
LETTER

A Note on the Identification of Ortho-, Meta- and Pyro-Phosphates by Means of Paper Chromatography⁽¹⁾

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(Received December 18, 1951)

It is often desired in the field of biochemical studies to identify easily ortho-, meta-, and pyro-phosphates in minute quantities. A char-

acteristic yellow coloration produced with ammonium molybdate instantly from the ortho-phosphahate and only after heating from meta- and pyro-phosphates, will be usefull for detecting them when combined with a development on filter paper. However, it is difficult to distinguish the spots clearly by this method unless the separation is quite complete, as they are all colored similarly. Only for the ortho-phosphate, several methods have been described for its identification, in which the phosphate was detected either on a paper without development by a blue stain formed by adding a drop of molybdate followed by that of benzidine⁽²⁾ (a

(1) The present paper was read by one of the authors (J. I.) before the 8th Meeting of this institute held on Sept. 29, 1951.

(2) F. Feigl *Z. anal. Chem.*, **61**, 464 (1922); **74**, 386 (1928); **77**, 299 (1929).

spot test), or also on a paper with development by the yellow color of phosphomolybdate or by the blue color formed further from the latter by the action of hydrogen sulfide.⁽³⁾ Nevertheless, none of these procedures has yet been applied for the identification of meta- and pyrophosphates.

The present authors have now developed a method of identifying the three types of phosphates accurately on paper chromatograms. About 0.005 cc. of 0.1 *M* aqueous solutions of sodium ortho-, meta- and pyro-phosphates, respectively alone or in their various mixtures, were developed on paper strips (1.8×20 cm. of the Toyo filter paper No. 2) at 25-30°C. with butanol-acetic acid-water (4:1:5 V/V) as a solvent, followed by spraying with a solution of ammonium molybdate in nitric acid⁽⁴⁾ and by heating, and then by further spraying with a solution of benzidine in acetic acid.⁽⁴⁾ On neutralizing in an atmosphere of ammonia, meta-, pyro- and ortho-phosphates were revealed successively as a blue spot with $R_F = 0$, a purplish red with $R_F = 0.15$, and a blue with $R_F = 0.21$. They were easily distinguished respectively, owing to the difference in color and also to the colorless boundary lines which always appeared among them.

Coexistence of arsenate or nitrate with phosphates was found not to disturb the present identification. Further experiments are now in progress, in which the development with a solvent mixture of modified ratio on the Toyo filter paper No. 50 and the influences of heavy metallic ions upon the chromatograms are respectively examined. Details will be published later elsewhere.

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(3) C. S. Hanes and F. I. Isherwood, *Nature*, **164**, 1107 (1949).

(4) Cf. F. Feigl, "Qualitative Analysis by spot Tests," 3rd English edition (translated by R. E. Oesper), New York, 1947, p. 251.