

CHROM. 5253

A mixed silica gel-cellulose support for thin-layer chromatography of non-volatile organic acids

Thin-layer chromatography has been used in this laboratory in a study of the non-volatile organic acids in guava¹. Difficulties were encountered in obtaining adequate resolution of the acids on cellulose and on silica gel supports. The effectiveness of mixtures of the two materials for TLC supports was investigated and is the subject of this note. Silica gel-cellulose supports have been used with varying degrees of success for the separation of amino acids^{2,3}. We found that a silica gel-cellulose mixture eliminated the streaking found on cellulose and showed better resolution of acid spots than did silica gel alone.

Materials and methods

Mixtures of MN Silica Gel G and Cellulose MN 300 (Macherey, Nagel & Co.)* were spread on glass plates 20 × 20 cm with a Desaga spreader. Silica gel was mixed at 20, 30, 40, 50, 60, and 70% levels with cellulose. Total volume of water added to 30 g of the mixture was 6 parts of water to each part of cellulose (w/w) plus 2 parts of water to each part of silica gel (w/w). The silica gel-cellulose mixtures were blended for 1 min in a Waring blender, spread at a thickness of 300 μ. Coated plates were left to dry and set overnight at ambient temperature and activated at 110° for 1 h. Plates were cooled and stored in a desiccator until used.

Three solvent systems were used. Solvent (I) anhydrous diethyl ether-88% formic acid-water (20:5:3); (II) *n*-butanol-88% formic acid-water (4:1:5); (III) benzyl alcohol-*tert.*-butanol-isopropanol-88% formic acid-water (24:8:8:1:8).

Mixtures of known organic acid solutions (50 mg/ml in 95% ethanol) were applied in 1-μl quantities to the plates under a stream of warm air. The plates were allowed to cool to ambient temperature and then were developed to a height of 10 to 12 cm. After development, the plates were dried overnight at ambient temperature. Bromphenol Blue reagent (0.04% in 95% ethanol and 0.05% sodium acetate) was used to detect the acids.

Results and discussion

The use of silica gel-cellulose mixtures corrected major problems which arose from use of either support individually. Mixtures with increasing percentages of silica gel progressively eliminated the streaking found with use of cellulose. In addition, supports of cellulose mixed with silica gel permitted resolution of spots previously showing poor resolution³ with silica gel as the sole ingredient. Plates with lower percentages of silica gel proved easier to handle and spot, the support layer being less brittle and more adhesive than higher percentage silica gel supports.

In order to determine the most satisfactory percentage of silica gel a mixture was sought which gave the best resolution, least streaking and easiest plate handling.

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

The 60% silica gel-40% cellulose mixture proved to be the most satisfactory support. Plates required no special handling, and no difficulties were encountered during application of samples. Spots were distinct and compact with no streaking. In addition, the incorporation of 60% silica gel with cellulose gave the best separation in terms of giving the maximum differences in R_F values between malic and citric and between ascorbic and tartaric acids in comparison to those from 20, 40, 50, and 70% levels of silica gel with cellulose.

TABLE I

R_F VALUES ($\times 100$) OF ORGANIC ACIDS ON 60% SILICA GEL IN CELLULOSE DEVELOPED WITH THREE SOLVENT SYSTEMS

Acid	Solvents		
	I	II	III
Fumaric	97	95	92
Adipic	97	92	91
Glutaric	94	90	94
Succinic	88	88	87
Lactic	84	77	78
Oxalic	82	69	70
Malic	59	61	64
Citric	51	57	55
Pyroglutamic	61	52	72
Ascorbic	31	45	58
Tartaric	36	36	46
Quinic	20	26	37
Galacturonic	07	09	18

Several non-volatile organic acids that are commonly found in plant material were spotted on thin-layer plates of 60% silica gel-40% cellulose and developed in three different systems (Table I). The elution order was the same under the three different solvent systems with the exception of glutaric and pyroglutamic acids.

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Received December 30th, 1970