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Silica gel or cellulose for the thin-layer chromatography of indole-3-acetic acid?

Silica gel layers have been widely used in the separation and determination of indole compounds by thin-layer chromatography[1-8]. However, there are some reports[9,10] showing that the indole derivatives cannot be recovered without considerable losses when chromatographed on silica gel, but these losses can be reduced by using cellulose layers. Thin layers of cellulose have been successfully employed for the separation of urinary indoles by SMITH and co-workers[11] also. In contrast, COLLET and co-workers[4], as well as TAFURI[7], have obtained excellent recoveries of added indole-3-acetic acid (IAA) from silica gel. Therefore, to explain these contradictions, a comparative investigation was carried out for the estimation of IAA chromatographed on both silica gel and cellulose layers.

Experimental

The thin-layer plates were prepared in the usual way using a Desaga apparatus, and Kieselgel H or HF_{254} (Merck) and MN 300 Cellulose (Macherey-Nagel) powders, respectively. The layers were 250 μ in thickness. The silica gel plates were activated for 30 min at 105°, and those coated with cellulose were air dried overnight. Both types of layers were used with and without previous washing with ethanol–HCl (1.06) (5:1). The IAA samples (Merck, always 120 μg) were dissolved in methanol and applied as small bands to the starting line with a calibrated blood pipet, under a stream of cold air. Then, after equilibration for an hour, the plates were developed with isopropanol–ammonia (0.88)–water (80:5:15) or isobutanol–ammonia (0.88)–water (10:1:1) as solvents, in the dark at room temperature. The solvents were renewed every second day. The chromatograms were dried in darkness for 2 h in a stream of cold air or 16 h in a fume-cupboard at room temperature.

After location of the IAA bands under a long-wave U.V. lamp, the layers containing IAA were scraped off into a mixture consisting of 4 ml of methanol and 2 ml of Salper-reagent[12], agitated for 30 min and filtered through a glass filter. The optical density of the filtrate was measured in a spectrophotometer at 530 mμ and the quantity of IAA read from a calibration curve. 120 μg IAA placed in a small Petri-dish and treated in the same way as the plates themselves, and a blank layer, respectively, served as controls in each run. All measurements were repeated three times or more.

Results and discussion

The effect of different layers and drying times on the IAA recoveries is shown in Table I. There was practically no detectable effect on the results due to the solvent or the washing of the layer, therefore the data referring to the solvent isobutanol–ammonia–water, as well as the washed layers, are omitted.

As can be seen from the results, if the drying time does not last more than 2 h, there is no difference between the IAA recoveries from the two media. The 20–25% loss of IAA during the chromatographic and determination processes can be regarded as normal[10,13]. The very high (95–98%) recovery figures reported by others[4,7] seem

TABLE I

IAA RECOVERIES FROM SILICA GEL AND CELLULOSE LAYERS

Solvent: isopropanol–ammonia–water (80:5:15). S = Kieselgel H or HF_{254}; C = MN 300 Cellulose. The applied quantity of IAA was 120 μg in every case. Numbers followed by the same letters are not significantly different at the 5% level.

Layer	*Drying time (h)*	*IAA measured (μg)*	*Recovery (%)*
S	2	93.5	77.9[b]
	16	57.0	47.5[c]
C	2	94.0	78.3[b]
	16	89.0	74.6[b]
Dish control	2	105.5	87.9[a]
	16	96.0	80.0[b]

to be exceptional ones. However, if the removal of the plates from the tanks is followed by prolonged drying, then the IAA recovery from silica gel layers decreases sharply, while the related changes in the case of the cellulose plates remain negligible.

The cause of the IAA-destruction on silica gel after the longer drying time *i.e.* 16 h in contact with air, and its existence even after washing is not clear. The adsorption of IAA on plaster of Paris suggested by OPIEŃSKA-BLAUTH and co-workers[10] can be excluded because this effect is not applicable in the case of Silica Gel H or HF_{254} which do not contain this binding material. Probably the large surface of the silica gel in the layer affords favourable conditions for the oxidation of IAA by atmospheric oxygen. The conclusion can be drawn, however, that for the chemical determination of IAA by means of thin-layer chromatography, silica gel is as good as the MN 300 Cellulose powder if the chromatograms are dried for not more than 2 h. On the other hand, if prolonged standing of the plates before the assay cannot be avoided, the use of MN 300 Cellulose instead of silica gel is to be recommended.

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