CHROMATOGRAPHY OF AMINO ACIDS ON SILICA GEL. I. TWO-DIMENSIONAL CHROMATOGRAPHY ON SILUFOL® THIN LAYERS

K. CHMEL

Research Laboratories, Kavalier Glassworks, Votice

Received September 29th, 1971

A series of solvent systems for chromatographic separation of amino acids on Silufol® thin layers was tested. A two-dimensional chromatographic method was found enabling the identification of any one of twentytwo amino acids in the investigated sample. It was observed that the R_F values of single amino acids in chloroform–methanol–ammonia (30 : 30 : 7·5) are dependent on the relative humidity of the air, especially in the 50–90% region. The time of the development is at most half of that necessary for thin-layer chromatography on cellulose.

A series of procedures[1–17] is described in the literature for chromatographic separation and identification on thin layers of silica gel of amino acids in mixtures. For the separation of more complex mixtures two-dimensional chromatography is usually employed[1–6,11,14]. In view of the fact that various silica gels give varying results of chromatography under otherwise identical conditions, it is not surprising that the application of published procedures for the separation of amino acids to Silufol® causes the amino acids to remain mostly near the start and not to separate. Hence, it was necessary to check known solvent systems, choose the most suitable ones and use them for two-dimensional chromatography of amino acids.

EXPERIMENTAL

Material: Silufol®, size 5 × 15 and 15 × 15 cm. Chromatography chambers, 2 µl application capillaries and 10 µl micropipette were from the TLC kit I and II (Kavalier). Solvents were of analytical grade, redistilled. Amino acids were purchased from Merck, BDH Chemicals Ltd., and Lachema. Standard solution of single amino acids: 0·1% in 1M-HCOOH (2 µl were applied). Mixture of amino acids: 0·02% solution in 10% 1-propanol (10 µl were applied). The solutions were kept in a refrigerator.

Development: Ascending, two-dimensional chromatography in an unsaturated chamber (*i.e.* its walls were not lined with filter paper) was used. The chamber was ready for use 30 min after the solvent was poured into it. One dose of solvent may be used four times, then it must be exchanged for a fresh batch. The chromatographed amino acids are applied onto the start 2 cm from the lower edge of the plate, the separation path is 13 cm long. After termination of the separation in the first direction the plate is dried, first in air and then for 10 minutes in an oven at 110°C.

Before the chromatography in the second direction the plate is allowed to stand in air for a minimum of 10 min (equilibration with the surrounding atmosphere). The temperature during the development was 22–25°C, relative air humidity 50–60%. Detection was carried out by uniform spraying of the chromatogram with a ninhydrin solution (0·6%) in ethanol and subsequent drying at 110°C, until the amino-acid spots are distinctly visible. When systems containing ammonia were used, the ammonia must be thoroughly eliminated before detection by heating the plate in a drying oven.

RESULTS AND DISCUSSION

In order to find the most suitable ratios of components of the solvent system the following mixture of amino acids was separated: arginine, proline, hydroxyproline, glutamic acid, aspartic acid, phenylalanine, and tryptophan. The separation was carried out on Silufol® plates of 5 × 15 cm dimension, and the solvent path was 10 cm. The results obtained were represented graphically, *i.e.* R_F values were plotted as functions of the solvent composition (Fig. 1). In this manner the most suitable solvent composition was determined and in the chosen solvent R_F values of single amino acids were determined by one-dimensional chromatography (13 cm solvent path), as averages of six measurements (Table I). The following solvent mixtures

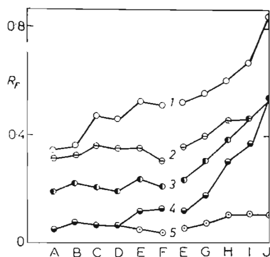

FIG. 1

Dependence of R_F Values on the Solvent System Composition

System phenol–water–ethanol: A (3 : 0·5 : 0); B (3 : 1 : 0); C (3 : 1 : 1); D (3 : 1 : 2); E (3 : 1 : 3); F (3 : 1 : 4); G (3 : 2 : 3), H (3 : 3 : 3); I (3 : 4 : 3); J (3 : 6 : 3). *1* tryptophan and phenylalanine, *2* proline, *3* hydroxyproline, *4* aspartic and glutamic acids, *5* arginine.

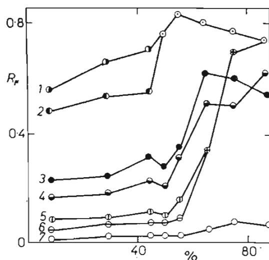

FIG. 2

Dependence of R_F Values of Amino Acids on Relative Air Humidity

System chloroform–methanol–ammonia (30 : 30 : 7·5); *1* phenylalanine, *2* tryptophan, *3* proline, *4* hydroxyproline, *5* glutamic acid, *6* aspartic acid, *7* arginine.

TABLE I

R_F . 100 Values of Amino Acids in the Investigated Solvent Systems I—VI

Solvent composition: I ethyl acetate–isopropyl alcohol–water–ammonia (20 : 20 : 25 : 1·5); II acetone–water–acetic acid–formic acid (50 : 15 : 12 : 3); III chloroform–methanol–ammonia (30 : 30 : 7·5); IV phenol–ethanol–water–acetic acid (25 : 25 : 25 : 2·5); V 1-propanol–water–ammonia (45 : 15 : 15); VI 1-butanol–water–acetic acid (25 : 25 : 25).

Amino acid	I	II	III	IV	V	VI
1 glycine	28	43	23	30	41	43
2 alanine	29	52	32	33	44	47
3 valine	42	62	42	47	55	56
4 leucine	55	68	63	56	64	69
5 isoleucine	50	68	52	55	62	62
6 β-alanine	21	53	17	35	35	50
7 serine	34	46	20	32	43	42
8 threonine	38	50	37	36	50	51
9 phenylalanine	70	71	75	60	65	70
10 tryptophan	70	73	60	58	64	74
11 proline	16	28	22	43	39	36
12 hydroxyproline	26	35	19	38	38	42
13 arginine	4	12	3	18	18	22
14 histidine	30	8	33	17	48	18
15 lysine	2	8	5	13	19	17
16 glutamine	34	37	20	36	48	43
17 asparagine	35	40	22	32	42	40
18 glutamic acid	33	62	10	37	35	55
19 aspartic acid	32	55	7	32	34	53
20 cystine	35	18	10	23	42	31
21 methionine	51	67	51	55	62	60
22 tyrosine	55	68	55	55	51	68

were tested: ethyl acetate–isopropyl alcohol–water–ammonia[6], acetone–water–acetic acid–formic acid[16], chloroform–methanol–ammonia[5,11,14,17], propanol–water–ammonia[1,4,9,10], butanol–water–acetic acid[3,4,8,13–15], phenol–water[1,3–5,8,11,14–16], ethanol–water[2,4,12,15], chloroform–methanol–acetic acid[6]. In phenol–water system too low R_F values were obtained (Fig. 1) and therefore ethanol had to be added to it in order to increase the solubility of water in phenol. Among the above mentioned solvent systems the two last ones are not suitable for chromatography on Silufol®. As it was found that the R_F values of single amino acids fluctuate the dependence of R_F values on relative air humidity[18] was also investigated. The R_F values of amino acids in systems I, II, IV, and VI are independent on the relative humidity of the ambient atmosphere, but the sharpness of the separation decreases sharply above

80% humidity. In system V a mild increase of R_F values with increasing relative humidity was observed, while the R_F values in system III are strongly dependent on relative humidity (Fig. 2), especially in the 50–90% range. This dependence of the R_F values on relative air humidity may explain the observed deviations of the positions of amino acid spots on plates and two-dimensional chromatograms, occurring sometimes. Silica gel is very sensitive to such effects[18]. However, if relative air humidity is controlled during the work, more constant results may be obtained.

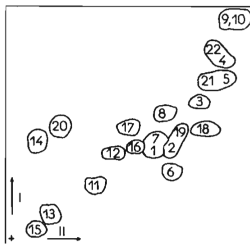

FIG. 3

Two-Dimensional Chromatogram of Amino-Acids Mixture

First direction: system I, second direction: system II; indication of amino acids as in Table I. The start and front was placed 2 cm from the edge of the plate (not illustrated in the Fig.).

From the R_F values of single amino acids (Table I) maps were plotted for two-dimensional chromatography in all combinations of systems. The most suitable of these were checked by chromatographic separation of all amino acids, and in ambiguous cases mixtures composed of a lesser number of substances were chromatographed. Three pairs of systems were selected as most suitable: I and II (Fig. 3), I and III, and II and III. Using these systems the number and the quality of amino acids in the sample may be determined. In the first pair of solvents the following amino acids cannot be distinguished: phenylalanine and tryptophan, tyrosine and leucine, methionine and isoleucine. In the pairs I–III and II–III phenylalanine and tryptophan, and leucine and tyrosine may be separated. Other amino acids may be identified on the basis of different positions on chromatograms, developed in all three solvent system pairs.

The author thanks Dr J. Hydánek for valuable advice and Mrs V. Růzhová for technical assistance.

REFERENCES

1. Nürnberg E.: Arch. Pharm. *292*, 610 (1959).
2. Mutschler E., Rochelmeyer H.: Arch. Pharm. *292*, 449 (1959).
3. Niederwieser A., Pataki G.: Chimia *14*, 378 (1960).
4. Brenner M., Niederwieser A.: Experientia *16*, 378 (1960).
5. Fahmy A. R., Niederwieser A., Pataki G., Brenner M.: Helv. Chim. Acta *44*, 2022 (1961).
6. Diamantstein T., Ehrhart H.: Z. Physiol. Chem. *326*, 131 (1961).
7. Brenner M., Niederwieser A.: Experientia *17*, 237 (1961).
8. Honegger C. G.: Helv. Chim. Acta *44*, 173 (1961).
9. Weicker H., Huhnstock K.: Klin. Wochschr. *40*, 44 (1962).
10. Pataki G.: J. Chromatog. *16*, 541 (1964).
11. Rokkones T.: Scand. J. Clin. Lab. Invest. *16*, 149 (1964).
12. Geiss F., Schlitt H., Klose A.: Z. Anal. Chem. *213*, 321 (1965).
13. Stahl E.: Z. Anal. Chem. *221*, 3 (1966).
14. Rosetti V.: Ann. Chim. (Rome) *56*, 935 (1966); Chem. Abstr. *66*, 397 D (1967).
15. Shellard E. J., Jollifee G. H.: J. Chromatog. *38*, 257 (1968).
16. Detterbeck F. C., Lillewik H. A.: Michigan Mental Health Res. Bull. *11*, 17 (1968).
17. Tyihak E., Vagujfalvi D.: J. Chromatog. *49*, 343 (1970).
18. Chmel K.: Chem. listy, in press.

Translated by Ž. Procházka.