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Polyamide-Silica gel Mixed Layer Chromatography of Vitamins

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Thin-layer chromatography of vitamins has been the subject of numerous investigations. The separation of water soluble vitamins on thin layers of silica gel[1-4], aluminum oxide[4], starch[5], polyamide[6] and fat soluble vitamins on thin layers of silica gel[7] and aluminum oxide[8] have been reported. But there is no report on separation of both water and fat soluble vitamins on the same layer.

In previous papers, polyamide-silica gel mixed layer has been successfully used for the identification of water soluble vitamins[9,10]. In this studies, this method was further applied to separate 12 fat and water soluble vitamins. For comparison, thin-layer chromatography using only polyamide and silica gel is also described. Separation on polyamide-silica gel mixed layer was found to be preferable.

Experimental

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

Preparation of polyamide-silica gel mixed layers. Eight g of polyamide chip were dissolved in 100 ml of 90% formic acid. After standing for over night and stirring, a homogeneous solution was obtained, then 52 g of silica gel G (E. Merck) were added. Of the previous solution 300 ml were poured into a dish (15 × 20 × 2.5 cm), and a glass plate (12×16×0.1 cm) was dipped into it. Both side of the glass were covered homogeneously. The glass was hung for 2 min. over the dish to let the excess solution drain off. It was then air dried 3 hrs. and heated at 100°C for 30 min. The plate was cooled in a desicator.

Preparation of polyamide layer. Twenty g of polyamide were dissolved in 90 ml of formic acid; then 10 ml of distilled water were added, and proceeding as described in the previous method, but without adding silica gel G.

Preparation of silica gel layer. Dilute slurries of silica gel G (45 g in 100 ml of water) were sprayed at 2 kg/cm^3 pressure from a distance of 20 cm onto 8 sheets of glass plate (12× 16 cm) in a horizontal position, then dried at 100°C for 30 min. The thickness of layers was about 250 μ.

Chromatographic procedure

A 0.5% alcoholic solution of vitamins were applied to the starting line 1.5 cm from the bottom of the layer, and the plate was developed in the dark by ascending techniques. The chamber had been equilibrated with the respective solvent for 30 min. before use.

Visualization. Riboflavin phosphate (sodium) can be recognized under UV light at 254mμ. The layers were sprayed with a 0.05% Rhodamine B alcoholic solution, and all the other spots could be observed under UV light at 254 mμ.

TABLE I

CHROMATOGRAPHIC DATA

Solvent I: ethanol–ether–cyclohexane–glacial acetic acid (23.5:6:21:2);
Solvent II: ethanol–dioxane–cyclohexane–glacial acetic acid (23:6:30:2).
P–S, Rf value obtained on polyamide–silica gel layer; S, silica gel layer; P, polyamide layer.

No. Substance	Solvent I			Solvent II			Color[b]
	P–S	S	P	P–S	S	P	
1 Vitamin D_2	0.97	0.91	0.96	0.97	0.93	0.97	V
2 Vitamin E	0.91	0.73	0.95	0.89	0.91	0.95	O
3 Vitamin A	0.85	0.70	0.94	0.85	0.90	0.94	O
4 Vitamin D_3	0.81	0.58	0.93	0.81	0.84	0.93	O
5 p–Amino benzoic acid	0.69	0.83	0.47	0.62	0.69	0.56	V
6 Nicotinamide	0.41	0.50	0.76	0.39	0.33	0.85	V
7 Dicethiamine hydrochloride	0.32	0.27	0.92	0.35	0.21	0.94	V
8 Vitamin C	0.24	0.71	0.17	0.31	0.64	0.09	V
9 Riboflavin phosphate (sodium)	0.18	0.03	0.26	0.22	0.53	0.41	—
10 Vitamin K	0.08	0.76	0.04	0.08	0.64	0.05	V
11 Thiamine hydrochloride	0.02	0.03	0.76	0.02	0.04	0.80	V
12 Thiamine acetate	0.02	0.03	0.76	0.02	0.04	0.80	V
Time required (min)[a]	100	120	240	105	160	270	

a : Time required to ascend 10 cm from origin.
b : Fluorescence under UV light after spraying Rhodamine B
O: Orange color; V: Violet color

Results and Discussion

Polyamide layer chromatography has been used for the separation of many compounds. Separation is based on the formation of hydrogen bonds between the CONH group of polyamide and the OH group of the sample. However, the method is not effective when the compounds for the separation are not forming a hydrogen bond with the carbonyl oxygen of Polyamide. For this reason, its application is rather limited and not suitable for general purpose. On the other hand, separation by silica gel is based on the mechanism of adsorption or partition. It has been most extensively used because of its highest capacity and strong activity. The polyamide–silica gel mixed layer has the advantage of both the polyamide layer and silica gel layer. The separation on the mixed layer is based on the formation of hydrogen bonds between

the polyamide and the sample and adsorption between the silica gel and the sample. The layer does not crack or peel and can be stored easily. Both sides of the glass plate are independent to each other and two chromatograms can be run simultaneously.

Rf values of polyamide–silica gel mixed layers, silica gel layers and polyamide layers with two solvent systems are given in TABLE I. It has been found that the results obtaining using the mixed layers show better separation and sharper spots. This method is suitable for the identification of various vitamins.

Riboflavin phosphate can be recognized only before spraying of Rhodamine B alcoholic solution and the spots show yellowish fluorescence. For better visualization of spots under UV light, the amount of spray should be carefully controlled. Over spraying of Rhodamine B solution easily spoil the orange fluorescence of Vitamin A, D_3, E. As vitamins are rather sensitive to light, the plate should be developed in the dark.

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中 文 摘 要

維他命之多醯胺—矽膠混合薄層分析

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12種水溶性及油溶性維他命應用 Polyamide-Silica gel 混合薄層，Polyamide 薄層，Silica gel 薄層等三種薄層分析法分別進行鑑別並檢討所得之結果爲 Polyamide–Silica gel 混合薄層分析法較好。