

to the inhibition caused by fatty acids released during digestion. The effect of phospholipase A on the oxidase activities of the electron transport particle and the release of cytochrome *c* from the particle are shown in Table 1.

These results (10) provide evidence that cytochrome *c* exists in the electron transport particle as a phospholipid-cytochrome *c* complex similar to insoluble complexes of cytochrome *c* which have previously been described (6).

KISHORE S. AMBE\*  
FREDERICK L. CRANE

Institute for Enzyme Research,  
University of Wisconsin, Madison

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  10. We wish to thank Dr. D. E. Green for his encouragement of this investigation. The valuable technical assistance of Mrs. Wanda Fechner is gratefully acknowledged. This work was supported in part by research grants RG-5506 from the Division of Research Grants and H-458 from the National Heart Institute, both of the National Institutes of Health, and by Atomic Energy Commission contract No. AT(11-1)-64, project 4. The animal tissue was generously furnished by Oscar Mayer and Company, Madison, Wis.
- \* Postdoctoral fellow, U.S. Department of State.

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### Multiple pH Levels in Chromatograms

**Abstract.** Lines of buffer solutions drawn longitudinally on chromatograms with a capillary pipette provide different levels of pH which affect the  $R_f$  values, color, fluorescence, and other properties of the chromatographed substances.

In chromatographic studies of the decomposition products of indole derivatives (1) we have observed the influence of pH and of the nature of buffer solutions on the following properties of the chromatographed substances: (i)  $R_f$  values; (ii) color, fluorescence, and absorption of short-wave ultraviolet light; (iii) changes in color, fluorescence, and absorption which result from exposure of the chromatograms to ultraviolet radiation; and (iv) color reactions with several reagents.

Most of those effects can be conveniently observed in a single chromatogram prepared as follows: On a 7- by 15-cm sheet of Whatman No. 1 filter paper,

four parallel, lengthwise lines are drawn with a straightedge and a capillary pipette filled with buffer solutions at convenient pH values. After the lines have dried, the solution to be chromatographed is applied with a pipette, crosswise to the strips of buffer solution, in a band 5 mm wide, over the whole width of the sheet.

Figure 1 shows a chromatogram, prepared according to this method, of a  $10^{-3}M$  aqueous solution of indole-3-acetic acid, decomposed by ultraviolet radiation. Citric acid (0.1M)-disodium phosphate (0.2M) buffer solutions at pH 3, 5, 7, and 8 were applied along the indicated vertical lines. Ascending chromatography with acetone and water (8/2) was used. The outline of the fluorescent zones of the completed chromatogram is shown. Except for zones IV and VI, which were not affected by the buffer strips and which are presumably nonionizable substances, all the zones have more or less wavy outlines. Zones IV, V, and VI overlap, and zone V is visible as an increase of the fluorescence of zones IV and VI, according to a wavy pattern.

An example of the graphic analysis to which the zones may be submitted is shown for zone II. Closed circles mark the middle of the zone at the level of the buffer strips and crosses that at the interval between strips. At low pH levels the substance is in the undissociated form if it is an acid and in the cation form if it is a base. Its  $R_f$  value for zone II at the pH 3 level is approximately 0.1, and the  $R_f$  value of the crosses is, on the average, only slightly greater. In between the buffer strips the substance is therefore in the same form as it is at the pH 3 level (2).

At high pH levels the substance is in the undissociated form if it is a base and in the anion form if it is an acid. At the pH 5, 7, and 8 levels, zone II has an  $R_f$  value in the neighborhood of 0.28, which may be taken as that of the base or anion. It is seen that the anion (or base) is more soluble in the solvent than the acid (or cation) of the substance of zone II. Traces of acidic or basic vapors in the chromatographic tank may shift the  $R_f$  value in between strips from the undissociated to the ionic  $R_f$  value or vice-versa.

Regions of more intense fluorescence are represented by heavier hatching. They were conspicuous in zones II, III, VII, IX, and XI. The dark areas of zone X (indoleacetic acid) due to the quenching of the blue fluorescence of the filter paper under short-wave ultraviolet light were more clearly visible at the lower pH values.

As a result of exposure of the chromatogram to ultraviolet radiation, the outline of zone X, in part overlapping zone XI, was made clearly visible,

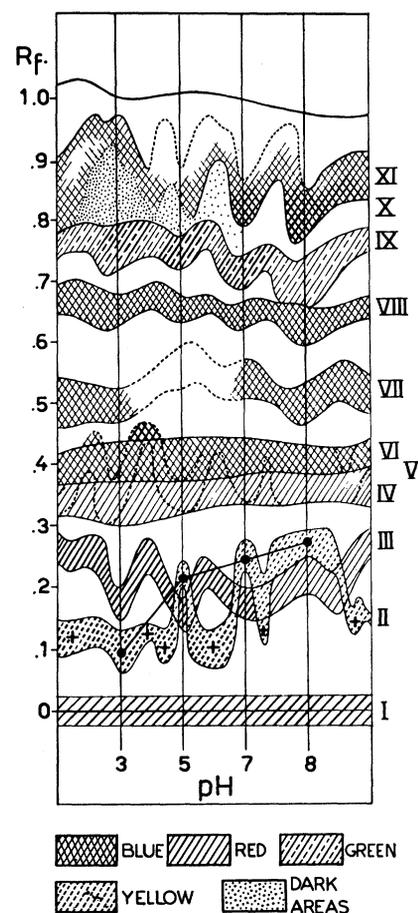


Fig. 1. Chromatogram of decomposition products (by ultraviolet radiation) of indole-3-acetic acid. Ascending chromatography. Acetone and water, 8/2. Citric acid-disodium phosphate buffer solutions (0.1M) at pH 3, 5, 7, and 8 were applied along the vertical lines before chromatography. The decomposed solution was applied at I. The approximate fluorescence color is indicated by conventional hatching.

thanks to its faint blue fluorescence at the lower pH levels and its strong turquoise fluorescence at the level of the pH 8 strip (3, 4).

A. A. BITANCOURT  
ALEXANDRA P. NOGUEIRA\*  
Plant Cancer Research Center,  
Instituto Biológico, São Paulo, Brazil

#### References and Notes

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  2. Difference in solubility of citric acid and disodium phosphate changes the pH of the strips as the solvent ascends. In short chromatograms, however, the change is not likely to be very important or to alter greatly the effect of pH on the chromatographed substances.
  3. A detailed analysis of this chromatogram and of other similar ones and the conclusions therefrom regarding the chemical identity of the decomposition products of indole-acetic acid is in preparation.
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- \* Research fellow, Brazilian Research Council.  
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