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Polyamide layer chromatography

XIX. Repeated use of polyamide layers

Polyamide layer chromatography has been used in the analysis of 1-dimethylamino-naphthalene-5-sulfonyl-(DANS)-amino acids[1], dinitrophenyl-(DNP)-amino acids[2], phenylthiohydantoins (PTH) of amino acids[3], lactones[4], nucleobases, nucleosides[5] and nucleotides[6] with satisfactory results. Recently we devised a method of washing used polyamide layers. The washed layers were found as good as new ones even after five repeated uses. This paper describes the method of washing acidic, neutral and basic substances from polyamide layers and shows their reproducibility.

Materials

The polyamide layers were prepared according to WANG *et al.*[7]. 2,4-Dinitrophenyl amino acids, inosine and coumarin were used as samples. These samples were the same quality as was used before (see refs. 2, 4 and 5). All solvents were purified to meet chromatographic requirements.

Methods

The chromatographic method was the same as was used in earlier work[2,4,5]. The chromatograms were washed with two kinds of wash solution; (A) acetone–ammonia water (29 % NH_3) (9:1, v/v) and (B) acetone–90 % formic acid (9:1, v/v). After soaking the used layers in either of the two wash solutions for 6 h, the layers were washed several times with purified methanol, then hung up and air dried. Afterwards, hot air (about 70°) was blown over the air dried layers for a few seconds only to make sure that they were completely dry; longer blowing caused damage to the polyamide layers.

Results and discussion

Table I shows the R_F values obtained for DNP-alanine (acidic), inosine (basic) and coumarin (neutral) after each washing. The R_F values on layers used five times repeatedly were almost the same. The developing times were also the same after each

TABLE I

THE R_F VALUES OF REPEATEDLY USED POLYAMIDE LAYERS

Samples	*R_F value for repeat no.*					*Solvent**	*Development time (min)*
	1	*2*	*3*	*4*	*5*		
DNP-alanine	0.50	0.50	0.50	0.50	0.50	I	60 ± 3
Inosine	0.54	0.55	0.55	0.55	0.55	II	95 ± 3
Coumarin	0.45	0.45	0.45	0.45	0.45	III	65 ± 3

* Solvent system: (I) Benzene–glacial acetic acid (4:1, v/v); (II) acetone–glacial acetic acid (9:1, v/v); (III) water–90 % formic acid (9:1, v/v).

washing. This meant that the polyamide layer did not change its sorption characteristics after several developing and washing processes.

The wash solution (A) was good for acidic substances. The solution (B) was excellent for basic and neutral types of samples. The dipping time in wash solution (A) should not exceed more than 6 h, otherwise the polyamide layers would peel off. In wash solution (B), the sheets were not harmed even when left for over 3 days.

It was found that the layers should be dipped into the wash solution immediately after development and location of the spots. The samples will be sorbed irreversibly after long standing (for example, overnight).

It is clear from these results that polyamide layers could be used repeatedly. In theory, they can be used an infinite number of times, but mechanical damage caused by handling and decomposition of polyamide resin by developing solvents will restrict it to somewhere around ten times. We have used washed layers in quantitative analysis of DNP-amino acids successfully[8].

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Simple preparative thin-layer chromatography

Numerous attempts have been made to exploit the advantages of thin-layer chromatography for preparative work. Such methods are inconvenient, quite apart from the increase in scale required. Thus to elute the components of mixtures directly from chromatograms needs special apparatus and procedures[1,2] and to recover components by extraction involves subsequent removal of fine adsorbent particles. For best results prior concentration "on the layer" by additional chromatographic steps[3,4] is needed. Consequently most workers still use thin-layer chromatography only for analysis and for preparative work move to classical column chromatography[5]