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Paper Electrophoresis of Carbohydrates in Glycerol-Boric Acid Buffer

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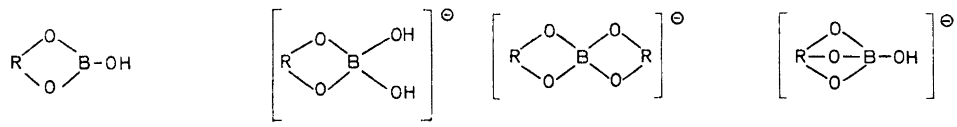
The paper electrophoretic mobilities of various monosaccharides and methyl ethers, disaccharides, furanosides, uronic and hexulosonic acids in glycerol-boric acid buffer, pH 6.8, have been examined and have been compared with those in borate, pH 10 and sulphonated phenylboronic acid, pH 6.5. The mobilities in the new buffer for most of the carbohydrates parallel those obtained in the sulphonated phenylboronic acid buffer. Advantages of the new procedure include more available chemicals used, higher absolute mobilities and sharper spots obtained.

Paper electrophoresis involves the migration of charged substances in a conducting solution under the influence of an applied electrical field with filter paper as support for the electrolyte. In order to make neutral carbohydrates migrate it is necessary to ionise them by using a solution of high pH or to complex them with some charged ionic species.

The reaction of polyhydroxy compounds with boric acid and borate ion has long been known and thoroughly studied.[1,2] Borate buffer of pH 10 has been used in paper electrophoresis of carbohydrates.[3,4] The complexes involved can be formulated as follows:



I II III IV

The ionic species II – IV migrate during electrophoresis. Of these, the tridentate complex (IV) is formed only under favourable steric conditions. These charged tetragonal boron complexes occur in low concentrations in

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aqueous boric acid. At higher pH their concentration is increased which results in increased electrophoretic mobilities of various carbohydrates.[3,5] The maximum mobilities of carbohydrates are generally in the region 9 – 10, a pH range which has been used much for paper electrophoresis. It is also important, however, that the relative mobilities of certain pairs of carbohydrates are dependent on pH. Thus, for instance, at pH 9 – 10 D-glucose has a mobility greater than that of D-fructose, but at pH 7 – 8 the reverse relationship is obtained. Both sugars, however, have considerably lower migrations at pH 7 – 8 than in alkaline borate. The migration of various types of carbohydrates in alkaline borate and its dependence on the spatial disposition of the hydroxyl groups have been discussed in detail in reviews.[3,4]

Thus, *cis*-1,2-diols of the five-membered ring compounds complex more readily with borate than the corresponding *trans* compounds, which is reflected by greater electrophoretic mobilities. In the zig-zag conformations of acyclic 1,2-diols the *trans* isomer, however, seems to react more strongly with borate than the *cis* isomer. In glucopyranosides the mobilities are attributed to borate complexes between the C-4 and C-6 hydroxyls.

No significant increase in the rate of mobility of sugars at pH 7 was found by using phenylboronic acid instead of boric acid.[6,7] Sulfonated phenylboronic acid at neutral pH-values, however, increased the mobilities for a number of carbohydrates and gave much more selective reactions than borate at pH 10. The new buffer was also more suitable for alkali-labile carbohydrates. The sulphonic group is believed to decrease the ionisation of the boronic acid group, thus leading to trigonal boronic esters (I) rather than tetragonal complexes. These migrate during paper electrophoresis due to the ionisation of the sulphonic acid group. With reducing sugars and glycosides, the largest contribution to the mobilities was found to arise from *cis*-1,2-diols of five-membered rings, but none from *cis*-1,2-diols of six-membered ring compounds or from such a diol group as that on C-4 and C-6 in D-glucopyranose. Important complex formation in this electrolyte occurs across a *cis*-1,3-diol grouping of axially disposed hydroxyl groups.[6,8]

More recently, paper electrophoresis of various carbohydrates in diphenylborinate at pH 10 was studied [9] and the electrophoretic mobilities were compared with those in borate. Only one type of charged complex, analogous to type II, can be formed with diphenylborinate. Nevertheless, the mobilities for the various polyols in this buffer closely parallel those obtained in borate at pH 10.

Various other complexes of inorganic ions are known to be formed with carbohydrates and their behaviour in electrophoresis has been studied.[4] Thus, at this laboratory, paper electrophoresis of sugars and carbonyl derivatives of carbohydrates in hydrogen sulphite buffers has been examined.[10]

Sugars can be separated chromatographically on an ion exchange resin as borate complexes and by elution with alkaline borate.[11,12] Earlier a modification was developed, which allowed separation of borate complexes of neutral sugars at neutral pH and elevated temperature, using a glycerol-boric acid buffer adjusted to pH 6.8 by addition of sodium hydroxide.[13] This system gave a higher degree of resolution of various sugars and higher recoveries than with the alkaline borate buffer.

In the present investigation the use of a similar buffer in paper electrophoresis has been studied. One would expect that the high content of glycerol in the electrolyte would favour a high concentration of a glycerol complex of type II, which is considerably more acidic than boric acid. The ionic strength will thus be increased and higher buffer capacity will be possible than with a boric acid-phosphate buffer.

As discussed above, electrophoresis at neutral pH-values has previously shown interesting differences compared to alkaline borate.[5,6] The high concentration of glycerol-type II complex will favour the formation of a complex of type III in the glycerol-boric acid buffer. Opposite to that, the type I complex is formed when the sulphonated phenylboronic acid is used.[6] One could therefore expect some differences between the two buffer systems because of structural reasons but similarities because of the pH.

In a preliminary experiment D-glucose and its monomethyl ethers in four different buffers based on glycerol, boric acid, and the requisite amount of sodium hydroxide for the desired pH were tested (Table 1). As a comparison,

Table 1. Glucose and its methyl ethers in different buffers.

$$M_G = \frac{\text{true distance of migration of the substance}}{\text{true distance of migration of glucose}}$$

Substance	Buffer A M_G	Buffer B M_G	Buffer C M_G	Buffer D M_G	Buffer E M_G
D-Glucose	1.00 (9.9 cm)	1.00 (10.5 cm)	1.00 (10.3 cm)	1.00 (17.9 cm)	1.00 (8.5 cm)
2-*O*-Methyl-D-glucose	0	0.04	0.07	0.14	–
3-*O*-Methyl-D-glucose	1.18	1.21	1.34	1.03	1.34
4-*O*-Methyl-D-glucose	0	0.10	0.29	0.19	0.12
6-*O*-Methyl-D-glucose	0.68	0.79	0.80	0.83	0.82

a related buffer containing mannitol was examined. The electrophoresis was run at 40° and 1500 V (about 30 V/cm) for 1.5 h. The spread of mobilities in the four glycerol-boric acid buffers was found to be greater than in borate and of similar order as in sulphonated phenylboronic acid,[6] but the absolute mobilities were higher than those reported for the latter.

Buffer B, pH 6.8, was chosen for further studies. Buffer A did not give as distinct spots as B and when buffer C, with the highest glycerol content, was used, the paper sometimes burned off outside the cooling area. Buffer D of pH 7.4 did not give as good a separation pattern as B, although the mobilities were higher. Buffer E containing mannitol, gave similar M_G-values as the glycerol buffers, but was not studied further.

The paper electrophoretic mobilities in buffer B, pH 6.8, sulphonated phenyl boronic acid, pH 6.5 and borate, pH 10.0, of some monosaccharides and their monomethyl ethers, oligosaccharides, and furanosides are shown in Tables 2–5. Electrophoretic mobilities of hexuronic and hexulosonic acids in buffer B, borate pH 10.0, and hydrogen sulphite pH 4.7 are given in Table 6.

Table 2. Monosaccharides.

Substance	Buffer B M_G	Sulphonated phenylboronic acid [6] pH 6.5, M_G	Borate pH 10 M_G
D-Xylose	1.60	1.8	1.00 [6]
D-Lyxose	1.24	2.3	0.71 [6]
L-Arabinose	1.28	2.4	0.96 [6]
D-Ribose	1.74	4.7	0.77 [6]
D-Glucose	1.00	1.00	1.00 [6]
D-Mannose	0.87	1.1	0.72 [6]
D-Galactose	1.06	1.8	0.93 [6]
D-Gulose	1.60	–	0.82 [17]
D-Idose	1.84	–	1.02 [17]
D-Talose	1.85	–	0.87 [17]
D-Allose	1.28	–	0.83 [17]
D-Altrose	1.64	5.8	0.97 [6]
D-Fructose	1.74	9.3	0.90 [6]
L-Sorbose	2.00	8.5	0.95 [6]
D-Tagatose	1.93	8.6	0.95 [6]
D-Allulose	1.99	–	0.79
D-Xylulose	2.12	–	0.67
D-Ribulose	2.32	–	0.80
D-*glycero*-D-*gulo*-Heptose	1.49	–	0.89
D-*glycero*-D-*ido*-Heptose	2.37	–	0.91
D-*glycero*-L-*gluco*-Heptose	1.72	–	0.98
D-*glycero*-L-*manno*-Heptose	1.00	–	0.73
D-*glycero*-D-*galacto*-Heptose	0.89	–	0.73
D-*glycero*-D-*talo*-Heptose	1.48	–	0.62

Table 3. Methyl ethers of xylose, glucose, and galactose.

Substance	Buffer B M_G	Sulphonated phenylboronic acid [6] pH 6.5, M_G	Borate pH 10 M_G
D-Xylose	1.60	1.8	1.00 [6]
2-*O*-Methyl-D-xylose	0.04	0	0.39 [6]
3-*O*-Methyl-D-xylose	1.49	2.9	0.66 [6]
D-Glucose	1.00	1.0	1.00 [6]
2-*O*-Methyl-D-glucose	0.04	0	0.23 [6]
3-*O*-Methyl-D-glucose	1.21	1.3	0.80 [6]
4-*O*-Methyl-D-glucose	0.10	0	0.24 [6]
6-*O*-Methyl-D-glucose	0.79	0.5	0.80 [6]
D-Galactose	1.06	1.8	0.93 [6]
2-*O*-Methyl-D-galactose	0.23	–	0.43 [18]
3-*O*-Methyl-D-galactose	0.86	–	0.63 [18]
4-*O*-Methyl-D-galactose	0.08	–	0.30 [18]

Table 4. Disaccharides.

Substance	Buffer B M_G	Borate pH 10 M_G
Sucrose, α-G-(1↔2)-β-Fru	0.08	0.10 [19]
Kojibiose, α-G-(1→2)-G	0.10	0.32 [20]
Sophorose, β-G-(1→2)-G	0.08	0.24 [21]
Laminaribiose, β-G-(1→3)-G	1.16	0.69 [21]
Maltose, α-G-(1→4)-G	0.26	0.32 [21]
Mannobiose, β-Man-(1→4)-Man	0.36	0.54
Cellobiose, β-G-(1→4)-G	0.29	0.23 [21]
Xylobiose, β-Xyl-(1→4)-Xyl	0.12	0.20
Lactose, β-Gal-(1→4)-G	0.31	0.38 [21]
Gentiobiose, β-G-(1→6)-G	0.74	0.75 [21]
Melibiose, α-Gal-(1→6)-G	0.91	0.76

Table 5. Furanosides.

Substance	Buffer B M_G	Sulphonated phenylboronic acid pH 6.5, M_G	Borate pH 10 M_G
1,2-*O*-Isopropylidene-α-D-glucofuranose	1.14	3.4 [8]	0.66 [6]
1,2-*O*-Isopropylidene-α-D-*ribo*-hexofuranos-3-ulose	0.94	0	0.39
1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose	0.78	0.88	0.57
1,2-*O*-Isopropylidene-α-D-*gluco*-hexodialdo-1,4-furanose	0.98	2.21	0.65
Methyl α-D-mannofuranoside	1.81	16.0 [6]	0.79

In spite of the fact that two different types of complexes, discussed above, are expected to predominate using buffer B and sulphonated phenylboronic acid, respectively, many similarities between the mobilities in the two systems were found and they were rather different from those in borate pH 10. On the other hand the mobilities for various carbohydrates in borate pH 10 closely paralleled those obtained in diphenylborinate [9] pH 10, in spite of the much more restricted complexing possibility in the latter buffer. This indicates that the pH of the boric acid-borate buffers may be the most important factor for most of the compounds studied. Advantages of the glycerol-boric acid buffer over the one based on sulphonated phenylboronic acid are the availability of the chemicals used in the former and the higher absolute mobilities and sharper spots obtained. A disadvantage is the impossibility, because of the glycerol present, to use the common silver and periodate-detecting reagents.

Table 6. Uronic and hexulosonic acids.

Substance	Buffer B [15] M_G	Borate pH 10 M_G	Hydrogen sulphite [15] pH 4.7 $M_{vanillin}$
D-Glucuronic acid	2.10; 2.88	1.19	0.91; 1.24
D-Galacturonic acid	2.50	1.16	1.33
D-Mannuronic acid	2.71	1.12	1.06; 1.45
L-Guluronic acid	2.70	1.02	1.41
L-Iduronic acid	3.07	1.04	1.05; 1.59
D-Altruronic acid	2.91	–	1.37; 1.49
D-Alluronic acid	3.22	–	1.35
D-*xylo*-5-Hexulosonic acid (5-Keto-D-gluconic acid)	3.22	–	1.29
D-*lyxo*-5-Hexulosonic acid (5-Keto-D-mannonic or 5-Keto-L-gulonic acid)	3.11	–	1.25
L-*ribo*-5-Hexulosonic acid (5-Keto-D-talonic or 5-Keto-L-allonic acid)	3.33	–	1.32
D-*arabino*-Hexulosonic acid (2-Keto-D-gluconic acid)	3.04	–	1.65
L-*xylo*-Hexulosonic acid (2-Keto-L-gulonic acid)	2.96	–	1.60

As shown in Table 2, buffer B is useful for the separation of the pentoses, hexoses, and heptoses. The differences in the mobilities between the ketoses and aldoses are not so great in buffer B as in sulphonated phenylboronic acid. For the sugar monomethyl ethers (Table 3) studied, buffer B seems to be the most useful of the three buffers. The same is true for oligosaccharides (Table 4). The limited results with furanosides (Table 5) indicate similarities between buffer B and sulphonated phenylboronic acid and that the former is a useful complement to the hydrogen sulphite buffer [10] for dicarbonyl sugar derivatives. The lack of migration of the 3-keto compound in the sulphonated phenylboronic acid is notable and is probably caused by a strong predominance of the hemiketal form of the compound.[14] Mobilities of various dialdoses and their corresponding monomethyl glycosides will be published elsewhere.

In the characterisation of hexuronic and hexulosonic acids, paper electrophoresis in buffer B has been useful and a good complement to the hydrogen sulphite buffer.[15] Both these buffers give more selectivity than borate pH 10 (Table 6) and often yields characteristic spots corresponding to both the lactone and the free acid form in the former systems.

EXPERIMENTAL

Substances. The substances used were available at this laboratory.
Composition of the buffers:

Buffer A: 0.4 M H_3BO_3, 0.6 M Glycerol, 0.060 M NaOH, pH = 6.8
B: 0.4 M H_3BO_3, 1.0 M Glycerol, 0.096 M NaOH, pH = 6.8
C: 0.4 M H_3BO_3, 1.4 M Glycerol, 0.134 M NaOH, pH = 6.8
D: 0.4 M H_3BO_3, 1.0 M Glycerol, 0.172 M NaOH, pH = 7.4
E: 0.4 M H_3BO_3, 0.5 M Mannitol, 0.290 M NaOH, pH = 6.8

Electrophoresis. The apparatus and technique of the paper electrophoresis are the same as described before by Foster.[16] The electrophoresis was run at 40° and the temperature was maintained with thermostated water circulating through the condensing block. The electrophoresis was run at 1500 V for 90 min. Hydroxymethylfurfural was used to indicate the endosmotic effect and glucose was the standard reference substance. The compounds were located by the anisidine hydrochloride spraying reagent. Also the non-reducing furanosides could be detected with this reagent after examination of the heated paper in UV-light (366 nm).

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