

rate, to affect the relative R_F values significantly. R_F values should not be regarded as absolute, but merely an indication of the degree of separation that may be achieved; comparison should always be made with authentic specimens. With care the spray reagent specified may provide further help in identification. For example, the 2-isomers appear as distinct brown spots. The remainder vary from brown/green to blue.

The concentration of the sugar sulphate applied should be near the limit of detection of the reagent to avoid streaking. The proportion of CPC in the solvent is critical and may have to be varied slightly to allow for variations between different commercial samples.

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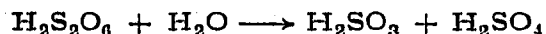
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Detection of the polythionates on paper chromatograms

Tests have been described for the detection of the polythionates in microgram quantities on paper chromatograms^{1,2}. These tests are not always suitable when detection must be followed by elution from the paper for further studies such as measurements of radioactivity. The dithionate ion can be especially difficult to detect in view of its chemical stability. In the test described by POLLARD, McOMIE AND JONES¹ the chromatograms formed are permanent, whereas in the method of GARNIER AND DUVAL² the spots cannot be eluted after detection by the reagents used in a form suitable for further studies. The need for an alternative method of detection arose out of our work on the sulphur metabolism of the thiobacilli.

Although dithionates are very stable, they can be hydrolysed. The salts can only be hydrolysed slowly at elevated temperatures but the free acid can be hydrolysed at 50° quite rapidly³ providing the basis for a test applicable on filter paper:



By spraying the paper with a mixture of hydrochloric acid and hydrogen peroxide followed by gentle warmth the dithionic and other acids are converted to sulphuric

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acid. Excess oxidising agent is detected by a starch-iodide spray leaving thionate spots as white areas. The sulphate formed can be eluted from the paper.

Method

Materials: Whatman No. 4 paper; 10 × 8 in. washed in turn by 0.2 N HCl, 0.2 N ammonia and distilled water followed by drying. Solvent system⁴: isopropanol 50 ml, acetone 20 ml, water 30 ml and sodium acetate 2 g. Sprays: (A) acetone 75 ml, 30 % w/v HCl 4.3 ml, 30 % H₂O₂ 0.17 ml and water to 100 ml; (B) 3 % w/v potassium iodide in 1 % w/v starch solution.

The test solutions of the polythionates were run by ascending chromatography until the solvent front had travelled 8 in. The chromatograms were dried in an oven at 50° for 1 h, and, after cooling, lightly sprayed with spray A. The chromatograms were returned to the oven at 50° for 40–50 min and then sprayed with spray B. White spots appeared against a blue background.

Sulphate formed can be eluted from the paper by shaking overnight with 0.2 N HCl. After filtering off the paper it was washed with some more of the 0.2 N HCl and the sulphate precipitated in the usual way as the barium salt for radioactive assay.

Results and discussion

Dithionate had an R_F value of zero in the solvent system used. The other thionates had R_F values close to those quoted by POLLARD^{1,4} and GARNIER AND DUVAL² in the solvent systems they used. Sulphite has an R_F value close to dithionate but does not interfere with the dithionate test since it is driven off before it is oxidised. It must be remembered that sulphite loses its SO₂ quickly after spraying with the HCl and H₂O₂ under cold conditions whereas the dithionic acid only liberates its SO₂ slowly under the higher temperature experienced after placing the chromatogram in the oven: the technique failed to detect sulphite spotted on to the paper at the same time as dithionate. A disadvantage of the method is that the chromatogram fades after 5 min but there is the advantage of being able to elute spots afterwards for radioactive assay. The sensitivity is comparable with that of the silver nitrate spray of POLLARD *et al.*¹ and the benzidine-permanganate spray of GARNIER AND DUVAL². 5 µg quantities of thiosulphate, dithionate, trithionate, tetrathionate and pentathionate are detectable but not sulphite or sulphate.

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