AN IMPROVED METHOD FOR IDENTIFICATION OF AMINO ACIDS IN DESCENDING PAPER CHROMATOGRAPHY[1, 2]

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ABSTRACT

A change in paper shape is described for use in descending paper chromatography. The "flask-shaped" paper provides greater reliability in the identification of amino acids in unknown mixtures. As many as three samples of unknown and/or known components can be run simultaneously on the same paper and under the same conditions. The method has been used by other investigators for the separation of pteridines and carbohydrates and has provided increased resolution and adaptibility.

Studies with extracts of yolk-albumen of the hen's egg in terms of their nutritional adequacy for differentiation of embryonic chick eyes in vitro led us into paper chromatographic analysis of the fractions. The active extract contains water- and alcohol-soluble substances, including unknown carbohydrate material and ninhydrin-positive substances. One of the ninhydrin-positive substances has a molecular weight of 10,000 or more. Since this does not move on chromatographic paper when 80 per cent phenol-water or butanol: acetic acid: water (5:1:4) are used as developers, it has been possible to investigate the remaining ninhydrin-positive substances which were presumed to be amino acids. Using Whatman #1 paper and the above developers, the usual types of one-dimensional descending and two-dimensional ascending chromatograms were developed (Block et al., 1955). At best, six spots were resolved using these methods. To answer the need for better resolution of the unknown mixture and for greater reliability in the identification of amino acids, a modification was made in the shape of the paper.

The suggestion for a change in paper shape originated from the report by Reindel

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and Hoppe (1953). Chromatogram "B" in figure 1 was modeled after their work. They found that a narrow neck, extending from the source of the developer, which flared out into the body of the chromatogram paper increased the resolution of amino acids. While searching the literature for these details of their work, we developed the paper shape shown by chromatogram "A" in figure 1. This "flask-

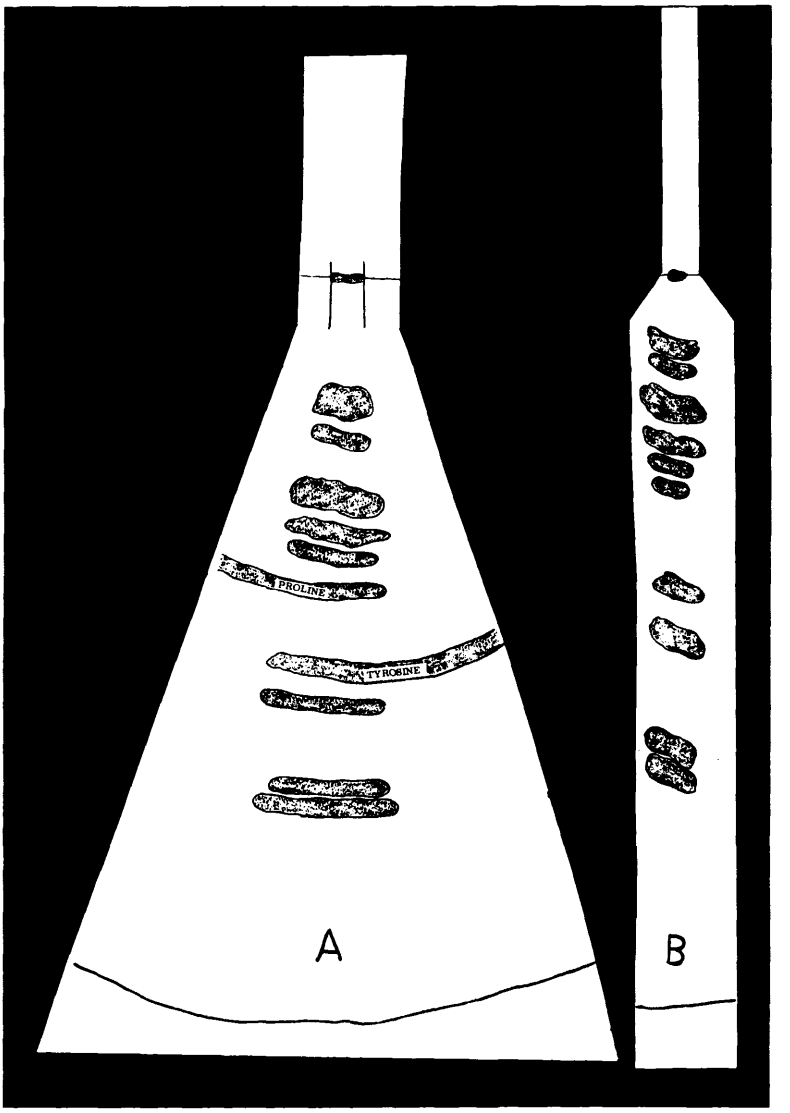

FIGURE 1. Comparison of two chromatographic paper shapes in the resolution of amino acids.

shaped" paper provides a somewhat improved resolution. More importantly, it provides a means of simultaneous application of more than one sample with the inherent ability to test more than one compound or mixture on the same paper in the same solvent under the same conditions. This is of significant value when one is dealing with the possibility that either of two amino acids with close R_F values

may be in the unknown mixture. It is possible to develop simultaneously the unknown mixture in the center with two known amino acids, one on each side of the unknown. In the "flask-shaped" chromatogram of figure 1, the unknown mixture from extraction of yolk-albumen, proline, and tyrosine were developed simultaneously. In this instance the chromatogram was sprayed with ninhydrin and, after the color had developed, individual spots were outlined and subsequently penciled in for photographic purposes. This chromatogram illustrates the use of the method in identification of unknown amino acids. The method is also useful in comparing the qualitative composition of two fractions prepared at different times.

We have adopted the following dimensions for the paper in our work: bottom of "flask" 22.8 cm; height of "flask" 38.2 cm; width of neck 3.5 cm; length of neck to flare 10.2 cm; point of application 1.8 cm above flare; and slit in the neck 0.5 cm above point of application and extending down to the beginning of the flare. Any slit in the neck separating different materials applied to the paper should extend at least to the beginning of the flare to provide best results.

Other investigators at Miami University have adopted this modified paper shape in their work. The method has been used for the separation of pteridines and carbohydrates. In all the work, increased resolution and adaptibility to simultaneous comparisons have been of significant value.

LITERATURE CITED

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