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Note

Low-fluorescence polyamide sheets for thin-layer chromatography

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The TLC-Ready Polyamide Sheets F 1700 Micro-Polyamide (Schleicher & Schüll, Dassel, G.F.R.) are especially useful for the micro-chromatography of dansylated amino acids and consist of a 0.2-mm thick polyester foil, coated on both sides with a layer of polyamide approximately 25 μm thick. The lowest limit of detection in a quantitative or qualitative analysis of such a chromatogram is determined by the background fluorescence: small amounts are visible or measurable only if their fluorescence is above the background fluorescence of the polyamide sheet.

Background fluorescence of TLC-Ready Polyamide Sheets F 1700 is visible under a UV lamp (excitation at 365 nm); it covers the whole range of visible light and can be used for measuring fluorescence quenching by UV-absorbing samples[1]. It consists of the fluorescence of the polyamide layer and that of the polyester foil; the latter part is highest under diffuse illumination and can be eliminated by the use of a non-fluorescent foil. The fluorescence of the polyamide layer can be reduced by intense UV illumination (bleaching).

For a quantitative comparison with TLC-Ready Polyamide Sheets (on polyester), Schleicher & Schüll supplied us with F 1700 Micro-Polyamide in layers on passivated aluminium foil*. Excitation was at 365 nm (Osram HBO 200 lamp with Schott BG38 and UG1 filters) and fluorescence was measured with an RCA 1P28 photomultiplier behind a Schott GG10 barrier filter. The diameter of the circular measuring field on the sheet was 0.12 mm, the travel of the measuring spot was 20 mm and values were recorded with a strip recorder. Table I shows the mean values of the fluorescence in arbitrary units for (1) TLC-Ready Polyamide Sheets F 1700 Micro-Polyamide on polyester (Schleicher & Schüll), (2) F 1700 Micro-Polyamide on aluminium foil and (3) F 1700 Micro-Polyamide on aluminium foil bleached for 20 min. Four kinds of illumination were used: (a) vertical epi-illumination, illuminated field 0.3 mm in diameter; (b) vertical epi-illumination, illuminated field 1.5 mm in diameter; (c) vertical epi-illumination, illuminated field 4 mm in diameter; and (d) diffuse illumination at 45°.

Variation in the thickness of the layer and bleaching during measurement causes a standard deviation of 10% in the figures given in Table I.

* Now commercially available from Schleicher & Schüll under the name TLC-Ready Polyamide Sheets A 1700 Micro-Polyamide.

TABLE I

FLUORESCENCE OF POLYAMIDE SHEETS (ARBITRARY UNITS)

Sheet	*Illumination*			
	Vertical			*45°, diffuse*
	Field 0.3 mm	*Field 1.5 mm*	*Field 4 mm*	
Polyamide on polyester	3.4	7.0	11	30
Polyamide on aluminium	3.2	3.5	3.3	5.3
Bleached polyamide on aluminium	0.6	0.7	1.0	1.0

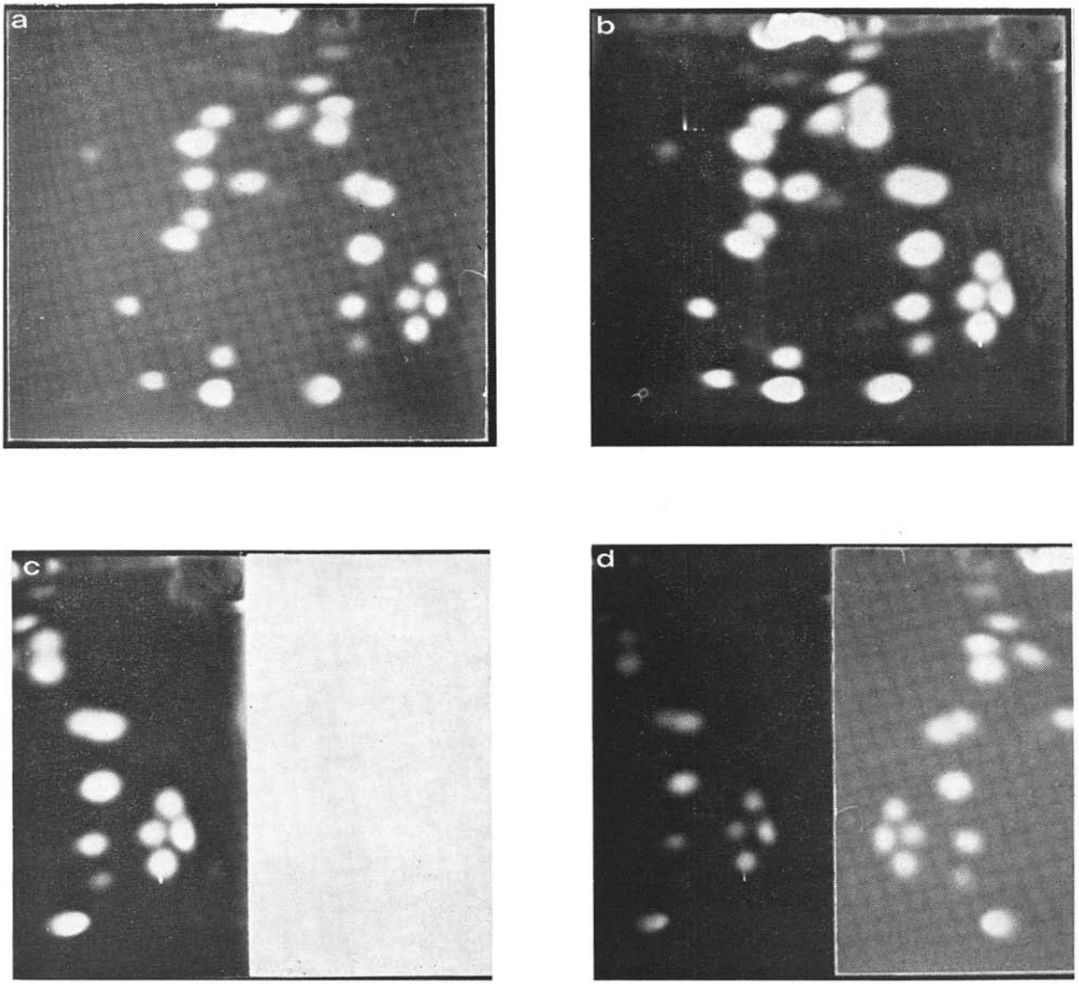

Fig. 1. Micro-chromatograms (3 × 3 cm) of Dns amino acids as described by Schulze and Neuhoff[3]. (a) F 1700 micro-polyamide on polyester; (b) the same layer on passivated aluminium; (c) and (d) two mirror-like halves of chromatograms on aluminium foil (left) and polyester foil (right) photographed simultaneously. Correct exposure for the chromatogram on aluminium (c) or on polyester (d) results in over- or under-exposure of the other half of the chromatogram.

The fluorescence of the polyamide sheets on polyester depends strongly on the size of the illuminated field. Under diffuse illumination, the aluminium foil reduces the background fluorescence to almost one sixth and improves the detection limit in the same proportion. Diffuse illumination is normally applied during visual inspection or photography of chromatograms. In photometry with small illuminated fields, the aluminium foil offers no advantage over polyester foil with respect to background fluorescence, but with reflection it increases the measurable part of the fluorescence emitted from a spot.

Bleaching of the polyamide layer requires UV illumination for 5–20 min with an HBO 200 lamp at a distance of 10 cm. Table I shows that the effect of bleaching is almost independent of the kind of illumination. The background fluorescence of bleached sheets recovers during storage for 1 week. If increased sensitivity of polyamide sheets is necessary for special applications, the sheets have to be bleached and evaluated within 1 day.

The chromatographic properties of TLC-Ready Polyamide Sheets are not changed by the passivated aluminium foil[2] or by bleaching. Fig. 1 shows microchromatograms of Dns amino acids. The same amounts were developed by the same method[3], but in the first dimension mirror images on a regular TLC-Ready Polyamide Sheet F 1700 Micro-Polyamide on polyester (Fig. 1a) and on an F 1700 micropolyamide layer on an aluminium foil bleached for 20 min (Fig. 1b) were obtained. For better comparison, the chromatogram in Fig. 1a is reproduced inverted. Fig. 1c and 1d show the corresponding halves of chromatograms, the left half an aluminium foil and the right half a polyester foil, photographed simultaneously in order to eliminate the influence of the photographic procedure. A correct exposure of one half results in over- or under-exposure of the other half. A comparison of all pictures shows that the spots in the chromatogram on aluminium foil are as well separated as on polyester foil. In Fig. 1b, the spots appear to be larger owing to low concentrations, which are detectable on the dark background but are lost in the high background fluorescence in Fig. 1a. The original size of the chromatograms was 3 × 3 cm. The following conditions were used: illumination, HBO 200 lamp at 45°; excitation filters, 2 mm BG38 and 3 mm UG1; barrier filter, 1 mm GG10; film, Ilford PAN F; exposure time, 1s for Fig. 1a and 1d and 2s for Fig. 1b and 1c.

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