PAPER ELECTROPHORESIS OF SUGARS WITH CETYLTRIMETHYLAMMONIUM BORATE*

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Paper electrophoresis with borate buffers is a widely used procedure for the separation of sugars[1]. The present paper reports the finding that when cetyltrimethylammonium (CTA) borate is substituted for sodium or potassium tetraborate, a striking change takes place in the relative mobilities of sugars. Thus some separations are obtained that cannot be achieved with the alkali borates.

EXPERIMENTAL

Most of the substances used were commercial samples.

2,3,4,6-Tetra-O-methyl-D-glucose was kindly provided by Prof. E. L. HIRST, and tetramethylammonium chloride by Dr. J. H. COMIN. 1,2,5,6-Diisopropylidene-D-glucofuranose was synthesized according to RECONDO AND RINDERKNECHT[2], and D-glucose 1,2-cyclic phosphate as described by KHORANA *et al.*[3].

Buffers

CTA bromide ("Cetavlon") was a generous gift of Industrias Químicas Argentinas Duperial. The commercial drug contains, in addition to the cetyltrimethylammonium salt, a mixture of dodecyl- and tetradecyl-trimethylammonium derivatives. The free base was obtained from the bromide by passage through a Dowex-1 column in the hydroxyl form. Boric acid was then added to give a pH of 9.6 at a final concentration of 0.1 *M* CTA (*ca.* 1 g of boric acid for each 100 ml of 0.1 *M* CTA). A similar procedure was applied for tetramethylammonium borate.

A 0.1 *M* solution of CTA hydroxide was also used for preparing CTA sulfate and CTA carbonate at pH 9.6, the former by addition of sulfuric acid and the latter by bubbling CO_2.

Paper electrophoresis

Electrophoresis was carried out with Whatman No. 1 paper and the apparatus of MARKHAM AND SMITH[4]. Toluene was the cooling liquid and platinum electrodes were used. In some cases the paper was enclosed between glass plates, according to KUNKEL

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AND TISELIUS[5]. In most runs a tension of 1,000 V (20 V/cm) was applied during 3 h with CTA borate and during 2 h with potassium borate. With a paper strip 15 cm wide, at this potential, the former buffer gave 6 mA and the latter 20 mA.

The separations were somewhat disturbed by the flow of buffer from the electrode compartments into the paper, especially from the anode chamber. Best results with the MARKHAM AND SMITH arrangement were obtained by the following procedure. After applying the substances, about 3 cm of the anodic end of the paper strip were dipped briefly into a collodion solution (4 % "Parlodion" in 1:1 alcohol–ether) and the solvent was allowed to evaporate. The rest of the strip was then moistened with buffer by sliding it against a filter paper wick dipped in buffer. The paper was then placed in the electrophoresis apparatus and the voltage was connected 10 min later, in order to allow for diffusion of buffer through the collodion membrane. Collodion can also be applied on both ends of the paper strip, but in this case the paper may run dry at some places, thus interrupting the current flow. This does not occur with the paper enclosed between glass plates, and accordingly, with this arrangement, both ends of the strips were coated, in order to obtain more precise determinations of the endosmotic flow. This treatment effectively prevents the flow of buffer into the paper, as shown by the fact that equal distances were traveled by substances spotted at different places along the paper strip, and by the constancy of the electric current during the run.

A few experiments were carried out with glass fiber paper (Hurlbut Paper Co. No. 334–AH).

Detection of spots

Sugars were revealed with silver nitrate, according to TREVELYAN *et al.*[6], modified as described previously[7]. The revelation of 2,3,4,6-tetra-O-methyl-D-glucose was greatly improved by a previous heating of the paper in an oven at 110° during 5 min. In order to detect diacetone glucose it was necessary to spray the paper previously with 10 % trichloroacetic acid in ethanol and to place it in an oven at 110° for 5 min.

Caffeine, creatinine, nucleotides, phthalic and benzoic acid were detected by examination under an ultraviolet lamp.

Sugar phosphate spots were located according to BURROWS *et al.*[8].

Electropherograms on glass paper were revealed for sugars with *p*-anisidine–hydrochloric acid[9].

Moving boundary electrophoresis

The experiments were performed with a Kern model LK-30 apparatus, using 0.05 *M* potassium tetraborate at pH 9.2 or 0.06 *M* CTA (borate) at pH 9.6. A gradient of 10 V/cm for the potassium buffer and 6 V/cm for the CTA buffer was applied during 20 min at 21–22°. The substances were tested at a concentration of 1 %, except for the mixture of creatinine and glucose, which contained 0.5 % of each.

Determination of endosmosis

An approximate measurement of endosmosis was made directly with the simple arrangement sketched in Fig. 1. The horizontal tube was filled with a slurry of cellulose powder* in the appropriate buffer. The potassium and CTA borate solutions

* Obtained from Whatman ashless cellulose tablets.

employed were the same as those used for paper electrophoresis. Ten minutes after applying the potential to the electrodes, the difference in height between the two side arms (from 5 to 25 mm, according to the experiment) was recorded. By this time

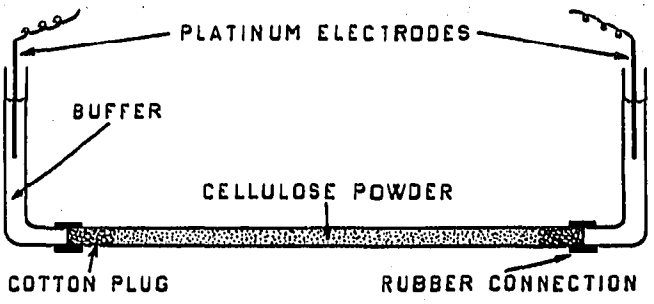

Fig. 1. Arrangement for direct measurement of endosmosis.

the liquid levels had become almost stationary. In one series of experiments the horizontal tube was 0.5 cm in diameter and 43 cm long and the applied voltage was 500 V. In another series the tube was 0.34 cm in diameter and 65 cm long, and the applied voltage was 1,000 V. In both cases several determinations were made and repeated after reversing the sign of the voltage. The results were reproducible within ± 15 %.

RESULTS AND DISCUSSION

Table I shows the results obtained with CTA borate, compared with those given by potassium and tetramethylammonium borate. The general effect of CTA borate may be roughly described as a reversal in the order of mobilities, as shown graphically in Fig. 2. For instance, the order of increasing mobilities is, in potassium borate, mannose–fructose–glucose, while in CTA borate the reverse holds true. It can also be observed that the methyl glucosides and the disaccharides run very rapidly in CTA borate. Some separations that are not possible in potassium borate can be obtained with the CTA salt, as is the case for glucose and xylose or fructose and galactose, so that the four hexoses: glucose, galactose, fructose and mannose can be separated in a single run. On the other hand, the disaccharides tested, with the exception of melibiose and turanose, have all about the same mobility. Although many sugars give round spots, there is more tendency to "tailing" with CTA than with potassium borate. This drawback can be partly overcome by reducing the amount of sample to about 0.2 μmole of each sugar.

Further experiments were carried out, in an attempt to explain the remarkable behavior of sugars in paper electrophoresis with CTA borate. An early observation was that in standard runs with CTA borate, the first 11 cm of the paper from the origin were free from spots, whereas with potassium borate the spots were distributed from the starting line up to about 17 cm. This suggested the possibility that the endosmotic flow with CTA borate might be of different sign than with potassium borate.

The mobility of several endosmosis markers[1,10] was then measured in different buffers. The results are shown in Table II and it can be seen that with CTA salts, especially with the borate and carbonate, the substances moved towards the anode,

TABLE I

MOBILITIES OF SUGARS AND SOME OTHER SUBSTANCES WITH DIFFERENT BORATE SALTS

The values indicated are approximate and are given mainly for comparison. The mobility of 2,3,4,6-tetra-O-methyl-D-glucose is taken as 0, and negative sign denotes movement towards the cathode.

Reference number	Substance	CTA Borate*	0.05 M Potassium tetraborate, pH 9.2		Tetramethylammonium borate*
		Distance traveled** cm	Distance traveled*** cm	$u \times 10^6$ cm^2/V sec	$u \times 10^6$ cm^2/V sec
1	D-Fructose	17.2	14.8	124	107
2	D-Galactose	15.0	15.6	129	112
3	D-Glucose	12.2	17.0	142	122
4	D-Mannose	19.5	10.8	96	85
5	L-Sorbose	13.7	16.4	139	120
6	D-Arabinose	15.3	15.2	130	110
7	D-Lyxose	22.3	11.2	98	86
8	D-Ribose	23.5	12.0	107	92
9	D-Xylose	14.1	17.2	140	121
10	L-Fucose	14.0	13.3	115	101
11	L-Rhamnose	19.3	6.8	69	58
12	Cellobiose	19.5	1.0	30	26
13	Lactose	19.6	4.4	51	44
14	Maltose	19.5	3.0	42	36
15	Melibiose	11.2	12.4	107	89
16	Sucrose	18.2	0.2	22	18
17	D-Trehalose	18.5	—0.6	17	16
18	Turanose	15.1	9.8	89	78
19	*meso*-Inositol	19.3	6.7	67	59
20	D-Sorbitol	15.0	13.4	114	98
21	α-Methyl glucoside	18.5	—0.4	18	12
22	β-Methyl glucoside	19.1	0.1	22	18
23	1,2; 5,6-Diisopropylidene-D-glucofuranose	6.0	—3	0	0
24	2,3;4,6-Tetra-O-methyl-D-glucose	12.7	—3	0	0
25	Adenosine-5′-phosphate	2.9	19	—	—
26	Adenosine triphosphate	1.7	20	—	—
27	Fructose-1,6-diphosphate	3.0	25	—	—
28	Fructose-6-phosphate	4.1	22	—	—
29	D-Glucose-1,2-cyclic phosphate	8.0	14.5	—	—

* Prepared as indicated under EXPERIMENTAL.
** Distance from point of application of sample after 3 h at 20 V/cm.
*** Distance from point of application of sample after 2 h at 20 V/cm.

although at different rates. The other electrolytes gave the usual small flow towards the cathode.

In view of the lack of uniformity in marker mobility with CTA salts, a direct determination of endosmosis was attempted in the manner described under EXPERIMENTAL. The differences in level obtained with CTA borate were 3 to 5 times greater and of opposite sign to that with potassium borate. This reversal of the electrical charge of the paper with CTA salts is in agreement with other reports[11]. By comparing the results of the direct measurement of endosmosis with those of Table II, it would appear that the mobility of creatinine in CTA borate is mainly attributable to the

endosmotic flow, whereas with this buffer the other markers and the sugars would somehow be delayed in their movement towards the anode.

The possibility of a chromatographic effect was investigated by submitting glucose and β-methyl-glucoside to paper chromatography with CTA borate as solvent. Both compounds gave, however, the same R_F of 0.9.

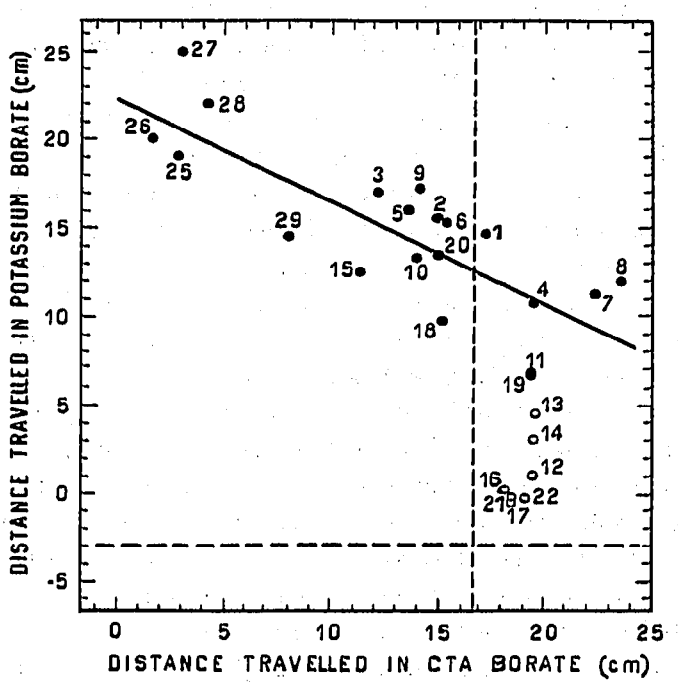

Fig. 2. Graphical representation of migration rates. The data and the reference numbers of the compounds are taken from Table I. The straight line was calculated by the least squares method. The correlation coefficient is —0.81. The values for several disaccharides and the methyl glucosides (empty circles) were not included in the calculation, since these substances are believed to be subject in CTA borate to the endosmotic flow only (see text). The broken lines represent the distance traveled by creatinine in the two buffers.

TABLE II

MOBILITIES OF ENDOSMOTIC MARKERS WITH DIFFERENT ELECTROLYTES

Mobilities are given in cm^2/V sec. × 10^6, and the positive sign denotes movement towards the anode. The mobility for sugars is given for those electrolytes where they all travel at the same rate.

Electrolyte	*Substance*				
	Caffeine	*Creatinine**	*Sugars*	*Diacetone glucose*	*2,3,4,6-Tetra-O-methyl-D-glucose*
0.15 *M* Ammonium acetate, pH 8.2	—11	—11	—11	—11	—11
0.05 *M* Ammonium sulfate, pH 8.0	—11	—11	—11	—11	—
CTA sulfate, pH 9.6**	+3	+28	+30	+3	—
CTA carbonate, pH 9.6**	+31	+61	+64	—	+50
CTA borate, pH 9.6**	+32	+77	—	+27	+60
Tetramethyl ammonium borate, pH 9.6**	—21	—21	—	—21	—21
0.05 *M* Potassium tetraborate, pH 9.2	—21	—21	—	—21	—21

* No decomposition to creatine occurred during the run. Creatine gave a different mobility.
** Prepared as indicated under EXPERIMENTAL.

The results obtained cannot be ascribed to a specific interaction with the support used, since electrophoretic runs of several sugars with glass fiber paper gave the same order of mobilities as with common paper.

The remaining possibility was that the observed mobilities were the net result of two contrary effects: an endosmotic movement towards the anode, superimposed on an opposite displacement towards the cathode.

In order to eliminate the endosmotic effect, some experiments of moving boundary electrophoresis were carried out. With potassium borate as electrolyte, creatinine and caffeine remained stationary, while glucose moved towards the anode. On the other hand, with CTA borate, creatinine only was immobile, whereas both glucose and caffeine traveled towards the cathode. In both caces mixtures of glucose and creatinine gave two distinct boundaries. It would appear therefore as if in the CTA buffer the negatively charged sugar borate complexes moved towards the pole of the same sign, the more so the higher their average charge, as shown in Fig. 2. Indeed, when a substance able to complex with borate bears in addition fixed negative charges, its displacement towards the cathode becomes greater, as shown by the low anodic mobilities found with several phosphate esters (see Table I and Fig. 2).

The long hydrocarbon chain of CTA is essential in order to give rise to the observed pattern of relative mobilities, since results with tetramethylammonium borate were similar to those given by potassium borate, as shown in Table I.

It is known that in solutions of detergents with long paraffin chains, like CTA salts, the cations aggregate, above a critical concentration, to form ionic micelles of considerable size, which bear the positive charges on their surface[12,13].

It seems then possible, that negatively charged ions may associate with the positively multicharged micelles and thereby be transported towards the cathode. The association may be expected to be stronger, the higher the negative charge of the ion, and this would explain the paradoxical result that the more highly charged anions show greater mobility towards the cathode.

In addition to the electrical forces, interaction between solutes and micelles may take place through Van der Waals forces. This would explain why different uncharged molecules travel at different rates, as shown in Table II. The association of molecules with the micelles would increase with increasing lyophobicity of the molecule, and in this connection it is worth mentioning that caffeine is less displaced towards the anode than the more water-soluble creatinine. When the negative charge and a strongly lyophobic group are present together in the same ion, the movement towards the cathode may become large enough to offset the endosmotic flow, as shown in the case of benzoic and phthalic acid, which show a mobility of approximately $-8 \cdot 10^{-6}$ cm^2/V sec in CTA borate and $-30 \cdot 10^{-6}$ cm^2/V sec in CTA carbonate.

The process taking place in zone electrophoresis with CTA buffers may then be summarized as follows. All the substances are transported at the same rate towards the anode by a rapid endosmotic flow; at the same time, association with the cathode-migrating CTA micelles to a variable extent will tend to carry the compounds in the opposite direction. The latter displacement will depend on the charge and/or the lyophobic character of each substance, thus giving rise to different apparent mobilities*.

The sugar derivatives that show low mobilities in potassium borate, such as the

* A reversal in electrophoretic mobility caused by CTA salts has also been reported for several bacteria[14].

methyl glucosides and most of the disaccharides, run at almost the same rate in CTA borate. This behavior could be explained, if it is assumed that the formation of borate complexes is somewhat depressed in solutions of the CTA salt, so that all the sugars with low affinity for borate would remain practically uncombined. They would then be affected only by the endosmotic flow, and, in view of their highly lyophilic character, it is perhaps not surprising that they move slightly ahead of creatinine.

The qualitative hypothesis outlined above seems to agree with most of the available facts, nevertheless some results remain unexplained. It is difficult to understand, for instance, why ribose and lyxose move well ahead of creatinine in CTA borate, when their mobility in potassium borate would indicate appreciable complex formation.

It is perhaps possible to apply the principle of the procedure described to the separation of other substances that differ from one another in charge and lyophobic character.

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SUMMARY

Paper electrophoresis of sugars and other substances with cetyltrimethylammonium borate at pH 9.6 gives rise to a pattern of mobilities different from that found with potassium borate, thus making certain separations possible that are not obtained with the potassium salt. There seems to be a general trend towards a reversal in the order of mobilities with the cetyltrimethylammonium buffer. The observed mobilities have been interpreted as the net results of two contrary effects: a strong and uniform endosmotic flow towards the anode and an opposite and variable movement towards the cathode. The latter displacement would take place by association of the solutes with the positively charged ionic micelles that are present in the solutions of cetyltrimethylammonium salts. Applications of this behavior might lead to the separation of other substances.

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