An improved technique for detecting spots on paper chromatograms with iodine vapor

The use of iodine vapor for detecting spots of various organic compounds on paper chromatograms has been a popular technique[1-5]. The disadvantage of this method lies in the fact that the paper background is also stained brown. For the examination of weakly stained spots as well as in the presence of tailing, the method was not found satisfactory.

On the assumption that the mechanisms through which the spots and the background are stained might be different from each other, attempts were made to reduce the staining of the background by treating the paper chromatogram with different reagents before being exposed to iodine vapor. It was found that filter paper impregnated with certain salts having acidic property, *e.g.* $(NH_4)_2SO_4$, $Al_2(SO_4)_3 \cdot 18H_2O$, McIlvaine's buffer of pH 2.2–4 (Na_2HPO_4 + citric acid), is far less stained than the untreated paper. Aluminum sulfate was found to be the most satisfactory; the impregnated paper strip remained virtually white even after being exposed to iodine vapor overnight. The spots, for example lipids like oleic acid, cholic acid or cholesterol, alkaloids including yohimbine or nicotine, and nitrogenous compounds such as *p*-anisidine and tryptophan, as well as the sulfur-containing compounds like methionine and glutathione, were stained as deeply as on untreated paper. The paper chromatogram treated in this way can be sprayed with starch solution whereby the iodine stained spots appear deep blue against a light blue background.

The procedure developed is as follows: The dried paper chromatogram is sprayed with a solution of aluminum sulfate in water (20 g $Al_2(SO_4)_3 \cdot 18H_2O$ in 100 ml water) on both sides until incipient dripping occurs. It is air dried and then hung overnight inside a jar containing some iodine crystals on the bottom. Exposure for 3 hours was found to be the shortest to obtain satisfactory results. The strip is again hung in air for 1 hour or longer to eliminate any superficially attached iodine and then sprayed with a 0.5% (w/v) starch solution. The background color becomes lighter as the strip dries. The color of the spots remains visible for over one month.

Squibb Institute for Medical Research, New Brunswick, N.J. (U.S.A.) S. C. PAN

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