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IMPROVED SEPARATION IN THIN-LAYER CHROMATOGRAPHY USING A SINGLE-COMPONENT SOLVENT IN AN UNSATURATED CHAMBER*

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SUMMARY

An unexpected observation was made during an interlaboratory investigation on R_F standardisation. Comparing the results which were found on silica gel layers obtained in saturated chambers with those in unsaturated chambers and using benzene as solvent, it was observed that, without exception, the unsaturated chambers gave better separations than the saturated ones.

INTRODUCTION

Recently our group has carried out an interlaboratory investigation in order to evaluate the usefulness in thin-layer chromatography (TLC) of the R_F correction formula of GALANOS AND KAPOULAS[1]. It has the form $R_F{}^c = aR_F + b$, where $R_F{}^c$ is the corrected R_F value and R_F is the uncorrected R_F value of the substance under consideration. The formula had originally been developed for paper chromatography.

Each of the participants in the investigation had received two sealed ampoules, marked 1 and 2. Ampoule 1 contained the test mixture of STAHL, as delivered by Desaga (Heidelberg, G.F.R.). The components of the mixture are indicated by Y (yellow), R (red) and B (blue). Ampoule 2 contained a mixture of the 2,4-dinitrophenyl-hydrazones of methanal and n-dodecanal, dissolved in ethyl acetate. The latter components are indicated by C_1 and C_{12}, respectively.

The following chromatographic conditions were prescribed: a silica gel layer,

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benzene as solvent, application 1.5 cm from the bottom of the plate and a 10-cm length of run. No further details were prescribed. In particular, the participants were left free to use saturated or unsaturated chambers, to use activated or non-activated layers, and to make their own choice of silica gel (*i.e.* type, manufacturer, homemade layers, prefabricated layers, plastic sheets, etc.). The data obtained for the corrected R_F values have been published elsewhere[2].

Apart from these results, however, an unexpected observation was made with regard to the use of unsaturated chambers, which, in our opinion, is worth mentioning. For the system under investigation, using a single-component solvent, unsaturated chambers generally yielded better separations than did saturated chambers.

TABLE I

R_F VALUES FOUND BY PARTICIPANTS, THE DIFFERENCE BETWEEN THE HIGHEST AND LOWEST R_F VALUES, THE CALCULATED a VALUES AND THE SATURATION IN THE INTERLABORATORY EXPERIMENTS
The R_F values are taken from ref. 2.

Expt. No.	R_F value					$R_{F(max)} - R_{F(min)}$	a	Chamber saturation
	C_{12}	Y	C_1	R	B			
I	0.590	0.467	0.342	0.207	0.132	0.458	—	+
II	0.646	0.510	0.369	0.207	0.085	0.561	0.790	+
III	0.482	0.372	0.260	0.167	0.100	0.382	1.23	+
IV	0.795	0.625	0.445	0.235	0.075	0.720	0.607	—
V	0.450	0.340	0.270	0.140	0.070	0.380	1.24	+
VI	0.555	0.445	0.360	0.225	0.105	0.450	0.986	+
VII	0.845	0.680	0.535	0.300	0.095	0.750	0.574	—
VIII	0.520	0.385	0.320	0.165	0.080	0.440	1.10	+
IX	0.880	0.750	0.590	0.360	0.170	0.710	0.577	—
X	0.450	0.355	0.280	0.140	0.060	0.390	1.13	+
XI	0.795	0.700	0.550	0.325	0.140	0.655	0.559	—
XII	0.615	0.540	0.435	0.295	0.190	0.525	0.959	+
XIII	0.955	0.935	0.855	0.660	0.410	0.545	0.637	—
XIV	0.59	0.44	0.36	0.18	0.07	0.52	0.906	+
XV	0.84	0.67	0.51	0.23	0.12	0.72	0.608	+
XVI	0.79	0.55	0.46	0.17	0.047	0.743	0.666	—
XVII	0.63	0.47	0.40	0.20	0.09	0.54	0.884	+
XVIII	0.54	0.42	0.30	0.165	0.085	0.455	1.00	+
XIX	0.52	0.39	0.25	0.14	0.05	0.47	0.986	+
XX	0.55	0.39	0.25	0.13	0.04	0.51	0.959	+
XXI	0.68	0.50	0.38	0.19	0.08	0.60	0.799	+
XXII	0.80	0.65	0.42	0.25	0.11	0.69	0.622	—
XXIII	0.79	0.58	0.37	0.18	0.05	0.74	0.631	—
XXIV	0.80	0.64	0.43	0.26	0.12	0.68	0.645	—
XXV	0.80	0.71	0.56	0.44	0.25	0.55	0.730	+
XXVI	0.82	0.67	0.49	0.24	0.11	0.71	0.600	—
XXVII	0.78	0.60	0.43	0.21	0.09	0.69	0.658	—
XXVIII	0.83	0.59	0.45	0.21	0.08	0.75	0.658	—
XXIX	0.82	0.67	0.47	0.24	0.11	0.71	0.598	—
XXX	0.90	0.77	0.57	0.31	0.12	0.78	0.516	—
XXXI	0.79	0.65	0.48	0.22	0.10	0.69	0.610	—
XXXII	0.82	0.56	0.38	0.17	0.03	0.79	0.633	—
XXXIII	0.73	0.61	0.45	0.23	0.12	0.71	0.684	— (S-chamber)
XXXIV	0.88	0.80	0.68	0.44	0.36	0.52	0.761	— (S-chamber)
XXXV	0.34	0.25	0.185	0.10	0.04	0.30	1.60	+
XXXVI	0.43	0.33	0.24	0.12	0.05	0.38	1.20	+.
XXXVII	0.64	0.48	0.39	0.18	0.07	0.57	0.818	+

This will be easily concluded from Table I, which gives the R_F values found by the various participants, the calculated a values and the saturation. The same conclusion will be derived from the plots in Fig. 1. Here, the various a values of the correction formula are plotted against the differences between the highest and lowest R_F values obtained in the corresponding chromatograms, *i.e.* $R_F(C_{12})-R_F(B)$. The overall spread for saturated chambers varies between 3.0 and 7.2 cm, the mean value being 4.8 cm, whereas the overall spread in unsaturated chambers varies between
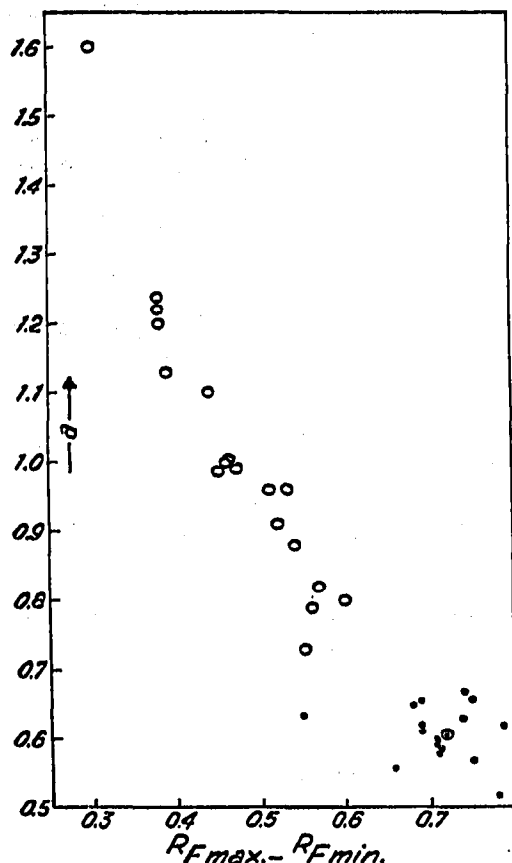

Fig. 1. Relationship between a and $R_{F(max)}-R_{F(min)}$, for saturated (O) and unsaturated (●) chambers.

5.4 and 7.9 cm, the mean value being 7.1 cm. Fig. 1 also shows that the a values for unsaturated chambers are grouped closely together (which may indicate that they have a better reproducibility within the group of unsaturated chambers) and that they have in general a distinctly lower value than those for saturated chambers. The mean value of a in unsaturated chambers is 0.614, whereas it is 0.968 in saturated chambers.
Since

$$a = \frac{R_F^o(Y) - R_F^o(B)}{R_F(Y) - R_F(B)}$$

where $R_F^o(Y)$ and $R_F^o(B)$ refer to the R_F values of the substances Y and B in the standard chromatogram (saturated chamber), and $R_F(Y)$ and $R_F(B)$ refer to the R_F

values of Y and B in an arbitrary chromatogram of which the R_F values are to be corrected, a lower a value thus indicates a better separation of Y and B in the arbitrary chromatogram than in the standard chromatogram. In general it appeared that unsaturated chambers yielded lower a values than did saturated chambers. It should be noted that a significant increase in the spot diameters in unsaturated chambers was not observed.

The above result is, in our opinion, quite remarkable. Although a possible explanation for the occurrence of improved separations when using multi-component solvents in unsaturated chambers has been given[3], an explanation is not yet available for single-component solvents. In unsaturated chambers solvent evaporation during the run will take place to a higher extent than in saturated chambers which require an extra solvent supply from the solvent reservoir. As a consequence of this solvent supplementation more solvent will be transported across the lower parts of the plate than across the higher parts, which, in turn, would be expected to result in a higher migration of the lower spots. Hence this "pushing up" would have an adverse effect on the separation, and on the spread of the spots. However, the findings described here indicate that other factors must also be involved, as the separation was distinctly improved by working with unsaturated chambers.

Further studies are being carried out to see whether similar observations can be made when other solvents and substances are used in unsaturated chambers.

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