Quantitative thin-layer chromatography of sugars on microcrystalline cellulose

The successful use in this laboratory of microcrystalline cellulose as an adsorbent for the qualitative thin-layer chromatographic separation of sugars[1] prompted us to study its application for the quantitative analysis of sugars.

Only a few quantitative estimations of unsubstituted sugars by thin-layer chromatography, on silica gel or kieselguhr, have been reported[2–5] and these were mainly devised to solve specific problems. As microcrystalline cellulose has many advantages[1] over silica gel for the thin-layer chromatography of water-soluble compounds, a quantitative method for the estimation of sugars separated on cellulose layers was desirable. The aniline hydrogen phthalate method[6] is widely used for the determination of reducing sugars on papergrams. This reagent gives a colored background when used for spraying "Avicel" plates. We have found that the microcrystalline cellulose can be reduced with sodium borohydride to give a modified cellulose that still maintains all of its former chromatographic properties but gives an almost white background with the aniline phthalate reagent[6], thus making it suitable for quantitative analysis.

Experimental

The microcrystalline cellulose (50 g, Avicel—Technical Grade, Avicel Sales Division of American Viscose Division, FMC Corp., Marcus Hook, Pa.) was stirred mechanically for 10 h at 25° with 1000 ml of an aqueous solution of 0.1 *N* sodium borohydride. After the solid had settled, the supernatant was decanted and the process was repeated with fresh borohydride. The cellulose was then filtered, washed to neutrality, dried over phosphorus pentaoxide in a vacuum desiccator and ground to a powder in a mortar. The chromatoplates were prepared by blending the reduced "Avicel" (100 g) with 430 ml of water for 30 sec at high speed. Smoother plates were obtained when the slurry was deaerated in a filter flask by applying vacuum for about 1 min. The homogeneous slurry was spread on glass plates (20 × 20 × 0.4 cm) with a Desaga applicator at 0.5 mm thickness. The plates were air dried overnight. The sugar solutions were applied in 1.25 μl quantities by means of a syringe microburet; if a larger volume was required, the plate was dried between applications until a total of 10 to 100 μg of sugar was spotted on the plate. The solvent systems reported for paper chromatography were satisfactory. The developed plate was dried with the aid of a commercial hair drier and was sprayed evenly with aniline phthalate reagent (prepared by dissolving 1.66 g of *o*-phthalic acid and 0.91 ml of pure aniline in 48 ml of 1-butanol, 48 ml of ethyl ether, and 4 ml of water)[6]. The plate was heated in an oven at 105–110° until the spots appeared (5–7 min). Rectangular areas around the spots were excised with a razor blade and transferred quantitatively from the plate to a test tube. The areas cut were the same for all the spots of each sugar. A blank was cut out from the plate at the height of the sugar spots. To the test tubes, 0.5 ml of the aniline phthalate reagent was added and the tubes were heated in an oven at 105–110° for 1 h. After cooling, the solid material was broken up with a thin glass rod and to this was added 4 ml of eluting agent, prepared by adding 4 ml of concentrated hydrochloric acid to 100 ml of acetone. The tubes were closed with Teflon stoppers and allowed to stand for 1 h with oc-

casional shaking. The tubes were centrifuged for 3 min and the supernatant was transferred to 1-cm quartz cells with a syringe. The absorbances were measured against the blank, in a Beckman DU spectrophotometer, at 390 mμ for the hexoses and rhamnose and at 360 mμ for the pentoses.

Results

D-Glucose, D-galactose, D-mannose, L-rhamnose, D-arabinose, and D-xylose gave a linear relationship between the light absorption and the concentration. It was established for D-xylose and D-glucose that this linearity holds within the range 0–150 μg. The amount of sugar in the unknown was determined by reference to a standard curve obtained from known sugars that were run on the same plate as the unknowns. Ethyl acetate–pyridine–water (2:1:2, v/v, upper phase)[7] was used in the major part of our study as it gave a good resolution in the shortest time. A mixture of D-glucose and D-xylose was separated in 75 min with this solvent. The coefficient of variation obtained for mixtures containing 20 to 100 μg, respectively, of each sugar was ± 3 %.

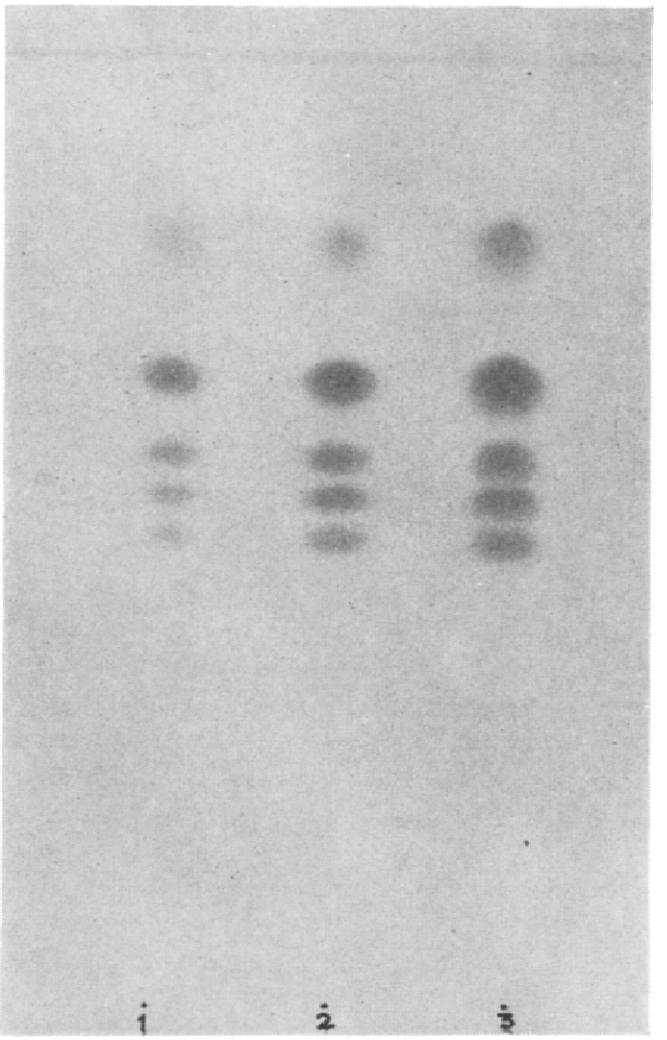

Fig. 1. Thin-layer chromatogram of a mixture of D-galactose, D-glucose, D-mannose, D-xylose, and L-rhamnose (ascending order) after four developments with ethyl acetate–pyridine–water (2:1:2, v/v, upper phase); from left to right: 10 μg, 30 μg, and 50 μg of each sugar, respectively.

Synthetic mixtures of equal amounts of D-galactose, D-glucose, D-mannose, D-xylose, and L-rhamnose in 10, 30, and 50 μg amounts of each were separated readily by four developments, with intermittent drying (hair drier, about 10 min), with ethyl acetate–pyridine–water (2:1:2, v/v); R_G 0.90, 1.00, 1.10, 1.30, 1.55, respectively (Fig. 1). A qualitative analysis of this complex mixture could be accomplished by a double development; for a quantitative determination, four developments afforded a satisfactory separation in 5 h; a similar separation by paper chromatography requires at least 24 h. A mixture of equal parts of D-galactose and D-glucose was determined with a 3 % error for D-galactose and 6 % for D-glucose when the concentration of each sugar per spot was maintained within the range 10 to 60 μg.

Acknowledgement

Acknowledgement is made to support from the Corn Industries Research Foundation.

Department of Chemistry, The Ohio State University, Columbus, Ohio (U.S.A.)

M. L. WOLFROM
ROSA M. DE LEDERKREMER
GERHART SCHWAB

1 M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *Chem. Ind. (London)*, (1964) 1065; M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *J. Chromatog.*, 17 (1965) 488.
2 G. PASTUSKA, *Z. Anal. Chem.*, 179 (1961) 427.
3 M. GEE, *J. Chromatog.*, 9 (1962) 278.
4 E. BAUCHER, H. SCHERZ AND K. KAINDE, *Mikrochim. Ichnoanal. Acta*, (1964) 652.
5 G. THOLEY AND B. WURTZ, *Bull. Soc. Chim. Biol.*, 46 (1964) 769.
6 C. M. WILSON, *Anal. Chem.*, 31 (1959) 1199.
7 M. A. JERMYN AND F. A. ISHERWOOD, *Biochem. J.*, 44 (1949) 402.

Received October 29th, 1965

J. Chromatog., 22 (1966) 474–476

Separation of polyphenyls by thin-layer chromatography

Recent interest in polyphenyls as reactor coolants has necessitated both qualitative and quantitative techniques for the separation of the isomers. DENTI *et al.*[1] have shown that sulfonation products of polyphenyls can be separated by paper chromatography. GEISS *et al.*[2] obtained a separation of the polyphenyl isomers by thin-layer chromatography (TLC) but their technique was complicated and reproducibility was difficult to achieve.

The purpose of this paper is to report the separation of the polyphenyl isomers by TLC using a single developing solvent system.

Experimental

Materials. Desaga TLC apparatus and tanks were used. Aluminum oxide G according to STAHL was used to coat glass plates 20 × 20 × 0.4 cm. Solvents were reagent grade. Polyphenyl isomers and azobenzene were obtained from Eastman