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PEAK BROADENING IN PAPER CHROMATOGRAPHY AND RELATED TECHNIQUES

VI. THE EFFICIENCY OF VARIOUS KINDS OF CHROMATOGRAPHY PAPER AND THIN-LAYER CELLULOSE POWDER FOR THE SEPARATION OF AMINO ACIDS

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SUMMARY

The efficiency of several chromatography papers and thin-layer cellulose powders for the separation of amino acids is investigated, using the minimum elution time for a given resolution as the criterion.

INTRODUCTION

Recently, DE LIGNY AND REMIJNSE[1] compared the efficiencies of ten kinds of Whatman paper and six kinds of thin-layer cellulose powder for the separation of amino acids by means of a 4:1:5 butanol–acetic acid–water mixture. In this study, they used the following criterion for the efficiency:

That paper or powder, which gives the best resolution under standard conditions (*i.e.*, standard positions of starting point and solvent front**) is the most effective one.

However, another quite different criterion is possible, namely:

That paper or powder, which gives some desired resolution in the shortest elution time is the most effective one.

As time plays a very important role in many separation problems this factor should not be neglected, so the second criterion will be the better one.

It is to be expected, that the order of efficiency by the first criterion will be different from the order of efficiency by the second. In general, a paper or powder having a high eluent flow velocity will get a better classification by the second criterion than by the first.

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** The distances of starting point and solvent front from the surface of the eluent in the tank were 1 and 11 cm, respectively, in thin-layer chromatography and 5–6, and 25–30 cm, respectively, in paper chromatography.

TABLE I

EFFICIENCY OF CELLULOSE POWDERS. $R = 4$[a]

Cellulose powder	R_F (1)	R_F (3)	$B \times 10^6$	C_M	k[b]	l_f	l_0	$t_{elution}$
M & N 300	0.21	0.30	3.07	1.27	0.017	13	0.9	9 600
Camag D	0.21	0.20						very long
Whatman CC41	0.13	0.20	3.56	4.67	0.029	28	3.0	28 000
S & S 144	0.22	0.32	3.01	3.24	0.037	19	3.0	9 300
S & S 142 dg	0.17	0.34	2.96	1.61	0.039	8	1.7	1 500
S & S 140 dg	0.24	0.41	2.81	3.10	0.069	15	3.5	3 400
	R_F (5)	R_F (6)						
M & N 300	0.36	0.48	2.71	0.70	0.017	8	0.8	3 600
Camag D	0.37	0.48	2.71	0.92	0.018	11	0.9	6 300
Whatman CC41	0.26	0.35	2.93	3.08	0.029	22	2.8	16 000
S & S 144	0.38	0.51	2.67	1.68	0.037	13	2.3	4 500
S & S 142 dg	0.40	0.53	2.65	0.82	0.039	9	1.7	2 300
S & S 140 dg	0.48	0.60	2.58	1.42	0.069	18	3.5	4 500
	R_F (6)	R_F (9)						
M & N 300	0.48	0.63	2.56	0.37	0.017	5	0.6	1 200
Camag D	0.48	0.64	2.53	0.44	0.018	5	0.6	1 100
Whatman CC41	0.35	0.50	2.68	1.82	0.029	10	1.9	3 600
S & S 144	0.51	0.64	2.53	0.91	0.037	11	1.8	3 200
S & S 142 dg	0.53	0.64	2.53	0.48	0.039	10	1.4	2 600
S & S 140 dg	0.60	0.72	2.50	0.70	0.069	14	2.8	3 000

[a] B in [$cm^2 \cdot sec^{-1}$]; B accounts for peak broadening by diffusion.
C_M in [sec]; C_M accounts for peak broadening by resistance to mass transfer in the mobile phase.
k in [$cm^2 \cdot sec^{-1}$]; k governs the eluent flow rate.
l_f in [cm]; l_f is the distance from the surface of the eluent in the tank to the solvent front.
l_0 in [cm]; l_0 is the distance from the surface of the eluent in the tank to the starting point.
$t_{elution}$ in [sec].
[b] Upward flow.

In the following, the efficiencies of the same six powders and ten papers are compared once more, now observing the second criterion.

For this purpose we can use the procedure described earlier[2] for calculating the minimum separation time for some desired resolution.

In this method the R_F values of the compounds to be separated, and data on peak broadening and eluent flow rate for the paper or thin-layer cellulose powder to be used are required. The calculations yield, for some specified value of the peak resolution*, the minimum elution time, the corresponding optimum position of the starting point, and the necessary distance of travel of the eluent.

Following this procedure the minimum separation times for the six powders and ten papers were calculated for the following combinations of amino acids**: L-α,γ-diaminobutyric acid (1) and L-aspartic acid (3); L-threonine (5) and L-α-aminobutyric acid (6); L-α-aminobutyric acid (6) and L-norvaline (9).

The peak resolution R was taken to be 4 for thin-layer chromatography and 3 for paper chromatography.

* The peak resolution R is defined as follows: $R = (l_A - l_B)/(\sigma_A + \sigma_B)$; where l = distance, travelled by the solute; σ = standard deviation of the solute distribution; A and B = faster and slower moving solute, respectively.
** For numbering of amino acids see ref. 4.

The R_F values were taken from the work of DE LIGNY AND REMIJNSE[3,4], the other data from DE LIGNY AND KOK[2]. Data for the papers W3 and W7 were determined by the present authors.

RESULTS

The results of the calculations are summarized in Tables I and II.

TABLE II

EFFICIENCY OF CHROMATOGRAPHY PAPERS. $R = 3$*

Chromatography paper	R_F (1)	R_F (3)	$B \times 10^6$	C_M	k**	l_f	l_0	$t_{elution}$
W1	0.09	0.14	5.60	15.5	0.032	67	3.6	142 000
W2	0.08	0.14	5.48	15.5	0.028	45	3.5	71 800
W3	0.14	0.22	2.38	12.8	0.031	32	4.2	32 200
W3 MM	0.10	0.17	4.12	14.4	0.040	38	5.1	36 700
W4	0.13	0.21	3.37	13.1	0.060	39	6.7	25 400
W7	0.19	0.26	3.90	11.5	0.036	49	4.4	65 800
W17	0.15	0.25	3.70	11.8	0.069	33	6.4	15 900
W20	0.07	0.13	4.83	15.9	0.016	43	1.9	116 000
W31 ET	0.20	0.33	3.17	9.4	0.106	30	6.6	8 700
W54	0.11	0.20	3.71	13.4	0.071	35	6.7	17 300
	R_F (5)	R_F (6)						
W1	0.20	0.33	4.40	9.4	0.032	20	3.0	12 600
W2	0.20	0.31	4.34	9.8	0.028	24	2.9	21 000
W3	0.21	0.33	1.34	9.4	0.031	21	3.3	13 900
W3 MM	0.23	0.35	3.24	8.9	0.040	24	3.7	14 200
W4	0.27	0.43	2.66	6.8	0.060	19	3.7	6 100
W7	0.27	0.41	3.49	7.3	0.036	19	2.9	10 400
W17	0.31	0.45	3.18	6.3	0.069	24	4.3	8 300
W20	0.18	0.31	3.53	9.8	0.016	16	1.8	16 000
W31 ET	0.39	0.51	2.86	5.0	0.106	34	5.9	10 600
W54	0.25	0.35	2.75	8.9	0.071	36	6.3	18 600
	R_F (6)	R_F (9)						
W1	0.33	0.49	4.11	5.5	0.032	16	2.1	8 000
W2	0.31	0.49	3.99	5.5	0.028	13	1.9	6 000
W3	0.33	0.48	1.67	5.7	0.031	16	2.5	8 500
W3 MM	0.35	0.50	2.99	5.2	0.040	18	2.7	8 200
W4	0.43	0.54	2.52	4.4	0.060	27	3.8	12 500
W7	0.41	0.54	3.32	4.4	0.036	22	2.4	13 400
W17	0.45	0.54	3.07	4.4	0.069	45	4.7	28 700
W20	0.31	0.45	3.24	6.3	0.016	16	1.4	16 700
W31 ET	0.51	0.64	2.74	2.7	0.106	28	4.2	7 200
W54	0.35	0.51	2.49	5.0	0.071	20	3.8	5 600

* For explanation of symbols see footnote to Table I.
** Downward flow.

DISCUSSION

It follows from Tables I and II that the elution times in paper chromatography are as a rule much longer than in thin-layer chromatography, even in spite of the fact that the resolution in paper chromatography is taken to be less complete than in thin-layer chromatography.

The efficiency varies appreciably with the pair of amino acids to be separated.

The orders of efficiency of the various powders and papers are given in Tables III and IV.

By summing the order numbers for the three pairs of amino acids we obtain a rough assessment of the efficiency of the various powders and papers. The order of efficiency in thin-layer chromatography is:

S & S 142 dg > M & N 300 > S & S 140 dg > S & S 144 ≈ Camag D > Whatman CC41;

and in paper chromatography:

W31 ET > W4 > W54 > W17 > W3 > W3 MM ≈ W7 > W1 > W2 > W20.

As expected, the orders of efficiency are different from those, found previously under standard conditions (namely: Camag D ≈ M & N 300 > Whatman CC41 and W3 > W7 ≈ W20 > W2). The main cause for this discrepancy is that in the present comparison of the efficiencies the flow rate of the eluent plays a large role, whereas it did not enter into the previous comparison.

In the case of paper chromatography the optimum values of l_0 generally do not differ much from the values used in practice (≈ 5 cm). If they do, the optimum values are so small that it is difficult to realize them experimentally.

In thin-layer chromatography, however, the optimum values of l_0 are often appreciably larger than the usual one (≈ 1 cm).

TABLE III

ORDER OF EFFICIENCY OF CELLULOSE POWDERS

Cellulose powder	*Order of efficiency*			*Sum of orders*
	1 + 3	*5 + 6*	*6 + 9*	
M & N 300	4	2	2	8
Camag D	6	5	1	12
Whatman CC41	5	6	6	17
S & S 144	3	4	5	12
S & S 142 dg	1	1	3	5
S & S 140 dg	2	3	4	9

TABLE IV

ORDER OF EFFICIENCY OF CHROMATOGRAPHY PAPERS

Chromatography paper	*Order of efficiency*			*Sum of orders*
	1 + 3	*5 + 6*	*6 + 9*	
W1	10	5	4	19
W2	8	10	2	20
W3	5	6	6	17
W3 MM	6	7	5	18
W4	4	1	7	12
W7	7	3	8	18
W17	2	2	10	14
W20	9	8	9	26
W31 ET	1	4	3	8
W54	3	9	1	13

The influence of the value of l_0 upon resolution in thin-layer chromatography, for a constant value of l_f, is shown in Fig. 1*.

All the graphs have a distinct maximum near $l_0 = 2.0$ cm. As this maximum was calculated to be at 2.3 cm for the acids 5 and 6, $l_f = 13$ cm and at 1.8 cm for the acids 6 and 9, $l_f = 11$ cm (see Table I) the agreement between theory and experiment is good.

The calculated optimum values of l_0 do not depend strongly upon the pair of

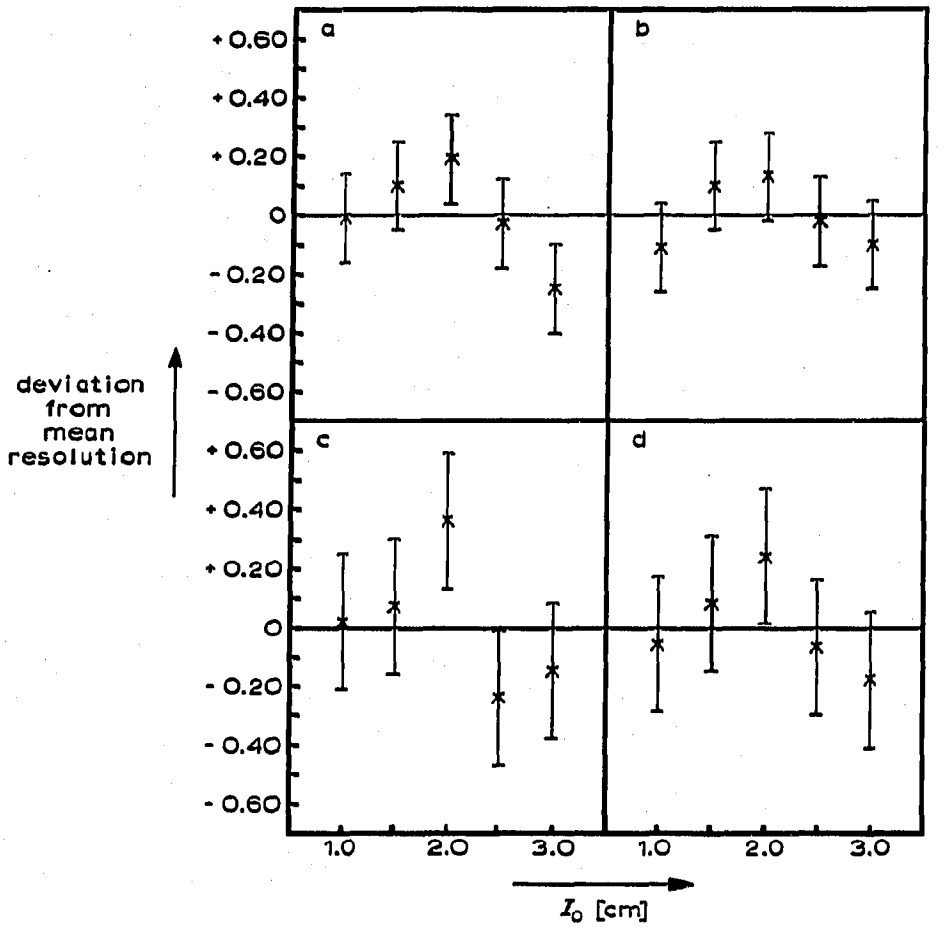

Fig. 1. (a) Resolution of amino acids 5 and 6 as a function of l_0, $l_f = 11$ cm; (b) resolution of amino acids 5 and 6 as a function of l_0, $l_f = 13$ cm; (c) resolution of amino acids 6 and 9 as a function of l_0, $l_f = 11$ cm; (d) resolution of amino acids 6 and 9 as a function of l_0, $l_f = 13$ cm. The results shown are mean values of 4 to 6 replicate experiments. The bars represent 90% probability intervals.

* The following procedure was used to obtain these data: After applying 1 μl of an aqueous solution, containing 1 μg of each of the amino acids 5, 6 and 9 to the ground edge of a glass plate (20 × 1 mm) this edge was then pressed on a thin-layer of S & S 144 cellulose powder. The mixture was applied five times to each thin-layer plate, the distances from the end of the plate varying from 1.0 up to 3.0 cm.

After equilibration with the vapour of the lower layer of a 4:1:5 butanol–acetic acid–water mixture, for at least 18 h at 21.5°, the upper layer was poured into the tank and the solvent was allowed to travel for a distance of 11 or 13 cm.

Then the plates were dried and stained with a ninhydrin solution and the densitograms obtained. From the peak width at half height and the distance between the peak maxima the resolution R was calculated.

amino acids to be separated and probably hold approximately for a much wider range of separations. Therefore, the values of l_0 given in Table I can serve as a rough guide for any separation problem in thin-layer chromatography.

CONCLUSIONS

The time needed to obtain a given resolution is much longer in paper chromatography than in thin-layer chromatography.

The efficiency of a paper or powder varies appreciably with the pair of amino acids to be separated. On the average, the order of efficiency by the criterion of minimum elution time for a given resolution is:

S & S 142 dg > M & N 300 > S & S 140 dg > S & S 144 ≈ Camag D > Whatman CC41 for thin-layer chromatography;
and

W31 ET > W4 > W54 > W17 > W3 > W3 MM ≈ W7 > W1 > W2 > W20 for paper chromatography.

If optimum separation in thin-layer chromatography is desired it is advisable to choose the following values for l_0, the distance between the starting point and the eluent in the tank:

M & N 300 ≈ 1.0 cm; Camag D ≈ 1.0 cm; Whatman CC41 ≈ 2.5 cm; S & S 144 ≈ 2.5 cm; S & S 142 dg ≈ 1.5 cm; S & S 140 dg ≈ 3.0 cm, instead of the usual value $l_0 \approx 1$ cm.

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