Photographic detection and documentation of U.V.-absorbent and fluorescent nucleotides on cellulose TLC plates

A recent note[1] has described the thin layer chromatography of nucleotides on U.V.-transparent glass in order to facilitate photographic recording of U.V.-absorbing components. This method is limited both by the size of such glass available and by its cost.

The present note describes a method for the detection and recording of both U.V.-absorbent and fluorescent compounds on the same photograph, using ordinary glass plates of any size, and has therefore the two advantages of versatility and cheapness.

Methods

Source of U.V. light. The source was similar to that described by MARKHAM[2,3] and consisted of an 80 W high pressure (*e.g.* Mazda MB/V) mercury lamp (removed from its enclosing glass bulb) the light from which was filtered first through a quartz flask filled with $CoSO_4$–$NiSO_4$ solution and then through one containing chlorine. This produced light of a wavelength of mainly 265 mμ.

Photography

Bromide paper (*e.g.* Kodak WSG. 3S) was used. The photographic paper was laid on a sheet of stiff cardboard or rubber and covered by the TLC plate (layer upwards); the latter was framed by a cardboard mask larger than the plate in order to prevent refraction of light round the edges of the glass (Fig. 1). The four components were clamped together with "bulldog" clips and suspended vertically about 80 cm from the U.V. source. Exposure times varied from 5–10 min depending upon the thickness of the plate and of the layer, as well as their distance from the source. Development of the bromide paper was carried out in the normal way.

TLC plates

The method has been used with cellulose or modified cellulose (DEAE or

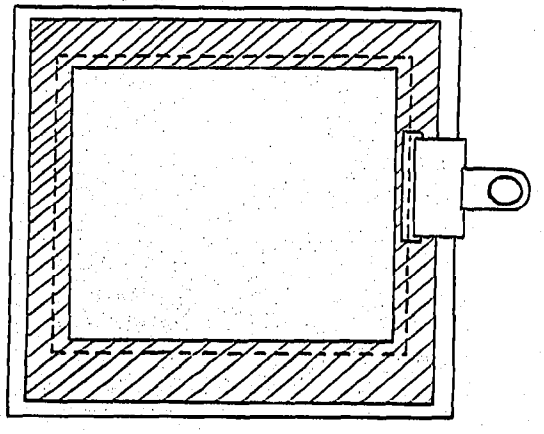

Fig. 1. Method of mounting the TLC plates for U.V. exposure, showing the mask (cross-hatched), extent of plate (dotted line) and one of the four clips.

Fig. 2a. Photograph of plate before exposure to MEK–NH_4OH vapour. Cellulose MN 300. Solvent: isobutyric acid–conc. NH_4OH–H_2O (6:3:1, v/v). 1,5: UMP; 2,6: AMP; 3,7: ADP and AMP; 4,8: NAD.

Fig. 2b. Same plate after exposure to MEK–NH_4OH vapour.

polymin*) layers on plates of "picture glass" (20 × 20 × 0.2 cm), the layers being spread with slit widths of 0.4 mm.

Discussion

The appearance of the resulting photographs is shown in Fig. 2a. With sufficient exposure, enough light penetrates the glass plates to give a grey background on which absorbent compounds appear as white spots. Apart from giving a permanent record, the method provides a very sensitive means of detecting such compounds. It has the additional advantage that fluorescent materials show up as dark spots. Thus, the absorbent spots 4 and 8 shown in Fig. 2a were shown to be due to NAD by exposure of the layer to methyl ethyl ketone–conc. ammonia (50:50) vapour and rephotographing (Fig. 2b). Similarly, guanosine derivatives may be detected by exposure of the layer to conc. hydrochloric acid fumes. The glass plate takes the place of the cellulose nitrate used by SMITH AND MARKHAM[4].

The technique is applicable to sheets of Chromagram Cellulose 6064 (Kodak Ltd., Kirkby, Liverpool), the exposure to U.V. light being much shorter.

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* Polyethyleneimine.