Reaction of sulfate ion with the Hanes-Isherwood reagent

In the course of a recent study we were interested in following the formation of pyrophosphate (PP_i) from orthophosphate (P_i); magnesium ions were present in the system in the form of magnesium sulfate. The reaction was followed paper chromatographically using well-known and extensively used procedures, *i.e.* the chromatograms were developed in an isopropanol–water–ammonia–trichloroacetic acid system[1], sprayed with the HANES-ISHERWOOD reagent, and finally exposed to hydrogen sulfide[2]. Blue spots with R_F values equal to, or very slightly lower than*, that of authentic PP_i were consistently detected in both the real and control experiments even though P_i was not present in some of the controls. Magnesium sulfate, and more specifically the sulfate ion, was found to be responsible for the false positive test.

There are reports in the literature that arsenate and silicate give colored spots with this reagent[3]. Further, sulfate, as well as sulfite and formate, are known to interfere (tailing) with the separation of mixtures of P_i and condensed phosphates; the HANES-ISHERWOOD reagent was used to visualize the phosphorus-containing spots in this study, but color formation with sulfate, sulfite, or formate was not reported[4]. In view of the fact that trace metals are frequently added to *in vitro* biological-type reactions in the form of their sulfates, the following observations concerning this unexpected color reaction are reported.

Whatman No. 1 paper which had been previously washed with 1 % aqueous ethylenediaminetetraacetic acid (disodium salt), distilled water, and ethanol was used in these experiments. The chromatograms were developed with EBEL's solvent[1], dried in the usual way, sprayed with the HANES-ISHERWOOD reagent, and the "phosphorus-containing" areas visualized by exposure to hydrogen sulfide. R_{P_i} values for a variety of compounds which gave a blue color under these conditions are summarized in Table I. Formation of the blue color was found to be independent of the agent

TABLE I

R_{P_i} VALUES[a] OF COMPOUNDS GIVING A BLUE COLOR[b] WITH THE HANES-ISHERWOOD REAGENT

Compound	$Na_4P_2O_7$	$MgSO_4$	Na_2SO_4	$ZnSO_4$	$(NH_4)_2SO_4$	$MnSO_4$
R_{P_i}	0.65	0.63	0.53	0.59	0.59	0.58
Compound	$Fe(NH_4)(SO_4)_2$		$CuSO_4$	$MgCl_2$	Na_2SO_3	$NaCHO_2$
R_{P_i}	0.58		0.54[c]	0.80[d]	0.60	0.93[d]

[a] Average values from three independent chromatograms, developed (ascending) during *ca.* 16 h with isopropanol–water–ammonia–trichloroacetic acid (75:25:0.3:5, v/v/v/w)[1]; NaH_2PO_4 was used as the reference compound.

[b] Amounts which were readily detectable: NaH_2PO_4 and $Na_4P_2O_7$, 0.05 μmole; Na_2SO_4, 0.25 μmole; $MgSO_4$ and Na_2SO_3, 0.5 μmole; $MgCl_2$, 1.0 μmole; and $NaCHO_2$, $\geqslant$ 0.5 μmole.

[c] Spot is brown if molybdenum complex is reduced with hydrogen sulfide, but blue with other reducing agents studied.

[d] Very weak.

* R_F values which are low are frequently observed in the presence of protein and/or high salt concentrations.

used to reduce the molybdate complex, *e.g.* exposure to hydrogen sulfide, ultraviolet radiation[5], spraying with $SnCl_2$ in dilute HCl[6], or spraying with 10 % aqueous ascorbic acid followed by heating at 37° for 5 min[7]. Color formation with ascorbic acid is immediate for P_1 and PP_1 but slower for sulfate. Reduction with H_2S gives blue spots for PP_1 and sulfate which can be distinguished in the presence of each other; the former is ultramarine while the latter is cobalt blue. Sulfate migrates at a distinctly different rate than P_1 and PP_1 in other solvent systems which have been used to separate P_1 and PP_1, *e.g.* isopropanol–isobutanol–water–ammonia (40:20:39:1, v/v/v/v)[4,8], isopropanol–ammonia–water (70:10:20, v/v/v)[9], and *tert.*-butanol–water–formic acid (80:20:5, v/v/v)[5].

It should be noted that this same problem is encountered in the paper electrophoresis of solutions containing these ions if the electropherograms are sprayed with the HANES-ISHERWOOD reagent. On the other hand, using the LOWRY-LÓPEZ procedure[7] for the quantitative determination of phosphate, following digestion with sulfuric acid, 0.5 μmole of magnesium sulfate or sodium sulfate did not give a detectable blue color (measured at 675 mμ) (0.01 μmole P_1 can be readily detected by this procedure).

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1 J. P. EBEL, *Mikrochim. Acta*, (1954) 679.
2 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
3 H. HETTLER, *J. Chromatog.*, 1 (1958) 389.
4 K. GASSNER, *Mikrochim. Acta*, (1957) 594.
5 J. CROWTHER, *Anal. Chem.*, 26 (1954) 1383.
6 D. N. BERNHART AND W. B. CHESS, *Anal. Chem.*, 31 (1959) 1026.
7 S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. III, Academic Press, New York, 1957, p. 845.
8 E. KARL-KROUPA, *Anal. Chem.*, 28 (1956) 1091.
9 R. W. CHAMBERS AND H. G. KHORANA, *J. Am. Chem. Soc.*, 80 (1958) 3749.

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Equilibrium separation of glucose, galacturonic acid, and sulfuric acid with a strongly basic anion exchange resin

Following acid hydrolysis of plant cell wall polymers, the strong acid used for hydrolysis must be separated from the rest of the mixture before colorimetric analysis of the sugar fraction or further chromatography. $Ba(OH)_2$ is often recommended for this step but did not give reproducible results in our hands.