Inexpensive adsorbents for thin-layer chromatography of carbohydrates

The usefulness of thin-layer chromatography (TLC) as a rapid and sensitive analytical technique has been firmly established. However, reports of separations of carbohydrate compounds by TLC have been relatively few compared with other classes of materials. STAHL AND KALTENBACH[1] and PASTUSKA[2] obtained resolution of monosaccharides on Kieselguhr G (Merck) and Silica Gel G (Merck) but oligosaccharides were poorly resolved by the solvent systems employed. Later WEILL AND HANKE[3] demonstrated excellent separations of glucose, maltose, and malto-oligosaccharides on Kieselguhr G (Merck) using combinations of *n*-butanol–pyridine–water as developing solvents. The only disadvantage of this system was that monosaccharide materials followed the solvent front.

Characteristic of the above publications is the use of the adsorbents Kieselguhr G and Silica G for all of the studies. Some of the drawbacks to the use of these materials have been noted above but in addition they are relatively expensive. We have found ordinary celite filter aid materials to be excellent adsorbents for the separation of carbohydrates by TLC and in some instances to give better resolution than that obtained with more expensive adsorbents.

Experimental

Apparatus. The thin-layer chromatography assembly manufactured by Research Specialties Company employing a fixed thickness spreader was used for preparation of the chromatoplates. Borosilicate glass plates (8 in. × 8 in.), were employed for all analyses. Battery jars or large glass cylinders 9 in. in diameter with glass covers were used as development chambers.

Materials. Solutions of anhydrous dextrose (National Bureau of Standards), maltose hydrate (Pfanstiehl Co. C.P.) and xylose, C.P. were prepared by dissolving 100 mg of each compound in distilled water and diluting to 10 ml.

Isomaltose and panose were prepared from maltose by the action of the enzyme, transglucosidase, present in fermentation broths of *Aspergillus niger* cultures according to the procedure of PAZUR AND ANDO[4]. The resulting hydrolysis mixture containing glucose, maltose, isomaltose, panose and minor unknown components was diluted with 2.5 volumes of distilled water prior to spotting the solution on the adsorbent.

n-Butanol, b.p. 116–118° and pyridine, b.p. 115–116° were used in developing solvents.

Adsorbents used were Kieselguhr G (Merck) obtained from Brinkman Instruments, Inc. and Johns-Manville's Hyflo Super-Cel and Filter-Cel.

Silver nitrate solution: 5 g of $AgNO_3$ dissolved in 95 ml of water and 6 ml of concentrated ammonium hydroxide.

Calcium sulfate, $CaSO_4 \cdot 0.5\ H_2O$.

Procedure. Thin-layer chromatoplates of the various Johns-Manville filter aid materials were prepared as follows: 0.8 g of $CaSO_4 \cdot 0.5\ H_2O$ was placed in a mortar, covered with 5 ml of distilled water and ground with a pestle for one minute. Fifteen grams of filter aid and 60 ml of distilled water was added and the mixture ground for an additional 1 to 2 min. The smooth slurry was poured into the spreader and the

prepared immediately. Five 8 in. × 8 in. plates could be obtained in this manner. After air drying 10–15 min the plates were dried at 100° for at least 30 min.

The Kieselguhr G plates were prepared in a similar fashion except no calcium sulfate was added and 20 g of adsorbent to 40 ml of water was employed.

Two μl of the solutions described above were placed 1 in. apart and 1 in. from the bottom of the adsorbents with the aid of a lambda pipette so that the solvent travelled either opposite or perpendicular to the direction of the spreader.

A soft stream of warm air from a hair dryer was directed at the spot to hasten evaporation of sample and keep the size of the spot to $^1/_4$ in.

The solvent front was allowed to proceed 8–10 cm and the development time was 30–80 min depending upon the particular adsorbent. The solvent employed was *n*-butanol–pyridine–water (75:15:10).

After development the plate was air-dried in a hood for 15–20 min and sprayed lightly with ammoniacal silver nitrate. Color development was carried out by heating 5 min at 100°.

Results

The R_F-values of the reference compounds employed and of the products obtained from the enzymatic digest of maltose are shown in Table I.

Discussion

Of the adsorbents employed Filter-Cel and combinations of Filter-Cel with Hyflo Super-Cel provided better resolution of monosaccharides than did Hyflo Super-Cel alone or Kieselguhr G. In the case of the latter two adsorbents glucose and xylose closely followed the solvent front and migrated as a single spot.

The substances detected in the enzymatic digest of maltose were also better resolved on Filter-Cel and formed more compact well defined spots than those obtained on the other adsorbents. However, solvent migration is considerably slower with Filter-Cel. Glucose and maltose in the enzyme digest material were identified by comparison of R_F values of reference compounds and by the rate of disappearance of maltose and the simultaneous formation of glucose due to the glucosidase activity of the enzyme. Isomaltose and panose have been previously identified by PAN, ANDREASEN AND KOLACHOV[5] and PAZUR AND ANDO[4] as the two major synthetic products resulting from the action of transglucosidase activity present in *Aspergillus niger* cultures on maltose. Although isomaltose and panose were not available to use as reference materials, the two major spots migrating below maltose were tentatively designated as isomaltose and panose since these were the only two other major spots detected in addition to glucose and maltose. The other unknown substances detected in trace quantities in the enzyme digest were not characterized but did separate as well defined spots. Their origin appeared to have been impurities in the maltose and enzyme materials employed.

Occasionally, specks of unground calcium sulfate remained in the slurry and tended to cause streaks during preparation of the filter aid plates. Although this was not a serious drawback it could usually be avoided by thorough mixing of the materials.

Thin-layer plates of Filter-Cel and Kieselguhr G could also be conveniently prepared by the spray technique described by BEKERSHY[6] which worked satisfac-

torily in our solvent systems. However, Hyflo Super-Cel due to its larger particle size tended to plug the nozzle of the chromatography spray bottle.

TABLE I

R_F VALUES OF REFERENCE COMPOUNDS AND PRODUCTS FROM ENZYMATIC DIGEST OF MALTOSE

	Adsorbent			
	Filter-Cel	*Hyflo-Super-Cel*	*Hyflo Super-Cel: Filter-Cel (6: 4)*	*Kieselguhr G*
Maltose digest				
Unknown	0.76	—	0.94	—
Glucose	0.67	0.94	0.76	0.96
Maltose	0.48	0.84	0.51	0.83
Isomaltose*	0.25	0.71	0.35	0.71
Panose*	0.15	0.50	0.16	0.55
Unknown	0.06	0.32	—	0.28
Unknown	0.00	0.00	0.00	0.00
Reference compounds				
Xylose	0.85	0.94	0.90	0.95
Glucose	0.67	0.94	0.76	0.95
Maltose	0.48	0.84	0.51	0.83
Development time (min)	80	30	50	50

* Tentative identification.

Conclusions

As a result of our work to date we have found the availability, low cost and high resolving power of ordinary filter aid materials make them excellent adsorbents for thin layer chromatography of carbohydrates. It is hoped similar results can be obtained with other classes of compounds.

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A method for the gas chromatographic separation of estrogens employing a solid injection system*

In recent communications from our laboratory, the gas chromatographic separation of synthetic mixtures of estrogens as well as their isolation from biological material

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