

THE DETECTION OF PHOSPHATES ON CHROMATOGRAMS

H. ROSENBERG

*Department of Biochemistry, John Curtin School of Medical Research,
Australian National University, Canberra, A.C.T. (Australia)*

A number of procedures have been described for the detection of organic phosphates on paper chromatograms. Most methods involve the use of an acid molybdate reagent, with subsequent reduction by one of a variety of reducing agents (*cf.* HETTLER¹). The most commonly employed are those of HANES AND ISHERWOOD², where the reducing agent is hydrogen sulfide, and of BANDURSKI AND AXELROD³, where reduction is accomplished by ultraviolet irradiation. A procedure for the detection of phosphate spots on chromatograms without the use of molybdic acid has been described by WADE AND MORGAN⁴.

The present communication deals with an alternative method for the chemical reduction of phosphomolybdate spots on paper; the method has proved satisfactory for the detection of a variety of phosphate compounds.

EXPERIMENTAL

Reagents

- (1) *Molybdate reagent*: 5% (w/v) ammonium molybdate solution 3 ml
5 N HCl 7 ml
acetone to 100 ml.

This reagent should not be kept for longer than one week.

(2) *Stock vanadyl chloride solution*. The solution was prepared by dissolving 2 g of vanadium pentoxide, V_2O_5 , in approximately 20 ml of boiling conc. HCl; boiling was continued for 10 min after the colour changed to blue-green. The volume was then adjusted to 37 ml by the addition of 6 N HCl, and the solution diluted with water to 400 ml. This stock solution, which keeps indefinitely, thus contains 0.5% of V_2O_5 in approximately 0.5 N HCl.

- (3) *Reducing reagent*: Stock vanadyl chloride solution 20 ml
acetone to 100 ml.

Approximately 250 mg of fine zinc powder is added to the green coloured reducing reagent and the mixture shaken in a stoppered flask until the solution is quite clear and brown in colour (about 10–20 sec). At this stage the zinc powder settles as a

References p. 489.

spongy precipitate and the clear reagent is recovered by decantation. This reagent should be prepared immediately before use.

Procedure for paper chromatograms

After development, the papers are thoroughly dried and then dipped in the molybdate reagent and allowed to dry in the air at room temperature, or in a stream of warm air. When the acetone has evaporated the papers are dipped in the reducing reagent and again allowed to dry in air. Blue spots are produced in about 2 min on a white background (an immediate blue background colour is produced, but this fades in 2 min). The background remains white for about 24 hours, after which period it slowly turns blue. If desired, the white background may be restored by dipping the chromatograms in a mixture of 1 part of 5 N HCl and 9 parts of acetone. The chromatogram is then allowed to dry in the air, whereupon the background fades slowly, leaving the spots unaffected.

Several papers in succession may be dipped in the reducing reagent, but it is ineffective once its colour has reverted to green. However, it may again be reduced by shaking with Zn powder as before.

The procedure described above was applied to a number of chromatograms. The following spots were detected after evaporation of the molybdate reagent at room temperature and subsequent reduction:

Inorganic orthophosphate (blue); pyrophosphate (purple); phosphocreatine (blue); ethanolamine phosphate (blue-green); adenosine triphosphate and adenosine diphosphate (faint blue). Adenosine monophosphate and glycerophosphate were not detected. Heat treatment of the paper (*cf.* ref.²) in the presence of the molybdate reagent would be required to hydrolyze the more stable esters.

Sensitivity

Since orthophosphate is probably required for the formation of the coloured complex, the sensitivity of the method was determined using successive dilutions of a solution of KH_2PO_4 . The lower limit of detection for orthophosphate was found to be $0.02 \mu\text{g}/\text{cm}^2$.

DISCUSSION

The method described above has been found convenient and rapid in use. The sensitivity limits obtained compare favourably with the other methods (*cf.* BANDURSKI AND AXELROD³ - $0.1 \mu\text{g P}$). There is no critical exposure time at any stage, nor is there any need for rigorous moisture control. The background is completely clear at least within 24 hours of development. The present modification involves the reduction step only; the existing hydrolytic procedures which liberate orthophosphate from the more stable compounds can be applied prior to reduction.

References p. 489.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. F. P. DWYER, D.Sc. of this School for his advice on the preparation of the reduced vanadium reagent.

Mr. B. THORPE provided skilled technical assistance.

SUMMARY

A new method, involving reduced vanadyl chloride in acetone for the identification of phosphates on paper, has been described.

The main advantages of the method are rapidity of use, colourless background and high sensitivity ($0.02 \mu\text{g P/cm}^2$).

REFERENCES

- ¹ H. HETTLER, *J. Chromatog.*, 1 (1958) 389.
- ² C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
- ³ R. S. BANDURSKI AND B. AXELROD, *J. Biol. Chem.*, 193 (1951) 405.
- ⁴ H. E. WADE AND D. M. MORGAN, *Nature*, 171 (1953) 529.

Received December 9th, 1958