Celite–starch for thin-layer chromatography

Silica gel G[1], silicic acid[2], cellulose powder[3], kieselguhr G[4] and aluminium oxide[5] have been used on chromatoplates for the separation of organic and inorganic compounds. Chromatoplates with these coatings have been effective in the separation of many hydrophobic compounds but only a few reports describe their use with hydrophylic substances. This report describes a low cost coating for chromatoplates which is particularly useful in the separations of hydrophylic compounds.

The coating is a mixture of Celite 535 and starch gelatinized in sodium hydroxide. After the coating is dry on the plate the alkali present is neutralized, in some instances, by spraying the coated and dried plate with acetic acid. In both cases the adhesion of the coating to the plate is good. Reducing sugars, methyl glycosides, amino acids, purines, pyrimidines and nucleosides are separated by using 90% aqueous methyl ethyl ketone (S_2) or 90% aqueous isopropanol (S_1) as irrigants.To detect the components on the chromatoplates the usual spray reagents for paper chromatography may be used.

TABLE I

R_F VALUES OF COMPOUNDS ON CELITE–STARCH CHROMATOPLATES

Solvents: S_1 = Isopropanol–water (90:10 v/v); S_2 = Methyl ethyl ketone–water (90:10 v/v); the time needed for S_1 to run a distance of 12 cm was 25 min, and for S_2 10 min. Detection: ASN = ammoniacal silver nitrate solution[6]; AT = saturated solution of antimony trichloride in chloroform[7]; NIN = 0.3% solution of ninhydrin in butanol[8]; U.V. = ultraviolet.

Compound	*Amount applied (μg)*	R_F		*Detection*	*Color*
		S_1	S_2		
D-Glucose	5	0.28		ASN	Black
Maltose	5	0.12		ASN	Black
L-Rhamnose	5	0.92		ASN	Black
D-Ribose	5	0.50		ASN	Black
D-Fructose	5	0.31		ASN	Black
Methyl α-D-glucoside	15		0.50	AT	Brown
Methyl α-D-galactoside	15		0.54	AT	Brown
Methyl α-L-rhamnoside	15		0.88	AT	Brown
Methyl α-D-xyloside	15		0.78	AT	Brown
Methyl α-D-mannoside	15		0.46	AT	Brown
L-Glycine	2	0.30		NIN	Purple
D-Alanine	2	0.51		NIN	Purple
L-Cystein	3	0.15		NIN	Purple
L-Valine	2	0.77		NIN	Purple
DL-Lysine	2	0.28		NIN	Purple
DL-Serine	3	0.32		NIN	Purple
L-Phenylalanine	4	0.85		NIN	Purple
Adenine sulfate	5	0.96		U.V.	
Dihydrouracil	5	0.74		U.V.	
Cytidine	5	0.55		U.V.	
2-Amino-4-hydroxy-pyrimidine	5	0.94		U.V.	
5-Methylcytosine HCl	5	0.88		U.V.	
Adenosine	5	0.77		U.V.	
Uridine	5	0.39		U.V.	
Inosine	5	0.21		U.V.	
Adenosine 5-phosphoric acid	5	0.15		U.V.	
Thymidine	3		0.85	AT	Blue
5-Methyl-deoxycytidine	3		0.64	AT	Blue
Deoxycytidine HCl	3		0.50	AT	Blue
Thymidine 5-monophosphoric acid	3		0.00	AT	Blue

For the preparation of the chromatoplates a suspension of 18 g of 200-mesh Celite 535 (Johns-Mansville Company) in 100 ml of 0.25 *N* sodium hydroxide solution was mixed with a suspension of 1.5 g of powdered potato starch in 20 ml of water and homogenized by mixing for 1–2 min. The mixture was immediately coated on clean and dry 20 cm × 20 cm glass plates with an applicator model S-11 (Brinkmann Instruments) which was adjusted to give a layer of 500 μ thickness. The plates were dried at 25° for 16 h. The chromatoplates were irrigated at 25° in chambers saturated with vapors of the solvents. Samples were applied 2 cm from the edge of the glass plate. The ascending irrigant technique was used and the best results were obtained when the plates were allowed to rest on a 5 mm thick layer of cotton soaked with the irrigant. Some results are shown in Table I.

Deoxy-D-ribose nucleosides were detected by spraying the plates with a saturated solution of antimony trichloride. A fairly stable deep blue color was produced upon heating the plates at 100° for 10 min. Under the same conditions D-ribose nucleosides produce a yellow brown color.

Since the optimum pH for the detection of amino acids with ninhydrin is about neutral[9], the chromatoplates for the separation of these acids were sprayed with 20 % solution of acetic acid and dried at 25° before use. Purines were not detectable on the chromatoplates by direct observation in U.V. light and another technique was employed for their observation. Chromatoplates containing these substances were dried and sprayed with water. Then a water wetted piece of Whatman No. 1 filter paper was applied to the chromatoplates and covered with glass plate. The plates were heated gently on steam bath for 2–3 min. The plates were separated and the paper pulled off and dried. Purines and pyrimidines were transferred to the paper and detected by a U.V. light (SL Mineralight, Model 2537 with a 253.7 mμ short wave filter).

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Received October 11th, 1963