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SEPARATION OF SUGARS BY CENTRIFUGAL MICROPARTICULATE BED CHROMATOGRAPHY

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SUMMARY

A method has been developed for the qualitative separation of mono-, di-, and trisaccharides by centrifugal chromatography on columns of microparticulate silica gels. Sugars, under the influence of centrifugal force, migrate through the gel as narrow bands. The distance traveled by each sugar is dependent on molecular size and structure, duration and magnitude of centrifugal force, and composition of the solvent systems employed. Advantages of this technique over older chromatographic procedures include speed of separation and technical simplicity.

INTRODUCTION

Separation of various classes of compounds soluble in organic solvents has been achieved by centrifugal chromatography on beds of microparticulate silica. PIEL[1] demonstrated that mixtures of dyes could be speedily resolved on beds containing silica particles approximately 120 Å in diameter. More recently, RIBI, PETCOFF, and coworkers successfully resolved mixtures of microbial lipids[2], steroids[3], and pharmacologically active ingredients of marijuana[4] with this procedure.

The present communication deals with the application of this technique to the resolution of mixtures of carbohydrates including pentoses, hexoses, amino sugars, and di- and trisaccharides. Our results, as outlined below, demonstrated that individual components of mixtures of sugars could be resolved by centrifugal microparticulate bed chromatography. The advantages of this procedure in comparison with other chromatographic methods are discussed briefly.

MATERIALS AND METHODS

Silica

Microparticulate silica (Type I G-30 Quso), average particle diameter 150 Å, was obtained from Ivan Sorvall, Inc., Instrument Research Laboratory, Hamilton, Mont.

Analysis

The equipment and procedures used were similar to those described earlier[2,3]. A slurry of silica (1.5 g silica/40 ml of appropriate slurry solvent) was packed in glass columns 3 mm in internal diameter and approximately 50 mm long (Ivan Sorval, Inc., Norwalk, Conn.) by centrifugation at 1500 × *g* for 10 min. Then 2.5 to 5 λ of solutions of the sugars (prepared by adding one volume of chloroform to one volume of dimethyl sulfoxide containing, usually, 10 mg sugar/ml) were added to a loading stopper which was placed on top of the bed. The reservoir above the stopper was filled with the developing solvent, and the apparatus was then centrifuged at 2500 × *g* for the length of time necessary to allow the sugars to migrate into and separate on the silica gel bed. After centrifugation the columns were extruded from the glass tubes and dried in an oven at 60° for about 10 min. The location of the individual sugars was determined by heating the columns after they had been sprayed with sulfuric acid saturated with sodium dichromate. Amino sugars were also detected by spraying with a commercially prepared ninhydrin spray (Gelman Instrument Co., Ann Arbor, Mich.).

Only reagent grade solvents were used in these experiments.

RESULTS

A variety of solvent systems employed for paper chromatography (PC) and thin-layer chromatography (TLC) was screened for suitability for separation of sugars by centrifugal chromatography. Certain of these solvents enabled the sugars to migrate through the gel bed as fairly narrow and discrete bands. These bands varied in width from 1–2 mm at the top of the column to 3–4 mm at the bottom. Migration distances (distance from the top of the column to the band front) of a number of sugars in several of the more useful solvent systems are presented in Table I. Separation of the sugars was generally achieved when migration distances differed by 2–3 mm for the slowly migrating sugars and 4–5 mm for the rapidly migrating sugars.

Not all of the sugars could be separated with a single solvent system, but behavior in several systems should be very helpful in identifying unknown sugars. As an example, both glucose and mannose migrated at the same rate in solvent system C (see Table I). However, in system B these sugars formed separate and distinct bands. Similarly, ribose and xylose migrated at about the same rate in system A, but their behavior was very different in systems B and C.

Examples of separations accomplished by centrifugal chromatography on microparticulate silica are presented in Figs. 1A and B. In each instance the column on the left illustrates the separation of sugars in a mixture and the other columns show the chromatographic behavior of each sugar when run individually.

The sensitivity of this technique was determined by chromatographing dilutions of mixtures of sugars. Amounts of 25 μg of each sugar were present in the solution applied to the columns shown on the extreme left in Figs. 2A and B. Serial two-fold dilutions of the starting mixtures were added to the remaining columns. Most sugars in quantities of 1.6–3.1 μg could be detected, but larger amounts of fucose and arabinose were necessary for visualization.

In general, results obtained on different occasions were very consistent but some variations did occur, particularly when a new lot of microparticulate silica was introduced. However, when repetitive analyses of mixtures were made with the same

TABLE I

MIGRATION DISTANCES OF SUGARS IN MICROPARTICULATE SILICA GEL COLUMNS

Solvent systems: (A) Chloroform–methanol–distilled water (65:25:4) for slurry and developing solvent; G-30 Quso (2% moisture). (B) Chloroform–methanol (90:10) containing 0.01 *M* sodium acetate (dissolved in methanol before addition of chloroform) for slurry, acetone–water–chloroform–methanol (40:4:40:16) for developing solvent; G-30 Quso (2% moisture). (C) Chloroform–methanol (65:25) containing 0.05 *M* sodium acetate (dissolved in methanol before addition of chloroform) for slurry, chloroform–methanol–distilled water (65:25:2) for developing solvent; G-30 Quso (6% moisture). The sample was centrifuged for (A) 13 min at 2500 × *g*, (B) 30 min at 2500 × *g*, and (C) 16 min at 2500 × *g*.

Sugar	*Migration distance*[a]		
	Solvent system A	*Solvent system B*	*Solvent system C*
Raffinose	5	1	1
Melezitose	7	1	4
Lactose	11	6	5
Trehalose	12	3	7
Maltose	14	5	8
Sucrose	18	7	11
Galactose	25	16	23
Glucose	27	16	26
Mannose	29	19	29
Sorbose	31	22	29
Fructose	32	24	29
Arabinose	38	30	34
Fucose	44	43	42
Rhamnose	46	[b]	40
Ribose	47	47	37
Xylose	48	30	41
Glucosamine	11	16	19
Galactosamine	14	12	15

[a] Distance (mm) from top of column to band front.
[b] Migrated off column.

TABLE II

AVERAGE DISTANCE OF MIGRATION OF SUGARS FOR TEN CONSECUTIVE TRIALS[a]

Sugar	*Migration distance* ± *S.D.* (*mm*)
Lactose	4.7 ± 1.0
Sucrose	11.3 ± 1.3
Galactose	22.6 ± 1.2
Glucose	26.1 ± 1.4
Arabinose	34.4 ± 1.0
Xylose	40.8 ± 1.4

[a] Slurry made with G-30 Quso (6% moisture) and chloroform–methanol (65:25) containing 0.05 *M* sodium acetate. Chloroform–methanol–distilled water (65:25:2) for developing solvent.

lot of silica and the same stock of solvent mixture, differences in migration distances were slight. The results of such an analysis are presented in Table II. In this example the maximum standard deviation for the migration distances for six sugars analyzed ten consecutive times was only 1.4 mm.

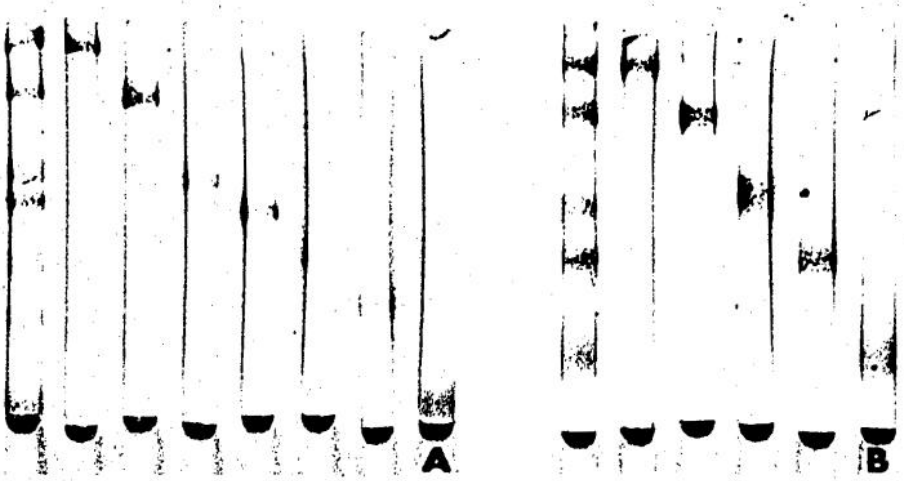

Fig. 1. Chromatograms of sugar mixtures resolved by centrifugal chromatography. (A) Sugars in left-hand column from top to bottom: lactose, sucrose, galactose, glucose arabinose, xylose, rhamnose. Sugars added individually in same order to remaining columns. (B) Sugars in left-hand column from top to bottom: melezitose, maltose, galactose, fructose, fucose. Sugars added individually in same order to remaining columns. System A (see Table I).

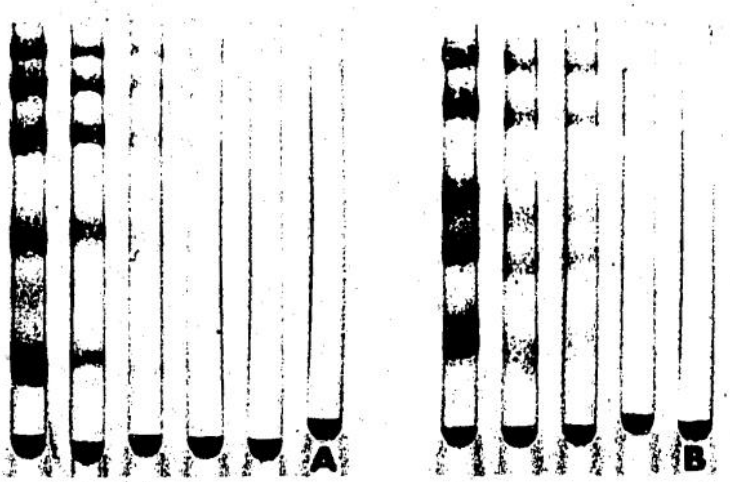

Fig. 2. Chromatograms of sugars analyzed in varying quantities with System A (see Table I). (A) Quantity (μg) of each sugar added to each column from left to right: 25, 12.5, 6.3, 3.1, 1.6, 0.8. Sugars from top to bottom: raffinose, lactose sucrose mannose, arabinose, rhamnose. (B) Quantity (μg) of each sugar added to each column from left to right: 25, 12.5, 6.3, 3.1, 1.6. Sugars from top to bottom: melezitose, maltose, galactose, fructose, fucose.

DISCUSSION

The above results demonstrate that a variety of sugars can be qualitatively separated with the aid of centrifugal force on columns prepared from gels of micro-

particulate silica. The molecules of each sugar migrate as a narrow band through the column independently of other sugar molecules; the position of each band depends upon the type of solvent, conditions of centrifugation, and upon substances, such as sodium acetate, which may be incorporated in the solvent. In general, migration distance is a function of molecular size; pentoses migrate more rapidly than hexoses and hexoses more rapidly than di- and trisaccharides. No attempt has been made to assay the sugars quantitatively, but it seems probable that methods applied to PC and TLC procedures could be adapted to the quantitative estimation of sugars in these columns.

Centrifugal chromatography offers several advantages over other methods of chromatographing sugars. The principal advantage is the rapidity with which assays can be completed. Analysis time depends on the complexity of the sugar mixture, but for simple mixtures all operations, including packing of the column, addition of sample, centrifugation, drying, and visualization may require 45 min or less. In contrast, PC and TLC procedures may take up to hours longer. Overnight development is common for resolution of sugars by PC[5]. Current procedures for TLC are also lengthy because the supporting medium is often impregnated with substances such as sodium acetate[6] or monosodium phosphate[7] before the plates are prepared, dried, and activated. Gas chromatographic procedures are about as rapid as centrifugal chromatographic methods, but the former technique is more complicated in that sugar derivatives must be prepared before analysis[8]. Also, the apparatus can analyze only one sample at a time, whereas with present centrifugal chromatographic equipment up to four samples can be analyzed simultaneously.

In addition, centrifugal chromatography is technically simple and relatively inexpensive. Less manual dexterity is required for the addition of a sample to a loading stopper than to a prescribed point on paper or thin-layer plates. Although the equipment for centrifugal chromatography may not be less expensive than that for PC and TLC, the investment is much less than that for gas chromatography. Taken together, these various advantages recommend this technique to those who are concerned with the separation and identification of sugars in mixtures.

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