

The preservation of paper chromatograms sprayed with ninhydrin

Excellent colour differentiation on chromatograms of amino acids is obtained by spraying with a solution of ninhydrin in chloroform containing a small amount of collidine¹ using mild heating conditions for development (about 2-3 min at 80°). Under these conditions, colours are seen which differ in many instances both in hue and intensity from the purple colours given by the majority of amino acids in the absence of collidine. These characteristic colours, which are often of use in confirming the presence of known substances or in revealing lack of homogeneity of incompletely resolved spots, usually fade to a uniform purple colour within 5-10 min under normal laboratory conditions. For this reason comparisons between large numbers of chromatograms are difficult and the comparing of chromatograms prepared and sprayed at different times is also precluded. It is possible to retard considerably the colour changes by sealing the chromatogram in a polythene bag immediately it has been developed. This effectively reduces loss of residual collidine, the presence of which appears to be essential both for the differential colouring and the stability of the colours. At the same time the chromatogram is protected from the atmosphere of the laboratory, which often hastens colour destruction. It has not been found satisfactory to develop the chromatogram in the bag, nor is there any advantage in increasing the collidine content of the spray beyond 0.2%. The stability of the colours, though much improved by this technique is still affected by light and raised temperature. However, if the chromatograms are stored in the dark at about 4° it is possible to retain the original colours for up to 14 days. After this period the spots gradually assume the more usual purple colours, but here again, continued storage in the cold and away from light leads to a considerable increase in stability. Chromatograms have been successfully stored in this way for up to 3 months with little change in the intensity or colour of the spots. The spray we have found most successful is prepared by adding ninhydrin (200 mg) to collidine (0.2 ml), in which it dissolves completely, and making to 100 ml with chloroform. The method of development is critical, the chromatogram being removed from the heating oven immediately visual inspection indicates colour differentiation is optimal. At temperatures much above 80° this stage is reached so rapidly that it is difficult to obtain satisfactory results. The chromatograms are then placed immediately in the polythene bags which are closed by turning over the tops and sealing with adhesive tape.

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