second direction. This effect may explain, to some degree, the systematic error of the TLC system.

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Detection of carboxylic acids on thin-layer chromatograms by their reaction with iodide–iodate and amylose

Selective detection reagents provide a useful adjunct to R_F data in identification of compounds separated by thin-layer chromatography. Although a number of reagents for visualizing carboxylic acids have been reported[1–7], interference by residual solvents often limits their sensitivity. This report describes a spray reagent composed of iodide, iodate and amylose which can be used to detect carboxylic acids on silica gel or cellulose after chromatographic separation in acid or basic systems.

Iodide is oxidized rapidly and quantitatively by iodate in the presence of acid by the reaction,

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

NOVAK AND DLASK[8] exploited this reaction for detecting acids on paper chromatograms after development in chloroform–acetone–water–formic acid or ethanol–ammonia–water systems. Chromatograms from the acid system were dried for 16 h at room temperature, and the acids were immediately visible as brown spots after spraying with a mixture of potassium iodide, potassium iodate and starch. The papers developed in the basic system were dried for 1 h at room temperature; brown spots developed 1–2 h after spraying. Their reagent contained about 0.01% starch, equivalent to about 0.002% amylose. It has been pointed out[9] that it is the amylose content of starch which affords the characteristic intense blue color of the starch–iodide complex. The reagent described here contains 0.33% amylose. This may account for differences in the results observed. The higher concentration of amylose affords visualization of the acids as blue spots which appear almost immediately after development in either acid or basic systems.

Procedure

Test solutions of each acid were spotted in 10-μl volumes of 5 mg/ml acetone solutions 3 cm from the bottom of 20 × 20 cm Analtech Uniplates®, which consisted

of 0.25 mm layers of microcrystalline cellulose or Silica Gel G on glass. Chromatography chambers were lined on three sides with filter paper and equilibrated with the chosen solvent system 30 min before use. The most satisfactory systems were:

(I) *n*-butanol–anhydrous ethanol–conc. ammonia–water (60:60:60:15) with microcrystalline cellulose plates,

(II) ethanol–conc. ammonia (112:16) with cellulose plates, and

(III) chloroform–acetone–water–90% formic acid (90:90:5:5) with silica gel plates. The chromatograms were developed until the solvent front had ascended about 12 cm from the starting line. Those developed in systems I or II were dried at 105° for 1 h, plates developed in system III were air-dried overnight at room temperature. The separated acids were revealed as dark blue spots on a white or light blue background by spraying the plates with a freshly prepared mixture of equal volumes of 8% potassium iodide, 2% potassium iodate and 1% amylose (Mallinckrodt IndicatAR®). The presence of acid vapors in the laboratory air vitiates the detection by coloring the background.

Results and discussion

Detection of acids was satisfactory with either adsorbent developed in any of the three systems; however, chromatograms on cellulose developed in the formic acid system were streaked. A glacial acetic acid system *viz.*, benzene–methanol–acetic acid (75:18:2) gave good separations with Silica Gel G thin-layers, but a blue background was obtained after spraying even after the plates had been dried at 100° for 2 h in a vacuum oven.

The spots appeared almost immediately after spraying the plates. The background gradually turned brown on standing; however, the acid spots remained easily discernible. NOVAK AND DLASK[8] reported a minimum detection limit of 10 μg for benzoic acid after paper chromatography in the basic system, and they were unable to detect 24 μg after acid system development, ascribing this result to sublimation from the paper during drying. In contrast, we found benzoic acid easily detectable on thin

TABLE I

CHROMATOGRAPHIC RESULTS WITH ACIDS

Acid	pK_a	R_F *values*		
		System I	*System II*	*System III*
p-Aminobenzoic	4.92	0.75	0.48	0.78
Anthranilic	5.00	0 87	0 69	0 88
Benzoic	4.20	0.90	0 90	0 90
Citric	3.08	0 33	0 00	0 17
Fumaric	3.00	0 59	0 20	0 68
Mandelic	3 37	0 88	0.69	0 65
Oxalic	1 19	0 40	0 00	0 09
Phthalic	2 90	0 63	0 19	0 61
Picric[a]	0.82	0.95	0.95	0.45
Salicylic	3.00	0 89	0 78	0.89
Tartaric	2.96	0 15	0 08	0 12

[a] Not detected with the spray reagent.

layers in all three systems. The detection limit using system I was determined to be about 2 μg for a 50-μl spot application. Table I shows that benzoic acid exhibits an R_F value of about 0.9 in all three systems, thus one would expect that less diffuse spots of acids with lower mobility and comparable pK_a values should be detected at least as sensitively. Picric acid, the only noncarboxylic acid of the eleven in Table I and the strongest acid in the group, was the only one which was not detected at the 50-μg load. Some other noncarboxylic acids were tested by spot-test technique after application of 50 μg to Silica Gel G thin layers. Of these, saccharin (pK_a 1.62) was not detected at all, and the bisphenol, bithionol (pK_a 4.82), was barely visualized. Cyclamic and bis (2-ethylhexyl) phosphoric acid were easily detectable. Although not specific, the reagent appears to be highly selective for carboxylic acids.

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