 | -0.045 | -0.026 | -0.105 | +0.024 |

TABLE III

 ΔR_M (stand. cellulose (Merck)) VALUES OF SOME CARBOHYDRATES ON DIFFERENT LAYERS USING THE SAME SOLVENT MIXTURE⁰

| Layer | Glucose | Fructose | Sorbose | Xylose | Sucrose |
|-------------------------------|---------|----------|---------|--------|---------|
| Cellulose (stand.) (Merck) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Silica gel (Merck) | -0.275 | -0.270 | -0.270 | -0.271 | -0.272 |
| Alumina (Merck) | -0.114 | -0.115 | -0.116 | -0.114 | -0.114 |
| Cellulose (Eastman) | -0.475 | -0.473 | -0.475 | -0.476 | -0.473 |
| Silica gel (Eastman) | -0.337 | -0.341 | -0.339 | -0.341 | -0.338 |

It is possible to calculate the R_M value of a substance Y on a layer Z in the given solvent mixture when the following data are known: a , $R_{M(\text{stand.subst.})}$ on the standard layer; b , $\Delta R_{M(\text{stand.subst.})}$ of the substance Y on the standard layer; and c , $\Delta R_{M(\text{stand.layer})}$ for the standard substance on the layer Z.

Example

Standard substance: D-glucose

Standard layer: cellulose pre-coated plate (Merck)

Substance Y: D-xylose

Layer Z: silica gel pre-coated plate (Eastman)

$$a = +0.027 \quad b = -0.101 \quad c = -0.337$$

By substituting these values into the expression:

$$R_{M(YZ)} = b + c - a$$

one obtains $R_{M(YZ)} = -0.411$

which only slightly differs from the experimental value:

$$R_{M(YZ)}(\text{exp}) = -0.415$$

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DISCUSSION

BRENNER: The use of the quantities R_M and ΔR_M implies that the underlying R_F values are physically meaningful. A given spot to be treated by the R_M concept should have migrated throughout in a mobile phase of constant composition extending from the start beyond the position of the spot. Only under these conditions are R_F values between 0.3 and 0.7 liable to yield useful R_M figures and R_F has to be measured with respect to the slowest moving solvent front when the mobile phase in the solvent chamber is a mixture.

BALENOVIĆ: Certainly what you say is true and our treatment could sometimes bring erroneous results. In the systems we examined, however, the effectiveness of the treatment was apparent. Every time we made some predictions, they were always found to coincide with the actual results, within the experimental errors.

HAIŠ: If you chromatographed substances belonging to different chemical

classes and if the carrier sorbent (silica gel, cellulose) played an active role in the distribution process, then your formula would be hardly applicable. It is not of general validity and deviations from it (greater differences between calculated and found values) could indicate an active participation of the carrier.

BALENOVIĆ: I share your opinion. The behaviour of substances described was experimentally proved only in our particular case, with substances which belong to the same chemical class.

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