A simple method for photographing ultra-violet absorbing spots on paper chromatograms

Recent studies in this laboratory have necessitated the purification of folic acid derivatives. These compounds may be observed when run on paper chromatograms by virtue of their ultra-violet absorption properties. In order to have a permanent and readily observable record of these chromatograms the following procedure for photographing was devised.

The chromatogram to be photographed was placed on a piece of Agfa "Copyrapid negativ" paper, and the two kept in contact by placing a piece of sheet glass on top of them. The chromatogram was exposed, through the glass, to a U.V. source by scanning with a U.V. lamp (in this case a Mineralight, model SL 2537) at a height of about 12 inches. The correct exposure time depended necessarily on the thickness of the chromatogram and the sheet of glass. It was in the order of 5–20 seconds. The exposed negative was developed in a "Copease Book Copier" machine using Agfa "Copyrapid developer" and Agfa "Copyrapid positiv" paper. The whole procedure of exposing and developing was carried out in a semi-darkened room.

The final positive consisted of a completely white background with the absorbing areas black. With short exposures any fluorescent spots present will appear as grey areas, with longer exposures they will be absent.

As many laboratories possess some model of a photocopying machine, and hence the necessary positive and negative material, developer and means of developing, the method described provides a very simple and convenient way of photographing U.V. absorbing areas on paper chromatograms.

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A simple chromatographic technique for the removal of dinitrophenyl-amino acids from excess dinitrophenyl-artifacts

In the dinitrophenylation (DNP) method[1] of N-terminal analysis of a protein, excess fluoro-dinitrobenzene (FDNB) is generally removed from the DNP-reaction mixture by washing the acidified solution with organic solvents prior to the acid hydrolysis of the DNP-protein. Small amounts of bound FDNB produce dinitrophenol (DNP-OH) on acid hydrolysis and this is normally removed from the ether-soluble DNP-amino acids by either cold finger sublimation[2] or column chromatography[3,4] before the DNP-amino acids can be estimated by paper chromatography[5]. The method of LI AND ASH[3] employs a silicic acid column containing water as the stationary phase and chloroform equilibrated with water as the mobile phase. The most soluble

DNP-amino acids in the mobile phase, such as di-DNP-lysine and DNP-leucine, travel close to the artifacts of which DNP-OH is almost colourless at this pH. In the presence of excess artifacts these fast moving DNP-amino acids can become masked and either discarded with the artifacts or the resultant DNP-amino acid mixture may contain enough DNP-artifacts to prevent a clean fractionation of the DNP-amino acids by paper chromatography. The present technique is a modification of the LI AND ASH[3] method which readily separates all the ether-soluble DNP-amino acids from excess artifacts (DNP-OH, DNP-NH_2, etc.) due to the selective binding of the DNP-amino acids on an alkaline column. Silicic acid (Mallinckrodt 100 mesh) 5 g is carefully ground with 2.5 ml 0.067 *M* Na_2HPO_4 and then made into a fine slurry with chloroform which is then poured into a column 1 cm × 30 cm and gently packed with a hand operated perforated piston.

The ether-soluble DNP-extract after concentration to dryness by vacuum distillation is dissolved in chloroform and applied to the top of the column. The column is first developed with chloroform equilibrated with 0.067 *M* Na_2HPO_4, the artifacts are yellow at this slightly alkaline pH and only partially ionised which enables the chloroform to wash them off the silicic acid. The DNP-amino acids on the other hand have their carboxyl group in the ionised form at this pH and consequently are insoluble in the organic phase and remain bound to the aqueous phase near the top of the column. The DNP-amino acids are then eluted with chloroform containing 1 % v/v glacial acetic acid which lowers the pH of the aqueous phase with the resultant suppression of the ionisation of the DNP-amino acid carboxyl group and solution in the mobile phase. The eluate is concentrated by vacuum distillation and DNP-amino acids can then be quantitatively estimated by the conventional paper chromatographic techniques[5].

This technique has been developed to study the N-terminal residues of a mixture of non-protein nitrogen components physically associated with soluble collagen[6]. In an analysis of this type it is not possible to free the DNP-reaction products from excess FDNB by acidification and solvent extraction without removing DNP-non-protein nitrogen components which are partially released from collagen under these conditions. The total DNP-reaction mixture must first be acidified, concentrated to dryness and then hydrolysed, with the production of excess DNP-OH. All the steps in this technique (apart from vacuum distillation) may be performed in the dark since it is not necessary to watch the columnar fractionation at all.

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