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A Versatile Spray Reagent

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Abstract

A spray reagent is described, based on the Guyard reaction between Mn^{II} and Mn^{VII} ions. It is useful after paper electrophoresis of carbohydrates, polyhydroxy compounds, hydroxy acids and unsaturated compounds.

In 1959 we described a useful spray reagent based on potassium permanganate.[1] It was mainly designed for use after paper electrophoresis in aqueous electrolytes, as residual traces of solvent made it unreliable for paper chromatograms.

The reagent consisted of a freshly made aqueous mixture containing 0·2% (w/v) chromium trioxide and 0·1% potassium permanganate in 1·6 N sulphuric acid. When it is sprayed lightly on filter paper the initial purple quickly fades, and after a few minutes a uniform brown stain of manganese dioxide spreads over the paper. During the next half hour or so the brown fades as the Mn^{IV} is reduced to Mn^{II} under the strongly acidic conditions. The sprayed paper must be stored between glass plates or plastic film during development in order to stop it from drying out.

Readily oxidizable compounds tend to reduce the purple straight through to colourless Mn^{II}; with others the brown colour first develops, but is reduced faster by the soluble organic compound than by the paper support. When detectable compounds are present on the pherogram a series of white or pale spots appear on the brown background. Development is stopped when the contrast is judged to be best, by neutralization with sodium carbonate, washing and drying. The record is permanent if the strip is protected from bright light and organic vapours. Some strips have been preserved for twenty years.

The reagent is not highly sensitive but has proved to be very useful for a wide range of compounds. Olefinic compounds (unsaturated acids, pyrrolizidine alkaloids) react well,[2] as do many reducing sugars,[1] alditols,[1] and hydroxy acids.[3] Cyclitols usually react well, but many methyl glycopyranosides react only poorly.[1] Many aromatic amines and phenols show up well.[4,5] The reaction is non-discriminating, as all reactive compounds show the same kind of spot. Some aromatic compounds may cause transient blue or grey colours during reduction of the permanganate ion.

[1] Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1959, **12**, 65.
[2] Frahn, J. L., *Aust. J. Chem.*, 1969, **22**, 1655.
[3] Frahn, J. L., *J. Chromatogr.*, 1968, **37**, 279; 1971, **56**, 87.
[4] Frahn, J. L., and O'Keefe, D. F., *Aust. J. Chem.*, 1971, **24**, 2189.
[5] Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1964, **17**, 265.

One disadvantage is the presence of chromic acid in the mixture, which is a health hazard. We are indebted to Dr R. L. M. Synge for pointing out the danger of chromium(VI) in sprays. Another is that if a compound will not reduce manganese(IV) within 10 min in strong acid it cannot be detected. These disadvantages have been overcome.

The formation of manganese dioxide results from the Guyard reaction[6]

$$2Mn^{VII} + 3Mn^{II} \rightarrow 5Mn^{IV}$$

That is, part of the permanganate is quickly reduced to Mn^{II} ions by the paper, and the latter then reduces the remainder of the permanganate to manganese dioxide, which is deposited on the paper. For some reason the manganese dioxide reacts only slowly with pure cellulose fibre in 1·6 N sulphuric acid, but reacts with soluble organic polyhydroxy compounds.

The presence of Cr^{VI} is not necessary; indeed it can inhibit oxidation by Mn^{VII}. Furthermore, the reduction of manganese dioxide by paper in the absence of Cr^{VI} is much slower, and it is now always possible to leave sprayed strips to develop for 30–50 min. Periods of 2 h or more have been achieved, and very sluggishly reacting compounds can be detected. Fortunately, the Guyard reaction shows an induction period of about 3 min so that it is possible to mix measured quantities of Mn^{VII} and Mn^{II} salts and spray a paper strip with the mixture, so that the manganese dioxide is produced under controlled conditions.

The spray reagent is quite sensitive. Its limits have not been defined but it is certain that an application of 5–10 μg of many common carbohydrates could be detected as spots after electrophoresis in borate in the usual way. Alditols, aldonic acids, reducing sugars and many glycosides show up well.

When a paper strip is sprayed four things can happen. (*a*) Olefinic compounds show up instantly, as white spots, in the first pass across the paper. (*b*) Highly reactive compounds quickly bleach the permanganate and appear as pale spots which persist throughout the developments. (*c*) Less reactive compounds, such as 1,2-*O*-isopropylidene-D-glucose, may initially bleach enough permanganate to show up faintly, then become obscured by the spread of the brown background, and only slowly reappear as the compound hydrolyses and reduces the manganese dioxide. (*d*) The spot may first become detectable after some time, even several hours.

The sensitivity for olefins is in principle very high because of the manner in which they appear; it is not known whether the sensitivity extends to very hindered double bonds, as found in some steroids and triterpenoids.

The reaction fails in the presence of phosphate ions, presumably because these stabilize some Mn^{III} compounds and inhibit the Guyard reaction, and the brown oxide does not form. Great care is needed if any of the equipment is also used for spraying aniline phosphate.

I have to give up active research and leave to others the study of this interesting reagent. Its scope ought to be at least as wide as that of the original reagent containing chromium.*

* *Editorial postscript.*—Dr John Mills, a valued contributor whose first paper on the electrophoresis of carbohydrates appeared in the pages of this Journal in 1959, died after a long illness on 31 July 1977.

[6] Stewart, R., 'Oxidation by Permanganate' in 'Oxidation in Organic Chemistry' (Ed. K. B. Wiberg) Part A, Ch. 1, p. 9 (Academic Press: New York 1965).

Experimental

The reagent is prepared as two stock solutions: (*a*) 0·1% (w/v) $MnSO_4,4H_2O$ in aqueous 2 N sulphuric acid, and (*b*) 0·5% (w/v) $KMnO_4$ in water. These are stored in a refrigerator.

Initially the solutions were mixed in the ratio (*a*) : (*b*) = 4 : 1 but more recently, 17 : 3. The ratio depends partly on technique, partly on darkness of background desired. The quantity of manganese dioxide initially formed is probably about 5–8 μg per 1 cm^2 of paper (Whatman No. 1).

The procedure for electrophoresis has been described.[1,5] The reagent is applied with a National Jet-Pak spraycan unit, in which the liquids orifice of the atomizer is constricted by the insertion of a glass capillary. It should not be too greatly constricted, however, as spraying time is unduly prolonged.

The dried electrophoresis strip is quickly, evenly and lightly sprayed with the freshly mixed reagent, alternately on front and back, until it is uniformly coloured and just starting to show a wet surface. This should not take more than 2 min from mixing.

The sprayed paper strip is quickly wrapped in a freshly washed and dried polyethylene film and weighted with a glass plate, in order to retard evaporation. The progress of development can be noted on the glass plate.

When development is complete the paper is rinsed in water, then completely neutralized in 5% sodium carbonate solution, washed well and dried at a moderate temperature. The papers are stored in the dark, away from organic vapours.

The film of manganese dioxide that forms on all spraying and measuring equipment is easily rinsed off with a little dilute sodium metabisulphite solution acidified with acetic acid.

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