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APPLICATION OF R_F CORRECTION IN THIN-LAYER CHROMATOGRAPHY BY MEANS OF TWO REFERENCE R_F VALUES

II. RESULTS OBTAINED WITH A POLAR MULTI-COMPONENT SOLVENT SYSTEM

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SUMMARY

Results of the inter-laboratory experiment described in this paper show that the GALANOS AND KAPOULAS equation can be applied satisfactorily to correct R_F values obtained on thin-layer chromatograms in a polar multi-component solvent.

Addition of Kieselguhr to the silica gel gives R_F^c values different from the R_F^c values found on silica gel alone, the deviation being independent of the amount of Kieselguhr added.

INTRODUCTION

In a previous paper[1] the results were reported of the application of the R_F correction method of GALANOS AND KAPOULAS[2] to a non-polar single component solvent (benzene) and silica gel as sorbent. The extraordinary gain in reproducibility of the R_F values thus corrected prompted the "TNO Discussion Group on Chromatography"* to set up a second investigation into the applicability of the method when using a polar multi-component solvent system.

* An informal discussion group in The Netherlands.

As in the previous inter-laboratory investigation, the experiments were carried out by members of the above-mentioned group.

EXPERIMENTAL

Each of the participants obtained two sealed ampoules, marked 1 and 2, respectively. Ampoule 1 contained a mixture of 1.5 mg of tryptophan and 0.15 mg of glycine per ml of methanol–water (1:1). Ampoule 2 contained a mixture of 0.6 mg of dimethyl yellow, 0.6 mg of naphthol yellow S and 0.6 mg of cochenille red per millilitre of methanol.

The components of these mixtures were indicated by: T (tryptophan), G (glycine), D (dimethyl yellow), N (naphthol yellow S), and C (cochenille red).

The following chromatographic conditions were prescribed: a layer of silica gel, with *n*-butanol–acetic acid–water (5:1:2) as solvent system, point of application 1.5 cm from the lower edge of the plate, 10 cm length of run and a 3-μl sample. A solution of ninhydrin had to be used as a spray for staining the amino acid spots. No further conditions were imposed. Twelve laboratories participated in the investigation, most of which carried out more than one experiment. Those carried out under essentially different conditions (tank saturation, activation, etc.) were regarded as independent experiments.

Table I shows the experimental details and Table II the R_F values as reported by the participants.

TABLE I

DETAILS OF THE THIN-LAYER CHROMATOGRAPHIC EXPERIMENTS RECORDED BY THE PARTICIPANTS

Expt. No.	*Silica gel used*	*Activation*	*Chamber saturation*
I	Silica Gel G, Merck	not activated	+
II	Bakerflex IB-F	not activated	+
III	Silica Gel G, Merck	not activated	sandwich
IV	Silica Gel G, Merck	not activated	+
V	Silica Gel G, Merck	110°, 1 h	sandwich
VI	Silica Gel G, Merck	110°, 1 h	+
VII	Silica Gel F_{254}, Merck	110°, 30 min	—
VIII	DC Fertigplatten, Merck	not activated	—
IX	DC Fertigplatten, Merck	100°, 1 h	—
X	DC Fertigplatten, Merck	not activated	+
XI	DC Fertigplatten, Merck	100°, 1 h	+
XII	Fluka H	not activated	+
XIII	Fluka H	230°, 30 min	+
XIV	Silica Gel G, Merck	110°, 1 h	+
XV	Silica Gel G, Merck	not activated	+
XVI	DC Fertigplatten, Merck	not activated	+
XVII	Silica Gel G, Merck	110°, 15 min	+
XVIII	Silica Gel G, Merck	not activated	+
XIX	DC Fertigplatten, Merck	not activated	+
XX	DC Fertigplatten, Merck	110°, 30 min	+
XXI	DC Fertigplatten, Merck	110°, 30 min	—
XXII	DC Fertigplatten, Merck	not activated	+

TABLE I (*continued*)

Expt. No.	*Silica gel used*	*Activation*	*Chamber saturation*
XXIII	DC Fertigplatten, Merck	not activated	—
XXIV	DC Fertigplatten S, Merck	not activated	+
XXV	DC Fertigplatten S, Merck	not activated	—
XXVI	DC Fertigplatten S, Merck	110°, 30 min	+
XXVII	DC Fertigplatten S, Merck	110°, 30 min	—
XXVIII	Silica Gel G, Merck	110°, 30 min	+
XXIX	Silica Gel G, Merck	110°, 30 min	—
XXX	Silica Gel G, Merck	not activated	+
XXXI	Silica Gel G, Merck	not activated	—
XXXII	Polygram Sil N-HR	110°, 30 min	+
XXXIII	Polygram Sil N-HR	110°, 30 min	—
XXXIV	Polygram Sil N-HR	not activated	+
XXXV	Polygram Sil N-HR	not activated	—
XXXVI	Silica Gel G, Merck	120°, 22 h	+
XXXVII	Silica Gel G, Merck	120°, 22 h	—
XXXVIII	Silica Gel H, Merck	110°, 60 min	—
XXXIX	Silica Gel G, Merck	not activated	—
XL	Silica Gel G, Merck	not activated	+
XLI	Silica Gel G, Merck	110°, 20 min	—
XLII	Silica Gel G, Merck	110°, 20 min	+
XLIII	Silica Gel G, Merck	100°, 20 min	—
XLIV	Silica Gel G, Merck	100°, 20 min	+
XLV	Silica gel G, Merck	not activated	+
XLVI	DC Fertigplatten, Merck	not activated	—
XLVII	DC Alufolie, Merck	not activated	—
XLVIII	DC Fertigplatten, Merck	not activated	+
XLIX	DC Alufolie, Merck	not activated	+

RESULTS AND DISCUSSION

As can be seen from Table III uncorrected R_F values show a better reproducibility in the multi-component solvent system used in this investigation, as compared to the R_F values found for other compounds in our previous investigation[1] in which a single-component solvent was used.

The corrected R_F^c values were calculated as previously published[1] using the GALANOS–KAPOULAS equation[2]:

$$R_F^c = aR_F + b.$$

From Table II the values of dimethyl yellow and cochenille red of experiment 1 were taken as standard values.

The results of these calculations are recorded and summarized in Table IV. Two groups of R_F values in this table show some deviation, *i.e.* those from the experiments 24, 25, 26, 27 and those from 32, 33, 34 and 35, which have substantially higher values than in the other experiments.

As in our earlier study[1] it appears that by using the correction procedure of GALANOS AND KAPOULAS, a gain in reproducibility has been obtained. However, it seems that this gain is less than that in our earlier study, which may be caused by the better uncorrected R_F values reproduced in this study.

TABLE II

R_F VALUES FOUND

Expt. No.	*Components*				
	T	*G*	*D*	*N*	*C*
I	0.47	0.14	0.79	0.34	0.09
II	0.56	0.18	0.865	0.45	0.11
III	0.50	0.18	0.76	0.36	0.11
IV	0.56	0.20	0.82	0.38	0.14
V	0.51	0.14	0.78	0.33	0.10
VI	0.54	0.18	0.86	0.38	0.12
VII	0.56	0.20	0.95	0.45	0.17
VIII	0.58	0.18	0.97	0.46	0.18
IX	0.57	0.18	0.98	0.45	0.17
X	0.45	0.12	0.80	0.32	0.11
XI	0.49	0.11	0.81	0.33	0.11
XII	0.55	0.23	0.91	0.42	0.12
XIII	0.55	0.23	0.87	0.41	0.12
XIV	0.658	0.330	0.941	0.571	0.235
XV	0.697	0.318	0.961	0.569	0.243
XVI	0.492	0.128	0.895	0.380	0.116
XVII	0.51	0.21	0.84	0.41	0.15
XVIII	0.63	0.19	0.90	0.48	0.18
XIX	0.45	0.11	0.86	0.36	0.11
XX	0.449	0.118	0.816	0.344	0.116
XXI	0.567	0.181	0.971	0.436	0.154
XXII	0.444	0.117	0.825	0.336	0.114
XXIII	0.554	0.162	0.974	0.438	0.155
XXIV	0.696	0.250	0.923	0.566	0.218
XXV	0.777	0.300	0.995	0.633	0.263
XXVI	0.671	0.244	0.901	0.532	0.194
XXVII	0.752	0.309	0.978	0.624	0.261
XXVIII	0.488	0.189	0.773	0.389	0.132
XXIX	0.584	0.251	0.881	0.475	0.182
XXX	0.540	0.189	0.829	0.401	0.138
XXXI	0.637	0.252	0.949	0.501	0.189
XXXII	0.648	0.181	0.795	0.496	0.162
XXXIII	0.750	0.234	0.883	0.612	0.217
XXXIV	0.709	0.188	0.836	0.610	0.188
XXXV	0.787	0.223	0.884	0.622	0.231
XXXVI	0.50	0.22	0.84	0.40	0.14
XXXVII	0.59	0.25	0.95	0.45	0.16
XXXVIII	0.645	0.225	0.985	0.465	0.145
XXXIX	0.59	0.25	0.89	0.48	0.22
XL	0.58	0.25	0.89	0.46	0.19
XLI	0.58	0.23	0.96	0.46	0.15
XLII	0.54	0.22	0.81	0.39	0.12
XLIII	0.58	0.22	0.97	0.45	0.17
XLIV	0.53	0.21	0.85	0.42	0.13
XLV	0.49	0.20	0.80	0.38	0.14
XLVI	0.565	0.200	0.95	0.415	0.15
XLVII	0.530	0.180	0.95	0.465	0.145
XLVIII	0.44	0.12	0.795	0.315	0.105
XLIX	0.43	0.115	0.78	0.32	0.10

TABLE III

STATISTICAL VALUES CALCULATED FROM TABLES II AND IV

Compound[a]	*From Table II*		*From Table IV*	
	Mean R_F	s[b]	*Mean* R_F[c]	s[b]
T	0.541	0.070	0.469	0.036
G	0.192	0.052	0.138	0.032
D	0.878	0.070	0.790	—
N	0.416	0.059	0.350	0.026
C	0.145	0.035	0.09	—

[a] Abbrevations, see text.
[b] Standard deviation for a single observation.

TABLE IV

CORRECTED R_F VALUES (R_F[c]) DERIVED FROM TABLE II

	Components				
Expt. No.	*T*	*G*	*D*[a]	*N*	*C*[b]
I	0.47	0.14		0.34	
II	0.507	0.155		0.405	
III	0.519	0.164		0.368	
IV	0.522	0.156		0.337	
V	0.511	0.171		0.378	
VI	0.487	0.145		0.335	
VII	0.439	0.116		0.349	
VIII	0.444	0.090		0.338	
IX	0.436	0.100		0.332	
X	0.435	0.098		0.303	
XI	0.420	0.090		0.310	
XII	0.471	0.188		0.356	
XIII	0.492	0.193		0.361	
XIV	0.491	0.165		0.403	
XV	0.533	0.163		0.408	
XVI	0.427	0.100		0.327	
XVII	0.454	0.150		0.353	
XVIII	0.528	0.100		0.382	
XIX	0.407	0.090		0.323	
XX	0.423	0.092		0.318	
XXI	0.441	0.113		0.332	
XXII	0.416	0.093		0.305	
XXIII	0.432	0.097		0.334	
XXIV	0.566	0.122		0.436	
XXV	0.580	0.123		0.444	
XXVI	0.562	0.139		0.424	
XXVII	0.570	0.137		0.444	
XXVIII	0.479	0.152		0.370	
XXIX	0.492	0.148		0.383	
XXX	0.541	0.142		0.356	
XXXI	0.503	0.150		0.380	
XXXII	0.627	0.112		0.460	
XXXIII	0.627	0.099		0.501	
XXXIV	0.602	0.099		0.546	
XXXV	0.687	0.083		0.510	
XXXVI	0.450	0.170		0.350	

(*Continued on p. 288*)

TABLE IV (*continued*)

	Components				
Expt. No.	*T*	*G*	*D*[a]	*N*	*C*[b]
XXXVII	0.472	0.172		0.348	
XXXVIII	0.508	0.158		0.357	
XXXIX	0.477	0.122		0.362	
XL	0.480	0.150		0.360	
XLI	0.461	0.150		0.357	
XLII	0.517	0.193		0.365	
XLIII	0.448	0.133		0.335	
XLIV	0.478	0.167		0.371	
XLV	0.462	0.154		0.345	
XLVI	0.454	0.135		0.323	
XLVII	0.426	0.191		0.367	
XLVIII	0.431	0.109		0.306	
XLIX	0.428	0.104		0.315	

[a] D value in Expt. No. I to XLIX = 0.79.
[b] C value in Expt. No. I to XLIX = 0.09.

TABLE V

R_F VALUES FOR MIXTURES OF SILICA GEL AND KIESELGUHR

Kieselguhr/ silica gel (w/w)	*Compound*				
	T	*G*	*D*	*N*	*C*
20/5	0.820	0.481	0.964	0.732	0.461
15/10	0.691	0.325	0.882	0.570	0.273
10/15	0.618	0.268	0.886	0.500	0.211
5/20	0.587	0.223	0.849	0.454	0.163

TABLE VI

R_F^c VALUES CALCULATED FROM TABLE V

Kieselguhr/ silica gel (w/w)	*Compound*		
	T	*G*	*N*
20/5	0.591	0.120	0.469
15/10	0.568	0.124	0.427
10/15	0.511	0.148	0.379
5/20	0.514	0.163	0.388
R_F^c	0.546	0.143	0.416
standard deviation	0.040	0.017	0.040

Table I shows that experiments 24 to 27 were carried out on Merck plates: Fertigplatten schnelllaufend (code No. 5737/0025). According to the manufacturer's specification the layer consists of a mixture of silica gel and Kieselguhr. To study the influence of the addition of Kieselguhr on the R_F^c values one of the laboratories (Central Institute for Nutrition and Food Research TNO, Zeist) carried out a series

of experiments with the same solvent system and test compounds as in the inter-laboratory experiment, but with layers of mixtures composed of varying amounts of silica gel and Kieselguhr. The uncorrected and corrected R_F values are recorded in Tables V and VI, respectively. The same R_F^0 values were used as in the inter-laboratory experiment (Table II, experiment 1).

From the results recorded in Table IV it can be concluded that the correction formula of GALANOS AND KAPOULAS is likewise applicable to this particular group of mixed layers regardless of the amount of Kieselguhr present. The higher R_F values found in these experiments as compared with the results on silica gel alone may be due to different behaviour towards Kieselguhr of the standards and of the test compounds.

No explanation can be given for the higher values found in the experiments 32 to 35. The composition of the plates used was unknown to us, so a similar experiment to examine the influence of possible additives could not be performed. Contrary to our earlier findings with a non-polar solvent[3,4] the multi-component solvent system used in this investigation did not yield improved separations in unsaturated chambers.

REFERENCES

1 J. H. DHONT, C. VINKENBORG, H. COMPAAN, F. J. RITTER, R. P. LABADIE, A. VERWEIJ AND R. A. DE ZEEUW, *J. Chromatogr.*, 47 (1970) 376.
2 D. S. GALANOS AND V. M. KAPOULAS, *J. Chromatogr.*, 13 (1964) 128.
3 R. A. DE ZEEUW, H. COMPAAN, F. J. RITTER, J. H. DHONT, C. VINKENBORG AND R. P. LABADIE, *J. Chromatogr.*, 47 (1970) 382.
4 R. A. DE ZEEUW, H. COMPAAN, J. H. DHONT, R. P. LABADIE, F. J. RITTER, C. VINKENBORG, *J. Chromatogr.*, 61 (1971) 392.