831. *Ionophoresis of Carbohydrates. Part IV.* Separations of Carbohydrates on Fibreglass Sheets.*

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The behaviour of fibreglass sheets as electrolyte supports during ionophoresis of carbohydrates in borate buffer has been examined. The main advantages of the use of fibreglass, rather than filter-paper, are that non-reducing carbohydrates are much more easily detected, that the absorption of amylosaccharides is considerably reduced, and that the greater electro-endosmotic flow facilitates separations of sugars with similar absolute mobilities. There are only slight differences between the M_G values on fibreglass and on filter-paper.

The separation of carbohydrates as their borate complexes by zone electrophoresis (ionophoresis) on paper strips, with the enclosed-strip technique,[1] has been well studied,[2] and recently the ionophoretic behaviour, on paper, of the *N*-benzylglycosylamines has been described.[3] There are some disadvantages in the use of paper strips as electrolyte supports, *e.g.*, numerous non-reducing carbohydrates and polysaccharides are difficult to locate after migration and certain polysaccharides, especially amylosaccharides, tend to be adsorbed.[2] Recently a fibreglass sheet of suitable structure has become available commercially and we report herein its behaviour, in comparison with that of paper (Whatman No. 3), as an electrolyte support in the ionophoresis of carbohydrates.

The fibreglass sheets had capillary properties similar to those of paper but, when borate-soaked, had a lower electrical resistance. D-Glucose had a lower absolute mobility on paper ($3{\cdot}66 \times 10^{-6}$ cm.^{2} v^{-1} sec.$^{-1}$) than on fibreglass ($4{\cdot}92 \times 10^{-6}$ cm.^{2} v^{-1} sec.$^{-1}$) (Table 2), as did all the other carbohydrates listed in Table 1. However, the relative mobilities, as expressed by the M_G values,[2] showed only slight differences in the two cases (Table 1); for

Table 1. *Comparative M_G values (error $\pm 0{\cdot}02$) on paper and fibreglass strips.*

	M_G value			M_G value	
Substance	Paper	Fibreglass	Substance	Paper	Fibreglass
DL-Glyceraldehyde	0·79	0·75	Maltose	0·34	0·30
1 : 3-Dihydroxyacetone	0·78	0·75	Gentiobiose	0·72	0·69
L-Arabinose	0·96	0·94	Glycerol	0·44	0·39
D-Ribose	0·77	0·74	*meso*Erythritol	0·75	0·70
D-Xylose	1·00	1·00	D-Arabitol	0·90	0·86
2-Deoxy-D-ribose	0·33	0·28	D-Glucitol	0·89	0·82
L-Fucose	0·89	0·88	Galactitol	0·98	0·95
L-Rhamnose	0·52	0·49	D-Mannitol	0·90	0·85
D-Galactose	0·93	0·91	D-*glycero*-D-*gala*Heptitol	1·00	0·95
D-Glucose	1·00	1·00	D-*glycero*-D-*manno*Heptitol	0·92	0·87
D-Mannose	0·72	0·67	αα-Trehalose	0·19	0·16
D-Fructose	0·90	0·88	Sucrose	0·18	0·15
L-Sorbose	0·95	0·95	Raffinose	0·28	0·23
L-Galaheptulose	0·89	0·86	Melezitose	0·22	0·21
D-Mannoheptulose	0·87	0·83	D-Glucose-1 phosphate	1·10	1·10
Sophorose	0·33	0·28	D-Glucuronic acid	1·20	1·23
Nigerose	0·69	0·65	Myoinositol	0·53	0·48
Cellobiose	0·29	0·26			

substances with a lower mobility than that of D-glucose ($M_G < 1{\cdot}00$) the M_G values on fibreglass were 0—0·05 unit lower than on paper. Thus there is negligible selective adsorption of the migrating substances on either electrolyte support. It has been inferred from other results [2] that carbohydrates of low molecular weight are not adsorbed on paper during ionophoresis.

* Part III, *J.*, 1956, 30.

[1] Foster, *Chem. and Ind.*, 1952, 1050; Gross, *Nature*, 1953, **172**, 908.
[2] Foster, Newton-Hearn, and Stacey, *J.*, 1956, 30, and references cited therein.
[3] Barker, Bourne, Grant, and Stacey, *Nature*, 1956, **177**, 1125.

The electroendosmotic flow, as measured by the movement of 2 : 3 : 6-tri-*O*-methyl-D-glucose, was towards the cathode for both paper and fibreglass when borate buffer (pH 10) was the electrolyte, but it was exceptionally high with the fibreglass (Table 3). Thus on fibreglass it was necessary to locate the origin line near the anode—on paper it is usually placed near the cathode for neutral carbohydrates. Use can be made of the high electroendosmotic flow with fibreglass in separations of carbohydrates which have M_G values similar to that of D-glucose. On fibreglass the migration of D-glucose is strongly opposed by the electroendosmotic flow (Table 3), so that the apparent migration distance is low. Thus the effective length of the fibreglass strip is increased, *e.g.*, a separation of D-glucose and D-galactose which on paper requires an apparent migration distance of *ca.* 40 cm. can be achieved on fibreglass in *ca.* 10 cm. In theory the separation on fibreglass can be improved by continuing the ionophoresis for a much longer period, but unfortunately the spots tend to become more diffuse on fibreglass than on paper; however, diffusion effects may be reduced appreciably [4] by operating at very high voltage gradients, though then very efficient cooling is essential.

Because of its chemical inertness, the detection of carbohydrates on fibreglass is much easier than on paper. Reducing sugars can easily be located on either support. With non-reducing carbohydrates, much more drastic staining procedures can be applied to fibreglass (see Experimental section).

The ionophoretic behaviour of amylose on paper in the presence of borate has already been described.[2] The polysaccharide was strongly adsorbed; the pattern of migration depended on the amount of amylose introduced on the paper and on the manner in which it was introduced. Amylose was selected for ionophoretic study on fibreglass because it is the most strongly adsorbed, on cellulose, of all the polysaccharides so far examined. A large migration towards the cathode, with very weak adsorption in the path of movement and negligible adsorption at the origin, occurred when a solution of amylose in borate buffer, but not when an aqueous solution, was put on fibreglass and ionophoresis in borate buffer was subsequently performed. However, the M_G value (0·21) indicated a significant movement against the electroendosmotic flow towards the anode (cf. M_G 0·18 on paper [2]). It appears that there is considerably less adsorption of amylose on fibreglass than on paper under optimum conditions. Amylose was detected on fibreglass by means of iodine or ammoniacal silver nitrate. Although the sensitivity of the latter reagent was not examined in detail, it clearly revealed the small traces of amylose (as indicated by the iodine method of known high sensitivity [2]) left in the path of migration of the main zone. Ammoniacal silver nitrate is of potential value for the detection, on fibreglass, of polysaccharides which do not react with iodine. Attempts to detect amylose and other polysaccharides on paper with ammoniacal silver nitrate were mainly unsuccessful.

Amylopectin has an M_G value of 0·31 on fibreglass (cf. 0·25 on paper [2]). The migration pattern obtained on ionophoresis of an artificial mixture of amylose and amylopectin indicated some association and only a partial separation. This behaviour contrasts with that on paper.[2]

The ability of certain sugars to form complexes with aqueous calcium chloride indicated a possible new method for ionophoretic separation of sugars. However, with an aqueous solution of calcium chloride on both filter-paper and fibreglass supports, L-arabinose and D-mannose, which readily form complexes,[5] could not be separated from D-glucose, which does not. Thus it appears that the sugar in such a complex is not part of an ionic species. The ionophoretic behaviour on fibreglass of the *N*-benzylglycosylamines will be described elsewhere.[6]

Experimental

The fibreglass sheets were supplied by H. Reeve Angel and Co. and were used without pretreatment. Whatman No. 3 paper was employed for comparisons. The capillary properties of fibreglass and paper were similar in that equal volumes of liquid gave only slightly larger circular spots on the fibreglass.

[4] Gross, *Nature*, 1955, **176**, 362.
[5] Pigmann and Goepp, "Carbohydrate Chemistry," Academic Press, New York, 1948, p. 48.
[6] Barker, Bourne, Grant, and Stacey, unpublished results.

Ionophoresis.—The apparatus and technique employed have been described elsewhere.[1] Solutions of the migrating substances were introduced on to the smooth side of the fibreglass. The electrolyte was 0·2M-sodium borate (pH 10). For comparisons strips of fibreglass and paper (38 × 10 cm.) were used under a standard potential of 500 v (*i.e.*, *ca.* .15 v/cm. for the effective length of 34 cm.). The results in Table 2 are typical of many.

TABLE 2. *Ionophoresis of* D-*glucose* (1·5 *hours*).

	Final current (milliamps)	Absolute distance (cm.) of migration	Mobility (10^{-6} cm.^{2} v^{-1} sec.$^{-1}$)
Whatman No. 3 paper	15	9·9	3·66
Fibre glass	21	13·2	4·92

Slight variations in the mobilities (and in the M_G values in Table 1) were observed in duplicate experiments for a given system. Elimination of the variations is difficult experimentally since it would demand precise control of (1) the quality and thickness of the paper and fibreglass, (2) the blotting process in the preparation of the paper,[1] (3) the clamping of the glass plates which enclose the paper or fibreglass,[1] (4) the amount of the migrating substances introduced on to the paper, and (5) temperature. The relative positions of the spots on individual " pherograms " [7] was less variable.

The M_G values recorded in Table 1 were determined as previously described,[1,2] 2 : 3 : 6-tri-*O*-methyl-D-glucose being used to determine movement due to electroendosmotic flow, the extent of which is indicated in Table 3. In preparing the paper and fibreglass for ionophoresis 1 drop of a 1·6% aqueous solution of each substance was introduced on to each electrolyte support to give a spot *ca.* 0·4 cm. in diameter. After ionophoresis under the standard conditions the spots on paper had diffused to *ca.* 0·7 cm. in diameter and those on fibreglass to *ca.* 1·0 cm. in diameter.

TABLE 3.

	Observed movement of sugar derivative : *	
	on fibreglass	on paper
D-Glucose	2 cm. ⟶ cathode	8 cm. ⟶ anode
2 : 3 : 6-Tri-*O*-methyl-D-glucose	15 cm. ⟶ cathode	2 cm. ⟶ cathode

* The extent of the electroendosmotic flow must be taken into consideration when selecting the position of the origin line.

Detection of the Migrating Substances.—Detection was carried out as customary for the reagents described, unless otherwise stated.

(a) *Aniline hydrogen phthalate.*[8] Equally effective for reducing sugars on both fibreglass and paper.

(b) *Ethanolic sodium hydroxide–silver nitrate.*[9] This reagent would detect all reducing sugars, and many non-reducing carbohydrates, on both fibreglass and paper in the absence of borate. The sensitivity was reduced appreciably on borate-impregnated fibreglass and paper, *e.g.*, hexitols and pentitols could not be detected. On fibreglass the spots which appeared were black on a pale grey background.

(c) *Ammoniacal silver nitrate.*[10] All reducing and most non-reducing carbohydrates so far examined are detectable by this reagent at 7% strength. The tendency for paper itself to react with the reagent results in considerable background interference in the detection of non-reducing carbohydrates and only one application of the spray is possible. With fibreglass there is negligible background interference and the spray may be applied several times if necessary. Spots appear black on a pale grey background.

(d) *Alkaline permanganate.* By means of water containing 1·0% of potassium permanganate and 2·0% of sodium carbonate reducing and non-reducing carbohydrates showed on fibreglass as yellow spots on a purple background. The background slowly (3 days) became yellow at room temperature under the influence of light and rapidly (20 min.) at 100°. On paper the background became brownish-yellow at room temperature in 10 min. and immediately on heating.

[7] Bücher, Matzelt, and Pette, *Klin. Wochenschr.*, 1952, **30**, 325.
[8] Partridge, *Nature*, 1949, **164**, 443.
[9] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[10] Hough, *Nature*, 1950, **165**, 400.

(e) *Periodate–benzidine.*[11] All the periodate in the reagent reacts when paper alone is sprayed and heated (10 min. at 100°), whereas under similar conditions on fibreglass it is largely unchanged. The migrating zones on fibreglass appear as white spots on a blue background.

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[11] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.