CHROM. 3350

Separation of sugars by thin-layer chromatography

The utilization of thin-layer chromatography (TLC) for the separation of carbohydrates has been much less widespread than its use for the separation of other biological materials. This has been due principally to the poor separation of some sugars by TLC and to the low capacity of the chromatoplates[1-3]. Consequently the inherent advantages of TLC over paper chromatography such as speed and sensitivity have not been entirely realized. In an attempt to improve carbohydrate separations by TLC, a variety of adsorbents, and mixtures of adsorbents, as well as various solvent combinations have been studied (*e.g.* refs. 1–14).

In our experience, good separations of substantial quantities of sugars have been achieved with silica gel and a simple solvent system that, to our knowledge, has not previously been employed. The purpose of this paper is to report some examples of separations that have been obtained.

Experimental

Preparation of chromatoplates. 40 g of Kieselgel D-O (Camag, Muttenz, Switzerland), 5 g of calcium sulfate, and 95 ml of 0.02 *M* sodium acetate buffer were manually shaken in a glass stoppered flask for 1 min. The slurry was transferred to a Desaga adjustable applicator, and layers 0.5 mm thick were deposited on clean glass plates. The use of buffer is not essential, although it seems to yield slightly cleaner separations of certain sugars, for example, arabinose and fructose. The plates were allowed to dry overnight at room temperature, then were used without further preparation, except for scoring into lanes 1 to 2 cm in width.

Procedure. Aliquots containing appropriate quantities of sugars, or sugar mixtures, dissolved in water, were applied to the plates with a Hamilton microsyringe (Hamilton Company, Inc., Whittier, Calif.). Applications were made in small increments (less than 1 μl) under a stream of warm air from a heat gun to facilitate rapid drying, and thus to minimize spot diffusion.

Ascending chromatography was conducted in closed glass tanks lined with Whatman No. 4 paper saturated with the developing solvent, to a distance of 10 cm from the origin. First developments, made at ambient temperature, took about 75 min, subsequent runs took about 60 min. After a run, the plates were dried with the aid of a heat gun then were either returned to the glass tank for another ascension, or were sprayed with the detecting reagent.

Solvent system. A wide variety of solvent systems were surveyed including a number that have previously been utilized for both paper chromatography and TLC; most of these generally gave unsatisfactory results under our conditions. The system that produced distinctly superior separations was chloroform–acetic acid–water (3.0:3.5:0.5, v/v); this system was employed for the studies described in this paper, with one exception to be noted.

Detection of spots. Of a number of spraying agents compared, one containing diphenylamine[15] appeared to produce the best results. The reagent was made up by dissolving 1 g diphenylamine and 1 ml aniline in 100 ml of acetone; prior to spraying, 10 ml of the acetone solution was mixed with 1 ml of 85 % phosphoric acid. The

sprayed plate was heated for 10 min at 130°. Most of the sugars appeared as dark grey spots; pentoses, however, were light bluish-grey, and fructose and fructose-containing compounds were rosy brown (when freshly heated).

Densitometry. Tracings of chromatoplates were made by transmission densitometry, using a Photovolt Model 530 TLC densitometer equipped with a 520 A photometer and a 52 C light source. Filter 610 was utilized in the scanning slit. The TLC stage was driven by a 5138 synchronous motor drive.

Preparation of starch hydrolyzate. To a 20 % commercial wheat starch slurry was added 0.1 % (based on starch) HT-1000 bacterial alpha amylase (Miles Chemical Co., Elkhart, Ind.). The slurry was heated at the rate of 2°–4° per min from 25° to 100°, and held at the latter temperature for 15 min. One μl of the crude hydrolyzate was used for TLC.

Results and discussion

An example of the separations achieved with the procedure outlined above is provided in Fig. 1.

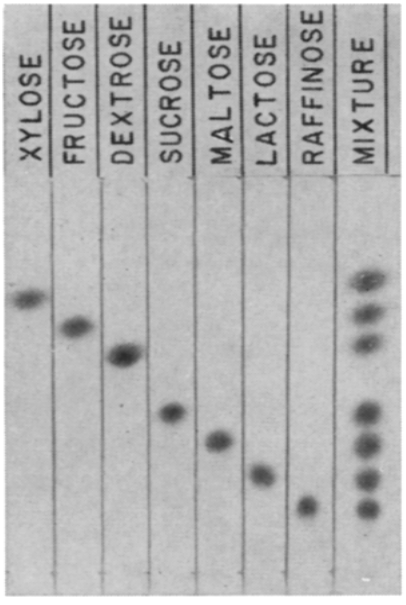

Fig. 1. Separation of seven indicated sugars (100 μg each) on buffered silica gel with chloroform–acetic acid–water (3.0:3.5:0.5, v/v); a threefold development was employed. Spots were visualized with aniline–diphenylamine.

TABLE I

R_G × 100 VALUES OF SUGARS

Xylose	126
Fructose	113
Glucose	100
Sucrose	74
Maltose	61
Lactose	47
Raffinose	32

Good separation of seven sugars of general biological interest, representing a pentose, two hexoses, three disaccharides, and a trisaccharide, may be noted. The system employed produced spots of desirable geometry: compact, circular spots were obtained for all the sugars, with no tailing or bearding evident. Combinations of other solvent systems and/or adsorbents studied in this laboratory yielded sugar spots of less satisfactory configuration, as manifested by tailing and diffusion, and distorted shapes.

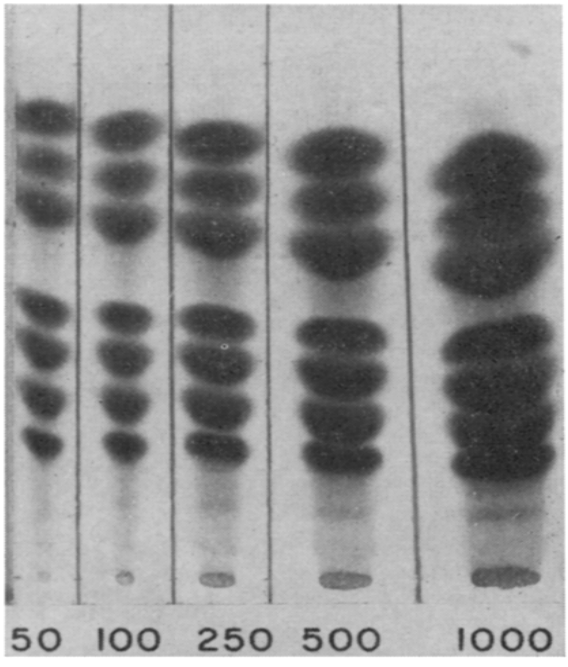

Fig. 2. Separation of indicated quantities (μg) of each sugar. Sugars and conditions same as in Fig. 1, except that a fourfold development was conducted.

The number of developments required to effect adequate sugar separations is largely dependent on the quantities of sugars involved. For the amount of material studied in Fig. 1 (100 μg of each sugar), two ascensions appear to be sufficient, but a threefold development enhances the separation.

The $R_G \times 100$ values of the sugars are summarized in Table I.

Aside from the ability to produce well-separated spots exhibiting good geometry, a desirable TLC procedure should have the property of handling relatively large amounts of material without overloading the chromatoplates. This feature is of particular importance when quantitation by elution of separated sugars followed by colorimetry is contemplated. That the combination of silica gel adsorbent and a chloroform–acetic acid–water solvent system may allow substantial quantities of sugars to be satisfactorily separated is indicated in Fig. 2.

Discrete separations were obtained when as much as 250 and 500 μg of each sugar was deposited on the plate. When larger quantities of sugars are being handled, additional development may be advisable; four developments were employed for this separation.

Such separations suggest that quantitation through elution and colorimetry should be quite feasible; work on this is currently proceeding in our laboratory.

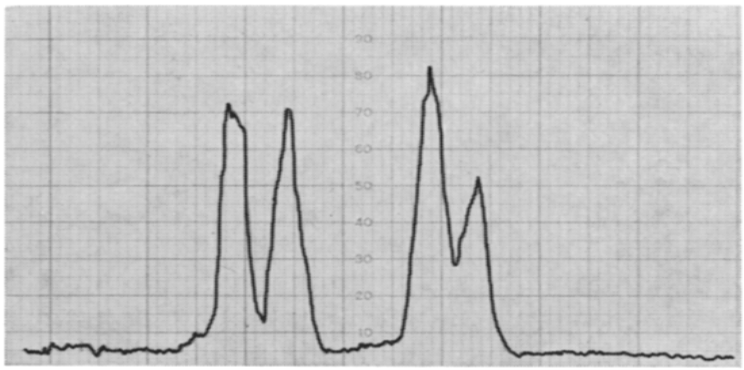

Fig. 3. Densitometer tracing of TLC plate. From right to left peaks represent fructose, glucose, maltose, and lactose; 100 μg of each sugar was deposited. Solvent and detecting system same as in Fig. 1.

Quantitation by densitometry is an alternative route, provided that adequate separations of sugars in a mixture are achieved. A densitometric tracing of a chromatogram of fructose, glucose, maltose, and lactose, separated under the conditions described above, is shown in Fig. 3.

Good peak definition and a steady base line are apparent. These characteristics indicate that a relatively rapid procedure for the quantitative evaluation of sugars in a mixture by TLC and densitometry should be possible.

Among the many applications for TLC in the field of carbohydrates is that of the analysis of commercial corn syrups[16], and of starch hydrolyzates in general. An example of a TLC separation of a crude wheat starch hydrolyzate is furnished in Fig. 4.

Separation of glucose units having D.P. values of from 1 to 9, and possibly to 10,

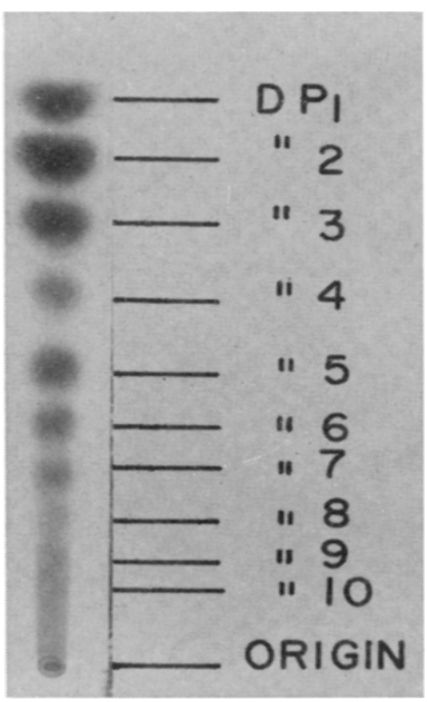

Fig. 4. Separation of oligosaccharides in crude starch hydrolyzate, with chloroform–acetic acid–water (10:79:11, v/v) and four developments. Spots were visualized with aniline–diphenylamine.

are indicated. To obtain this separation, the solvent system proportions were altered in the direction of greater polarity (chloroform–acetic acid–water (10:79:11, v/v)); four developments were conducted.

Research Laboratories, Continental Baking Co., Rye, N.Y. (U.S.A.)

V. A. DE STEFANIS
J. G. PONTE, Jr.

1 D. W. VOMHOFF AND T. C. TUCKER, *J. Chromatog.*, 17 (1965) 300.
2 YU. S. OVODOV, E. V. EVTUSHENKO, V. E. VASKOVSKY, R. G. OVODOVA AND T. F. SOLOV'EVA, *J. Chromatog.*, 26 (1967) 111.
3 J. P. MARAIS, *J. Chromatog.*, 27 (1967) 321.
4 E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.
5 C. E. WEILL AND P. HANKE, *Anal. Chem.*, 34 (1962) 1736.
6 V. PREY, H. SCHERZ AND E. BANCHER, *Mikrochim. Acta*, (1963) 567.
7 G. W. HAY, B. A. LEWIS AND F. SMITH, *J. Chromatog.*, 11 (1963) 479.
8 H. JACIN AND A. R. MISHKIN, *J. Chromatog.*, 18 (1965) 170.
9 P. G. PIFFERI, *Anal. Chem.*, 37 (1965) 925.
10 M. L. WOLFROM, R. M. DE LEDERKREMER AND G. SCHWAB, *J. Chromatog.*, 22 (1966) 474.
11 E. J. SHELLARD AND G. H. JOLLIFFE, *J. Chromatog.*, 24 (1966) 76.
12 E. GAROFALO, *Minerva Pediat.*, 18 (1966) 3.
13 A. LOMBARD, *J. Chromatog.*, 26 (1967) 283.
14 A. AFFONSO, *J. Chromatog.*, 27 (1967) 324.
15 I. SMITH, *Chromatographic and Electrophoretic Techniques, Vol. I*, Heinemann, London, 1962, p. 251.
16 C. N. HUBER, H. SCOBELL AND HAN TAI, *Cereal Chem.*, 43 (1966) 342.

Received November 30th, 1967

J. Chromatog., 34 (1968) 116–120

CHROM. 3305

A rapid method for the determination of *p,p'*-DDT to *p,p'*-DDE ratios in fish

We wish to describe a more rapid method of analyzing for the presence of DDT in fish than the commonly used method of MILLS, ONLEY AND GAITHER[1]. The following procedure was developed as the result of a need to routinely and rapidly determine the ratio of *p,p'*-DDT to its metabolites and although the recoveries of the pesticides from the tissues were not as high as those obtained using the above method, it was found to be satisfactory for obtaining the ratios of *p,p'*-DDT to *p,p'*-DDE.

Tissue samples ranging from 0.005 to 0.1 g were digested in 2–4 ml of formic acid and maintained at 60° for 1.5–2 h followed by extraction four times with hexane. The combined extracts were evaporated to dryness and the residue was washed with three 2 ml portions of acetonitrile. A small amount of alumina (20 mg, 80–200 mesh) was added and the sample was centrifuged. After evaporating the supernatant to dryness, the residue was dissolved in a small amount of ethyl acetate and spotted on a plate coated with aluminum oxide using the procedure described by KOVACS[2].

Since our samples contained ^{14}C-labeled DDT and its corresponding metabolites, DDE and DDD, a radio chromatogram scanner was used to detect the amounts