REPRODUCIBILITY OF R_k VALUES ON SILICA GEL IN THIN LAYER CHROMATOGRAPHY

M. S. J. DALLAS

Unilever Research Laboratory, The Frythe, Welwyn, Herts. (Great Britain)

SUMMARY

In thin layer adsorption chromatography R_F values and relative R_F values can be strongly dependent on adsorbent activity, which in turn can depend on the laboratory humidity.

A few experiments have shown that in general R_k values (where $R_k = R_M$ of sample substance minus R_M of standard on the same plate) are appreciably less dependent on adsorbent activity and grade, as claimed by BRENNER, PATAKI AND NIEDERWIESER. In particular, results with triglycerides on silica gel impregnated with $AgNO_3$ are described.

It has been pointed out by BRENNER et al.[1] that the function R_k, which is defined by the equation

$$(R_M)_i - (R_M)_{st} = (R_k)_{i/st} = \log (K_{st}/K_i)$$

where

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

st = some common standard or marker substance

i = some other substance examined under the same chromatographic conditions

is more reproducible than either R_F values or relative R_F values.

There is no doubt that R_F values for the less polar substances are quite strongly dependent on the activity of the adsorbent. It has also been shown[2] that relative R_F values are similarly dependent on adsorbent activity.

REICHEL[3] has shown that, in the thin layer chromatography of insecticides by the normal procedure, R_F values are quite strongly affected by the humidity of the laboratory atmosphere. But it is time consuming and not very convenient to bring the layer to a precise degree of activity[2,4-6] before development of the chromatogram. In the circumstances it was relevant to see to what extent R_k values are independent of the activity of the adsorbent.

If R_k values are independent of adsorbent activity over a wide range, then one may conclude that the mean energy of adsorption of a molecule of solute on the adsorbent surface is constant over a wide range of adsorbent activity.

R_F values have been measured here for certain simple dyes chromatographed on silica gel in benzene under a variety of controlled conditions and it is clear that the R_k values show considerably less relative variation than the R_F values themselves.

In particular the R_F values of certain glycerides have been examined under several different conditions. The results show that the R_k values can be independent of adsorbent activity over a useful range. It may also be seen, however, that in this case the R_k values are appreciably affected by temperature. It may also be added that the R_k values were greatly affected by the ratio of $AgNO_3$ to silica gel in the particular system described here.

TABLE I

R_k VALUES AND RELATIVE R_F VALUES (R_x) FOR TRIGLYCERIDES ON $AgNO_3$ IMPREGNATED SILICA GEL: EFFECT OF BOTH HUMIDITY AND TEMPERATURE

Plates developed in S-tank in benzene; adsorbent Silica Gel G–$AgNO_3$ (1:4).

Triglyceride[a]	Humidity				Temperature			
	15%		58%		10°		23°	
	R_x	R_k	R_x	R_k	R_x	R_k	R_x	R_k
SOS	58.5	+0.59	78	+0.60	51	+0.61	69.5	+0.57
OPS	49.5	+0.71	71	+0.73	39	+0.77	60.5	+0.69
SOO	23	+1.16	44	+1.16	17	+1.22	33	+1.13
OOO	7.5	+1.72	20	+1.67	3.5	+1.93	9.5	+1.78
SSS	100	0	100	0	100	0	100	0
R_F value SSS	0.755		0.905		0.685		0.84	

[a] O = Oleyl, P = palmityl, and S = stearyl.

This system was examined because it is apparently an adsorption system, where the R_F values are known to be greatly influenced by humidity and thus by the moisture content of the adsorbent. The results in Table I show clearly that in this case the R_k values are much more independent of environment than the relative R_F values, though the latter are often said to be more reproducible than the R_F values, from which they are derived.

Some cases have been found where the R_k values at constant temperature are influenced appreciably by the activity of the adsorbent, but these are cases of adsorption chromatography, where compounds of different class or structure have been compared.

It is probable, in general, that best results will be obtained where the substance chosen as standard has a structure fairly similar to that of the test substances. It should also be added that chromatographic conditions should be such as to ensure a constant ratio and composition of mobile phase along the whole of the distance of development[7].

Further tests will be necessary to see to what extent temperature control is necessary. It is clear from the work of KLEIN[8] that the pore size of the adsorbent may affect the R_k values, and thus R_k values could vary appreciably from one grade of adsorbent to another. The results of a few tests here on five grades of silica gel under standard conditions showed that R_k values were in fact appreciably more constant than the R_F values from which they were obtained.

REFERENCES

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DISCUSSION

GEISS: Are R_k values really more reproducible than R_F or R_M values referred to a standard? Isn't it only an apparent improvement obtained by a mathematical procedure?

HAIS: Expressing relative positions of the spots (of substances A) as differences of their R_M values against the reference standard is certainly to be recommended, if compared with R_X values, X being a reference standard. It should be borne in mind that $R_M = \log (1/R_F - 1)$, not $R_M = \log (1/R_F)$, thus $R_{M(A)} - R_{M(X)}$ is different from $-\log (R_{F(A)}/R_{F(X)})$. Only if R_F becomes very low and its reciprocal very high against 1, will $R_{M(A)} - R_{M(X)} = -\log (R_{F(A)}/R_{F(X)}) = -\log R_X$. Only then the influence of the cross-sectional ratio on the R_X value becomes negligible as pointed out by RACHINSKII*, at the first Liblice symposium, for substances with a low R_F value.

The cross-sectional ratio is a major and not easily controllable contributor to the variability. It is the advantage of the $R_{M(A)} - R_{M(X)}$ values that they eliminate the influence of this ratio. In the absence of gradients this greatly reduces the variation in partition chromatography.

SOCZEWIŃSKI: The advantages of the R_k coefficient depend on the functional relationship of the R_F and R_M values to the distribution coefficient α and cross-sectional area ratio A_{stat}/A_{mob}. For the relative R_F value

$$R_X = \frac{R_{F(A)}}{R_{F(X)}} = \frac{1}{1 + \alpha_A \dfrac{A_{stat}}{A_{mob}}} : \frac{1}{1 + \alpha_X \dfrac{A_{stat}}{A_{mob}}}$$

and thus R_X is a complicated function of α_A, α_X and A_{stat}/A_{mob}. On the other hand

$$R_k = R_{M(A)} - R_{M(X)} = \log \alpha_A \frac{A_{stat}}{A_{mob}} - \log \alpha_X \frac{A_{stat}}{A_{mob}}$$

$$R_k = \log \frac{\alpha_A}{\alpha_X}$$

* V. V. RACHINSKII, in I. M. HAIS AND K. MACEK (Editors), *General Problems of Paper Chromatography*, Publishing House of the Czechoslovak Academy of Science, Prague, 1962, pp. 47–52.

i.e., R_k is equivalent to the log relative net retention volume log $(V_{R(A)}/V_{R(X)})$ extensively employed in GLC and lends itself more easily to theoretical interpretations.

GEISS: On second thoughts, I should like to add further comments, *viz.*

(1) The R_k value indeed eliminates variations of the A_{stat}/A_{mob} "phase" ratio between *different* plates provided that this ratio is constant along each single plate. Hence it would not eliminate the influence of irregular spreading of layers.

(2) If, due to an external parameter, partition or adsorption coefficients of substances A and X change proportionally, then the R_k values remain constant, under assumptions as in (1). Here the R_X value does change. If the change of partition coefficients is not proportional, *e.g.* in the extreme case of inversions, then the R_k value must change, too.

Thus there are many practical instances in which the use of R_k values, though valuable in other well defined cases, does not help to decrease the variation in chromatographic results.

HAIS: In TLC, where adsorption chromatography prevails and gradient effects are very important, general applicability of R_k and similar values will of course be restricted.

DE ZEEUW (to Mr. DALLAS): Did you use single component or multi-component solvent systems?

DALLAS: Single component solvents because I used an S-chamber in which multi-component systems cannot be used if one is not to have solvent demixing.

DE ZEEUW: As far as I remember HONEGGER used chloroform with a small amount of ethanol. I wonder whether the results of HONEGGER can be explained by the fact that he actually works with a multi-component solvent system?

DALLAS: Yes, this is likely and would explain the shape of the indophenol spot he observed in the normal S-chamber.