CONCERNING THIN-LAYER ELECTROPHORESIS OF CARBOHYDRATES

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Separation of carbohydrates by paper electrophoresis (PE) in borate buffer is today a well established procedure[1,2]. Also several papers have been published dealing with thin-layer chromatography (TLC) of carbohydrates using borate buffer as a mobile phase[3–8]. Although thin-layer electrophoresis (TLE) is already an established method of separation[9–26], especially for substances of low molecular weight, it has not been employed systematically for separation of various compounds of the carbohydrate group.

It could be expected that TLE of carbohydrates would exhibit qualities inherent to TLE, namely shorter separation time, sharper zones, possibility of using aggressive spraying reagents, and wide variation in the composition of supporting media. Furthermore, TLE offers a new possibility for separation of substances which could be separated only with difficulty otherwise, and could also supplement TLC on the same plate in cases where insufficient separation is obtained by using only one of those procedures.

Our successful separation of some products of acid hydrolysis of the polysaccharide, evernan, several years ago[27] was the starting point for this investigation in TLE of carbohydrates.

MATERIALS AND METHODS

In all experiments Silica Gel G (Merck A.G., Darmstadt, Germany) was employed. Glass plates were of 200 × 200 mm size with a thickness of 3.3 mm. Thin-layer plates were prepared (thickness of the Silica Gel G layer was 0.25 mm) using Desaga stainless steel apparatus and adjustable spreader. Silica gel paste was prepared with abs. ethanol (35:65, w/v) and the plates were air dried. Buffer solution (pH 10.2) was prepared in the following manner: boric acid (7.22 g) was dissolved in 1 l of distilled water (solution A); sodium carbonate·H_2O (35.1 g) was dissolved in 2 l of distilled water (solution B). Solution A (582 ml) and solution B (1.418 ml) were combined, stirred 5 min with magnetic stirrer and used as a buffer. As a rule, fresh buffer solution was employed in each experiment. Carbohydrates were applied (using 10 μl Hamilton syringe), usually in 10 μg quantities (1 μg/μl of aqueous solution), 2 cm off the anodic end of the thin-layer plate. The plate was sprayed lightly and uniformly with the buffer immediately after the substances were applied, dried several minutes at room temperature, and then sprayed again in the same manner (total of approximately 2.5 ml of buffer is used). In a typical experiment weight of the air dried silica gel on the plate was 1.92 g, when sprayed to wetness with buffer, was 4.61 g, and with

addition of 13 ml of the buffer, the total weight was 17.5 g; in this condition the pH of the buffer, originally 10.2, decreased to 8.4. The plate was then accommodated in the Desaga (Brinkman) apparatus for thin-layer electrophoresis, using paper tongues and 200 ml of the buffer solution in each compartment. Electrophoresis was then performed at 400 V and 80 mA for 60 min unless otherwise indicated. All monosaccharides, disaccharides, and trisaccharides were obtained from one of the following companies in the best grades available: Mann Research Laboratories, New York, N.Y., K & K Laboratories, Palinview, N. Y., and Nutritional Biochemical Corporation, Cleveland, Ohio. All substances were used dissolved in distilled water. Lichenan and evernan were prepared as described by STEFANOVIĆ[28]; amylose was obtained from K & K Laboratories, dextran from Mann Research Laboratories and laminaran from Pierce Chemical Co., Rockford, Ill. Polysaccharides are employed dissolved in solution B. Tetramethylglucose was dissolved in chloroform. At the end of each run substances were usually identified by spraying with 50 % sulfuric acid followed by heating for 2 h at 120°. Several other sprays are also employed, *e.g.* aniline phthalate, phosphomolybdic acid and anisaldehyde–sulfuric acid[29]. The latter reagent is particularly useful because of the appearance of different colors with various carbohydrates.

The volume of electrolyte in the anode and cathode compartments after electrophoresis was measured colorimetrically (at 520 mμ) after addition of an equal quantity of red ink in each compartment, as well as by measurement of actual electrolyte volume.

RESULTS AND DISCUSSION

During our work on the isolation and structure determination of the polysaccharide, evernan, we have observed that products of acid hydrolysis of this polyglucan travel toward the cathode during thin-layer electrophoresis on Silica Gel G plates with borate buffer (pH 10)[27]. This direction of movement is unexpected, because migration of the carbohydrate during paper electrophoresis in the presence of borate ions results from the formation of weak, negatively charged, ionic species (I, II and III, Fig. 1)[30], which migrate during electrophoresis toward the anode. The concen-

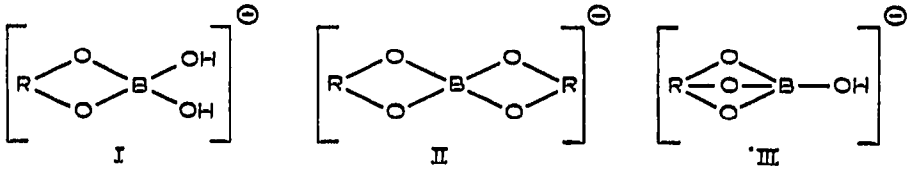

Fig. 1. Negatively charged ionic species produced by complexing of carbohydrates with boric acid.

tration of those ionic species in aqueous boric acid is low, and an increase in pH can raise their concentration and increase the electrophoretic mobility of the associated compounds. At pH 8.0 mobility of the carbohydrates is still proportional to borate content of the buffer, which indicates in this situation that some of the carbohydrates in borate solution are still incompletely complexed[1]. In our experiments, however, (pH of the buffer, 10.2) it could be expected that essentially all carbohydrates studied would complex with boric acid to a significant degree with the resultant, negatively charged species migrating toward the anode during electrophoresis. Nevertheless, this was not the case. All carbohydrates which we have examined by TLE on Silica Gel G

TABLE I

"THIN-LAYER ELECTROPHORESIS" OF CARBOHYDRATES

No.	*Compound*	*M_G value**	*Color with anisaldehyde–sulfuric acid reagent*
1	Dulcitol	0.41 ± 0.04	purple
2	D-(+)-Sorbitol	0.39 ± 0.04	blue-purple
3	L-(—)-Erythritol	0.88 ± 0.02	yellow
4	Adonitol	0.78 ± 0.01	purple-blue
5	Arabitol	0.69 ± 0.01	light brown
6	D-Glucose	1.00 ± 0.00	clear blue-green
7	L-Rhamnose	0.94 ± 0.06	green
8	D-(—)-Ribose	0.49 ± 0.02	prussian blue
9	L-(—)-Fucose	0.99 ± 0.01	blue
10	D-Mannose	0.96 ± 0.01	green-yellow
11	2-Deoxy-D-ribose	1.01 ± 0.01	purple-magenta
12	D-(—)-Levulose	0.51 ± 0.01	blue-green
13	D-(—)-Lyxose	0.89 ± 0.02	blue
14	L-(—)-Xylose	0.76 ± 0.01	pale-purple
15	Galactose	0.88 ± 0.02	grey-green
16	Nigerose	0.92 ± 0.03	blue
17	Turanose	0.93 ± 0.01	blue
18	D-(+)-Maltose	1.07 ± 0.02	blue-green
19	D-(+)-Trehalose	1.13 ± 0.01	light-blue
20	D-(+)-Lactose	1.10 ± 0.02	green
21	Cellobiose	1.11 ± 0.01	green-blue
22	Saccharose	1.11 ± 0.01	blue-green
23	D-Glucuronic acid	0.31 ± 0.02	pale maroon
24	Glucosaminic acid	0.42 ± 0.02	yellow-brown
25	Glucosamine·HCL	1.09 ± 0.02	yellow-brown
26	N-Acetyl-D-glucosamine	1.02 ± 0.01	brown-pink
27	D-Galactosamine·HCL	0.92 ± 0.01	yellow-brown
28	D-(+)-Melezitose	1.09 ± 0.02	blue-green
29	Amylose	1.12 ± 0.01	dark blue
30	Lichenan	1.11 ± 0.04	dark blue-green
31	Dextran	1.24 ± 0.01	blue-green
32	Evernan	1.16 ± 0.01	grey-blue
33	Laminaran	1.06 ± 0.01	grey-blue-green
34	2,3,4,6-Tetramethyl-D-glucose	0.80 ± 0.02	clear blue

* Mean and standard error of four determinations.

exhibit significant mobility, but in every instance these substances migrate toward the cathode. In Fig. 2 is presented the time and distance-traveled relationship for D-(+)-glucose and in Table I, M_G values for the series of monosaccharides, polyalcohols, oligosaccharides and polysaccharides. (Experimental conditions are as described in Section Materials and methods).

It is obvious from Table I, that M_G values of various sugars are reproducible. Experiments under various circumstances have established that reproducibility depends on several factors:

(A) Uniform thickness, homogeneity, and purity of the silica gel layer.

(B) Temperature at which the experiment is performed (temperature of the room, of the tap water used for cooling, etc.).

(C) The amount of the carbohydrate applied on the starting point and the size of the spot.

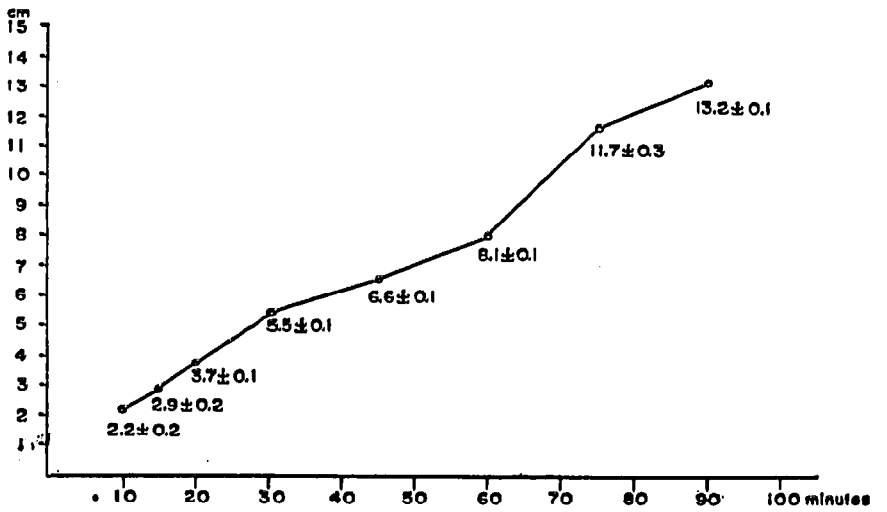

Fig. 2. Time and distance-traveled relationship in D-glucose. Experimental conditions as described in Section Materials and methods. Presented value is mean and standard error of four determinations.

(D) Buffer quantity and concentration in each compartment.
(E) Thickness of the glass plate on which silica gel was applied.
(F) The leveling of the apparatus for TLE.
(G) Uniform thickness of the paper wicks employed to connect compartments with TL plate.
(H) Uniformity and intensity of spraying with the buffer solution.
(I) Stability of the electrical current.

However, for most practical purposes strict adherence to all these points is not necessary since a comparison between known and unknown compounds is performed on a single plate.

Concerning the comparative mobilities of polyalcohols, monosaccharides and disaccharides, the results summarized in Table II were obtained.

TABLE II

STATISTICAL SIGNIFICANCE OF M_G VALUES OF POLYALCOHOLS, MONOSACCHARIDES AND DISACCHARIDES

Compound	M_G *value**
I. Polyalcohols: dulcitol, sorbitol, erythritol, adonitol, arabitol	0.63 ± 0.1
II. Monosaccharides: glucose, rhamnose, ribose, fucose, manose, levulose, lyxose, xylose, galactose	0.81 ± 0.07
III. Disaccharides: nigerose, maltose, turanose, trehalose, lactose, saccharose	1.05 ± 0.03

* Mean and standard error. Mean values for all three groups of compounds were calculated using the mean for every compound as reported in Table I.

Statistical significance: I *vs.* II no statistical significance; I *vs.* III, $P = < 0.001$; II *vs.* III, $P = < 0.05$.

It is obvious from Table II that the mobility of the carbohydrates can be expressed in the following manner: polyalcohols < monosaccharides < disaccharides. Regardless of the lower value of M_G for polyalcohols, no significant statistical differ-

ence was observed between them and the monosaccharide group. This was due to the highest spreading of M_G values for the compounds of the polyalcohol group; "TLE" is, therefore, the efficient method for separation of those compounds. Generally, it appears that the compounds more prone to complexing with borate buffer exhibited lower M_G values.

Concerning several compounds directly related to D-glucose, they can be arranged in order of decreased mobility in the following manner: glucosamine > N-acetylglucosamine > D-glucose > 2,3,4,6-tetramethylglucose > sorbitol > glucosaminic acid > glucuronic acid.

As could be expected, glucosaminic and glucuronic acids have the lowest migration values, most likely because of acquisition of negative charge due to the extensive dissociation of the carboxyl group in alkaline solution. Close to their M_G values is the migration value of sorbitol, which is significantly lower than M_G value of D-glucose (100.0). It is known that open chain carbohydrates, such as polyalcohols, interact strongly with boric acid, especially in alkaline conditions. Therefore, the relatively low mobility of sorbitol (and all polyalcohols in our experiment) could be explained by their preferential rate of complexing with boric acid and the resultant, higher negative charge of these compounds, which subsequently offers resistance to the buffer flow toward the cathode.

It could be expected that because of the lack of negative charge tetramethylglucose would travel more than D-glucose; however, a lower migration value was observed. This is most likely due to a dramatic difference between the solubilities of D-glucose and tetramethylglucose in aqueous solution.

The most important result of these experiments is our interpretation of the unexpected direction of migration of carbohydrates. The particular observations indicating to us that we are dealing not with TLE, but with electroosmosis, are summarized as follows:

(1) All carbohydrates, when in alkaline borate buffer migrate toward the cathode.

(2) 2,3,4,6-Tetramethyl-D-glucose does not form a complex with borate ions, and is therefore used as a marker for correction for electroosmosis in paper electrophoresis. In our experiments, this substance exhibited a significant rate of migration toward the cathode comparable to other carbohydrates.

(3) Measurement of the volume of electrolyte before and after electrophoresis in both compartments established that the volume of electrolyte at the cathode increased approximately for 6–8 % during one hour of electrophoresis. This increase in volume in the cathode compartment can result only with preferential movement of buffer solution from anode to cathode.

The foregoing three observations strongly support the hypothesis that during TLE of carbohydrates in borate buffer (pH 10.2) on Silica Gel G plates migration of sugars toward the cathode is the result of electroosmosis. As a consequence of the negative electrokinetic potential of the silica gel layer, the solvent (buffer) has acquired a positive potential, and is thereby dragged in the direction of the current (cathode). It is logical that this electroosmotic flow is especially pronounced at pH values favoring the ionization of negative groups in silica gel. However, at the same time alkaline conditions favor formation of a negatively charged complex between carbohydrates and boric acid. Final direction and rate of migration is, therefore, dependent

on several factors: degree of complex formation with boric acid, electroosmotic flow, adsorbtion on silica gel, "apparent volume" of the compound, hydrostatic flow, evaporation, and most likely a few other factors still not understood.

The pH value of our borate buffer which favors ionization of silicic acid evidently makes electroosmotic flow the predominant factor among the forces influencing migration of carbohydrates at this pH.

It is obvious from the reported results that this method can be employed for separation and tentative identification of some carbohydrates, and eventually also for other classes of compounds.

We suggest that in this situation, where it is established that the primary force deciding direction of the movement of compounds during TLE is electroosmosis, the procedure should be denoted thin-layer electroosmosis (TLEO). The range of mobilities for carbohydrates is not as great as that obtained by PC and PE; however, TLEO still appears to be a useful method for the separation of sugars in addition to or eventually combined with TLC, PC and PE. TLEO should be, therefore, looked upon as one more complementary tool for the analysis of carbohydrates.

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SUMMARY

A method is described for the separation and eventual identification of a series of carbohydrates using their migration on thin-layer Silica Gel G plates as a result of externally applied electrical field (400 V). Borate buffer (pH 10.2) was utilized as the electrolyte. Attention is drawn to the parameters determining migration toward the cathode and M_G values of examined compounds.

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