Polyamide mixed thin layer chromatography of amino acids

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The thin-layer chromatography of amino acids has been studied by numerous investigators. The separation of amino acid by thin-layer of cellulose (1-3) starch (4), silica gel (5-8), aluminum oxide (9), polyamide (10-13) and kieselguhr (14) has been reported. Recently, polyamide mixed layer has been successfully used for the separation of several types of compounds, e.g. polyamide-silica gel mixed layer for water soluble vitamines (15), and polyamide-kieselguhr mixed layer for antipyretics (16). Therefore, the mixed layer method was further applied to separate 12 amino acids. For comparison, thin-layer chromatography using only polyamide, only silica and only kieselguhr was performed under the same conditions, separation on polyamide mixed layer was found to be preferable. The method is suitable for identification of amino acids.

Experimental

Materials. The polyamide chip was Nylon 6, type 1022 B of UBE Industrial Ltd. (Osaka, Japan). The solvents were reagent grade of Wako pure chemical Industries, Ltd. (Osaka, Japan).

Preparation of polyamide-kieselguhr mixed layer.

Ten g of polyamide chip were dissolved in 100 ml of 90 % formic acid after standing for overnight, a homogeneous solution was obtained; then 50 g of kieselguhr G (E. Merck) were added. Of the previous solution 300 ml were poured into a dish (15 x 20 x 2.5 cm), into which a glass plate (12x 16x 0.1 cm) was dipped. Both sides of the glass were covered homogenerously. The glass was hung for 2 min. over the dish to let the excess solution drain off. It was then air dried for three hours and heated at 100°C for 30 min.

Preparation of polyamide-silica gel mixed layer.

Before proceeding as described in the previous method, 8 g of polyamide were dissolved; then 52 g of Silica Gel G (E.Merck) were added.

Preparation of polyamide layer.

Dissolved 20 g of polyamide in 90 ml of 90 % formic acid, then 10 ml of distilled water were added. After stirring, a homogeneous solution was obtained. The other steps are like the method described above, but without adding Silica Gel G or Kieselguhr G.

Preparation of kieselguhr or silica gel layer.

Dilute Slurries of Kieselguhr G or Silica Gel G (45 g in 100 ml of water) were sprayed at 1.5 kg/cm² pressue from a distance of 20 cm onto horizontal glass plate (12 x 15 cm) which were then dried at 100°C for 30 min. The thickness of layer was about 250μ.

Chromatographic procodure.

Two percent amino acid alcoholic solution was applied to the start line 1.5cm from the bottom of layer. The plate were developed by ascending techniques. The chamber had been equlibrated with the respective solvent for 30 min. before use.

Visualization.

After developing, the plate was sprayed with Ninhydrin solution (0.3g ninhydrin + 100 ml n-butanol + 3 ml glacial acetic acid) then dried in oven at 110℃ 10 min. The spots would produce violet color.

Rf values of polyamide-kieselguhr mixed layer, polyamide-silica gel mixed layer, kieselguhr layer and silica gel layer with four solvent svstems are given in Table I. It has been found that the results obtained using polyamide mixed layers show better separation and sharper spots. Spots on only polyamide layer sould not be detected and the results is omitted from Table I. This is due to the color reaction of Ninhydrin with amino acids is interfered by polyamide. Therefore, instead of amino acids, dinitrophenyl-amino scids DNP-amino acids have been used for the separation on polyamide layer by the previous investigators(10-13).

Table I Chromatographic data

Solvent I : MeOH-acetone-5% NH_4Cl-glacial acetic acid (50:10:10:2);

Solvent II : isopropyl alcohol-acetone-5% NH_4Cl-glacial acetic acid (50:10:10:2);

Solvent III: 5% sodium borate solution-n-butanol-glacial acetic acid (5:50:1);

Solvent IV : 5% sodium borate solution-n-butanol-acetone-glacial acetic acid-N, N-dimethylacetamide (5:50:10:1:1);

No.	Solvent / Sample	I		II		III		IV	
		P-S	S	P-S	S	P-K	K	P-K	K
1.	Cystein	0.08	0.15	0.21	0.22	0.27	0.30	0.30	0.00
2.	Asparatic acid	0.26	0.25	0.19	0.23	0.24	0.26	0.00	0.00
3.	Histidine	0.35	0.39	0.36	0.44	0.18	0.20	0.23	0.30
4.	Arginine	0.45	0.52	0.48	0.55	0.23	0.25	0.30	0.16
5.	Glutamic acid	0.50	0.51	0.67	0.54	0.03	0.06	0.04	0.05
6.	Lysine	0.54	0.41	0.40	0.45	0.14	0.17	0.17	0.13
7.	Hydroxyproline	0.57	0.57	0.53	0.62	0.43	0.59	0.51	0.59
8.	Alanine	0.63	0.56	0.57	0.63	0.47	0.60	0.49	0.59
9.	Methionine	0.66	0.53	0.66	0.73	0.69	0.83	0.77	0.76
10.	Tyrosine	0.68	0.65	0.51	0.74	0.80	0.90	0.71	0.85
11.	Phenylalanine	0.70	0.63	0.64	0.80	0.80	0.90	0.86	0.85
12.	Valine	0.73	0.59	0.69	0.73	0.75	0.86	0.81	0.79
Time required(min.)		90	30	120	50	220	110	210	90

P-S=Rf values of polyamide-silica gel layer; S= silica gel layer

P-K=polyamide-kieselguhr layer; K= kieselguhr layer

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〔中 文 摘 要〕

氨基酸之多酸胺混合薄層色層分析

12種氨基酸應用多醯胺—矽膠混合（1：6.5）薄層，多醯胺—硅藻土混合薄層（1：5）薄層，矽膠薄層，硅藻土薄層，多醯胺薄層等五種薄層色層分析法，分別進行鑑別，經檢討所得之結果，應用多醯胺混合薄層分離之結果較好。