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ELECTROPHORETIC SEPARATION OF SUGARS AND HYDROLYSATES OF POLYSACCHARIDES ON SILYLATED GLASS-FIBRE PAPER

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SUMMARY

The suitability of silylated glass-fibre paper as a supporting material for the electrophoresis of sugars and hydrolysates of polysaccharides has been studied. Because of the absence of molecular sieving effects by the supporting material, the separation is based only on differences in the molecular charge of the samples. The method described allows the separation of low- and high-molecular-weight substances in the same run.

A mixture of seven sugars was separated into single components within 45 min. To demonstrate the versatility of the method, various polysaccharide hydrolysates were separated electrophoretically. As electrophoresis is based on a different separation principle than chromatographic techniques, this method is considered to be an efficient alternative and/or complement to the chromatography of carbohydrates.

INTRODUCTION

Electrophoresis of carbohydrates has hitherto mainly been performed using paper (cellulose) or unsilylated glass-fibre paper as supporting materials[1–9]. Unfortunately some substantial disadvantages, *e.g.*, a high electroendoosmotic flow, leading to diffuse spots, and adsorption of sample material on the surface of the supporting material, have been demonstrated. Further, paper reacts with many carbohydrate detection reagents, leading to laborious staining procedures.

The aim of this investigation was to develop a simple electrophoretic method that could be used for the rapid identification of sugars and for the characterization of hydrolysates of polysaccharides. Particular attention was given to the choice of the supporting material. Jarvis *et al.*[10] developed an electrophoretic method for the separation of polysaccharides on silylated glass-fibre paper. This technique was successfully used to characterize gelling and thickening agents in foodstuffs[11,12]. Silylated glass-fibre paper does not show any molecular sieving effect, and exhibits a reduced sample adsorption on its surface and a small electroosmotic flow due to the treatment with dimethyldichlorosilane[10,13]. One further advantage compared to other supporting materials (*e.g.*, paper) is that even aggressive chemicals can be used for the detection of carbohydrates after separation.

The electrophoretic behaviour of different sugars and polysaccharide hydro-lysates on silylated glass-fibre paper is described in this paper. The separation is carried out in the presence of sodium tetraborate to form negatively charged carbohydrate–borate complexes. It is known that such complexes are preferentially formed with adjacent *cis*-hydroxyl groups[3,14]. The results obtained demonstrate the suitability of silylated glass-fibre paper as a supporting material for the electrophoresis of neutral sugar mixtures and of polysaccharide hydrolysates. The use of this supporting material makes it possible to separate low- and high-molecular-weight substances in the same run. The versatility of the method is demonstrated by analysing different hydrolysates of polysaccharides. The method turns out to be an alternative and an additional possibility to chromatographic techniques used for the characterization of carbohydrates.

EXPERIMENTAL

Equipment

Electrophoresis on a cooling plate was performed using an LKB (Bromma, Sweden), 2117 Multiphor apparatus.

Suspended-strip electrophoresis was carried out in a Shandon universal chamber (without cooling) for horizontal electrophoresis using a Vokam power supply (Shandon, London, U.K.).

Chemicals

Glass-fibre paper GF/C and filter-paper were obtained from Whatman (Maidstone, U.K.), 10-μl micropipettes from Brand (Wertheim, F.R.G.), Saran film from Dow Chemical (Horgen, Switzerland) and Triton X-100 and Tween 20 from Fluka (Buchs, Switzerland).

All sugars were of analytical-reagent grade and purchased from Fluka, except for the stationary marker 2,3,4,6-tetra-O-methyl-D-glucose (Koch-Light Labs., Colnbrook, U.K.). The polysaccharides were kindly provided by Obipektin (Bischofszell, Switzerland) (sodium pectate), Dow Chemical [methylcellulose Methocel A4C prem. (MC) and hydroxypropylmethylcellulose Methocel F4M prem. (HPMC)] and Kelco (San Diego, CA, U.S.A.) (gellan).

Chloroform, dimethyldichlorosilane, sodium tetraborate decahydrate, ethanol, trifluoroacetic acid (TFA), sulphuric acid (all from Fluka) and 1,3-naphthalenediol (Riedel de Haën, Seelze, F.R.G.) were of analytical-reagent grade.

Preparation of silylated glass-fibre paper

Silylated glass-fibre paper was prepared by modification of the method described by Jarvis *et al.*[10]. For electrophoresis on the cooling plate the glass-fibre paper was cut into strips 25.6 cm in length and for suspended-strip electrophoresis 16 cm in length. Organic material in the glass-fibre paper was removed by heating at 400°C for 2 h. Silylation was performed by immersion of the glass-fibre paper in 5% (w/w) dimethyldichlorosilane solution in chloroform for 24 h at room temperature. Before use the strips were rinsed in toluene and dried.

Hydrolysis of polysaccharides

Polysaccharide samples (10 mg) were hydrolysed with 2 M TFA (1 ml) in a nitrogen atmosphere at 120°C for 1 h in an oil-bath, using Pyrex screw-capped hydrolysis tubes (Auer-Bittmann-Soulié, Dietikon, Switzerland). After hydrolysis, TFA was removed by a stream of compressed air at room temperature. Residual TFA was removed from the hydrolysate by washing three times with 1 ml of deionized water and repeating the above procedure. Finally, the hydrolysate was dissolved in 0.5–2 ml of deionized water. Hydrolysates containing uronic acid lactones were treated with dilute sodium hydroxide solution (pH $\leqslant 8$) to yield the free uronic acids[15,16].

Sugar and marker solutions

Aqueous sugar solutions (0.2%, w/v) and an aqueous sugar mixture containing 0.2% (w/v) of each component (sucrose, maltose, rhamnose, mannose, glucose and galacturonic acid) were prepared. Aqueous marker solutions of MC and sodium pectate were prepared at a concentration of 0.25% (w/v).

Electrophoresis

Cooled plate method. Silylated glass-fibre paper was soaked for 1 day in a buffer solution (0.05 M sodium tetraborate solution, pH 9.2) containing 0.2% (v/v) Triton X-100. Excess of electrolyte was removed by slightly blotting the wet silylated glass-fibre paper before use. Wicks of filter-paper (Whatman No. 3) were wrapped in Saran film, taking care that no air bubbles remained trapped between the cooling plate and the glass-fibre paper. The system was pre-equilibrated for 15 min under the running conditions, then samples (5–15 μl) were applied to the supporting material as a fine uniform line (1–2 cm long) by the aid of a micropipette. Electrophoresis was performed at a constant current of 40 mA, resulting in a potential gradient of about 30 V/cm when a strip width of 8.5 cm was used. Cooling was achieved by running tap water of 8–10°C. Separation was achieved within 45 min. Glucose (M_{Glc}) was used as a mobility marker, whereas low-viscosity MC or 2,3,4,6-tetra-O-methyl-D-glucose served as a stationary marker.

Suspended-strip method. For the suspended strip technique the procedure described by Bettler *et al.*[12] was adopted.

Staining

A 0.2-g amount of 1,3-naphthalenediol was dissolved in 100 ml of ethanol; 4 ml of concentrated sulphuric acid were added before use. The separated carbohydrates were located by thoroughly spraying both sides of the hot air-dried electrophoresis strips (hair dryer) with the staining reagent. Heating at 110°C allowed the colour to develop in about 10 min.

Determination of the electrophoretic mobility, M_e

The electrophoretic mobility, M_e, was determined by calculating the ratio of the distance between the substance and stationary marker to the distance between the mobility marker [glucose (M_{Glc}) or sodium pectate ($M_{\text{Pect.}}$)] and the stationary marker.

RESULTS AND DISCUSSION

Electroendoosmosis, stationary and mobility marker

Stationary markers were used in order to take into account additional factors affecting the separation, such as electroendoosmosis of the dimethyldichlorosilane-treated support material or heating effects. Experiments with the stationary marker 2,3,4,6-tetra-O-methyl-D-glucose, which exhibits no complexation with borate, resulted in a migration on the cooling plate of approximately 3–5 cm/h towards the cathode. With the suspended-strip method, the endoosmotic flow was almost suppressed (0–1 cm/h) owing to the superimposed heating effect during the run. In comparative experiments tetramethylated glucose and low-viscosity MC exhibited the same migration properties. As MC showed a smaller diffusion, this polysaccharide was chosen as the preferred stationary marker.

For calculation of the electrophoretic mobilities of the separated sugars, glucose and sodium pectate were used as mobility markers. Glucose has commonly been used as a mobility marker to calculate the electrophoretic mobilities of mono- and oligosaccharides[17]. In this work, glucose was used as a mobility marker for the electrophoresis on the cooling plate, the mobility being 12.5 cm with respect to the stationary marker. On the other hand, sodium pectate has already been used for this purpose for the electrophoresis of polysaccharides on silylated glass-fibre paper[12]. Because of its minimal diffusion properties, this substance is especially suitable for suspended-strip electrophoresis. Comparison with other published data was possible by converting the $M_{Pect.}$ data from suspended-strip electrophoresis into M_{Glc} data by division by 0.91.

Electrophoresis of sugar mixtures

Glass-fibre paper shows a small electrophoretic resistance, thus allowing the use of higher potential gradients, leading to shorter analysis times compared with electrophoresis on other supporting materials. The small electrophoretic resistance allows the use even of simple suspended-strip electrophoresis (Fig. 1). Electrophoresis on a cooled plate allows much higher potential gradients to be used than in uncooled suspended-strip electrophoresis, thus leading to a better separation in a shorter time. The resolution is improved and diffusion is reduced (Fig. 2).

The mobilities obtained by electrophoresis on silylated glass-fibre paper (Table I) are comparable to those described for electrophoresis on cellulose paper and on unsilylated glass-fibre paper (reproducibility 4–7%[17]). The same mobility values are obtained by using electrophoresis on a cooled plate or the uncooled suspended-strip technique.

Electrophoresis of polysaccharide hydrolysates

One of the general methods for the partial characterization of polysaccharides is hydrolysis to their monomeric constituents and subsequent analysis by gas chromatography (GC) or thin-layer chromatography (TLC)[18–20]. As pointed out by Friese[20], it is very difficult to perform acid hydrolysis of polysaccharides quantitatively because of incomplete hydrolysis (different stability of the glycosidic linkages towards acids[21] and losses due to subsequent reactions. Further, many decomposition products undergo reversion reactions.
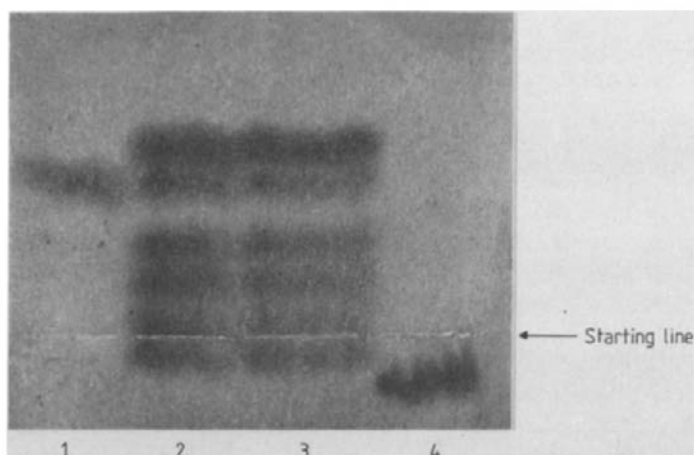
Fig. 1. Suspended-strip electrophoresis of sugars. 0.05 M borax (pH 9.2), 6 V/cm, 90 min, detection with 1,3-naphthalenediol reagent. 1 = Glucose (mobility marker), 10 μl; 2 and 3 = mixture of sucrose, rhamnose, maltose, mannose, glucose, 5 μl, and galacturonic acid, 10 μl; 4 = MC (stationary marker) 10 μl.

In contrast to most chromatographic techniques (*e.g.*, GC), electrophoresis on silylated glass-fibre paper detects most of the degradation products. This method permits the analysis of both low-(monosaccharides, oligosaccharides) and high-molecular-weight carbohydrates (polysaccharides[12]) in the same run. As electrophoretic separations are based on a different principle than chromatographic methods,
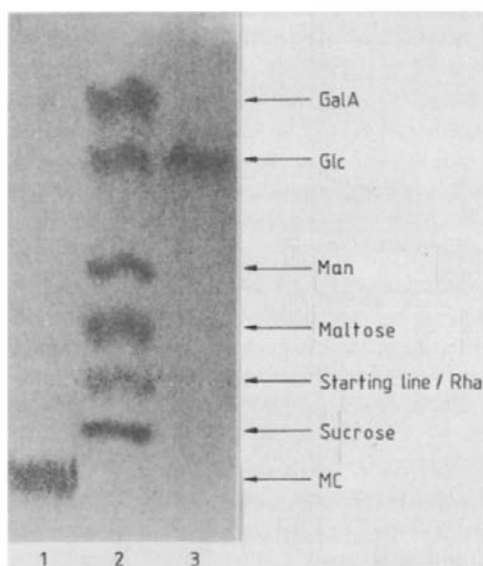

Fig. 2. Electrophoresis on a cooling plate of sugars. 0.05 M borax (pH 9.2), 40 mA, 45 min, detection with 1,3-naphthalenediol reagent. 1 = MC (stationary marker), 10 μl; 2 = mixture of sucrose, rhamnose, maltose, mannose, glucose, 5 μl and galacturonic acid, 10 μl; 3 = glucose (mobility marker), 10 μl.

TABLE I

ELECTROPHORETIC MOBILITIES OF CARBOHYDRATES ON SILYLATED GLASS-FIBRE PAPER EXPRESSED AS $M_{Pect.}$ AND M_{Glc}

Electrophoresis on a cooling plate, 0.05 M borax (pH 9.2) 40 mA, 45 min.

Mobility reference	$M_{Pect.}$	M_{Glc}
Mannose	0.63	0.69
Galactose	0.82	0.90
Lactose	0.38	0.42
Maltose	0.31	0.34
Sucrose	0.15	0.16
Glucose	0.91	1.00
Arabinose	0.83	0.91
Fructose	0.80	0.88
Xylose	0.91	1.00
Rhamnose	0.46	0.50
Galacturonic acid	1.09	1.20

the use of electrophoresis is suitable for a first characterization of polysaccharide hydrolysates especially for partially hydrolysed polysaccharides.

Electrophoretic patterns of MC and HPMC on the cooling plate are shown in Fig. 3. Commercially available MCs have degrees of substitution between 1.64 and 1.92[22]. In Table II the substitution pattern of MC is given together with the corresponding electrophoretic mobilities on cellulose paper as determined by Foster[1].
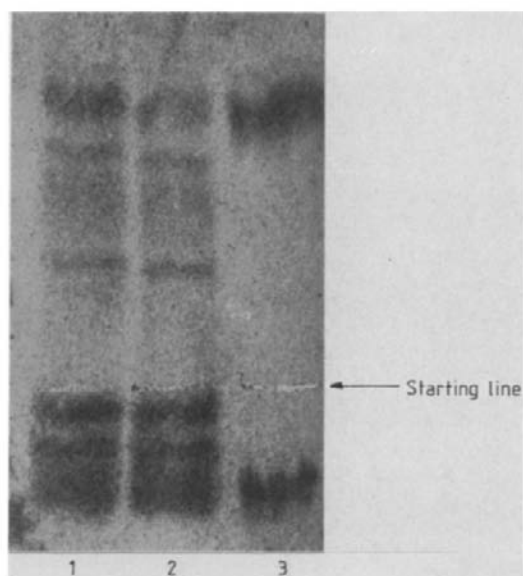

Fig. 3. Electrophoresis on a cooling plate of hydrolysates of cellulose derivatives. 0.05 M borax (pH 9.2), 40 mA, 45 min, detection with 1,3-naphthalenediol reagent. 1 = Hydrolysate HPMC, 10 μl; 2 = hydrolysate MC, 10 μl; 3 = mixture of MC (stationary marker) and glucose (mobility marker), 10 μl.

TABLE II

GLUCOSE ETHER SUBSTITUTION PATTERN OF MC WITH REFERENCE MOBILITIES (M_{Glc}) AND MIGRATION ZONES OF MC HYDROLYSATE

Conditions as in Table I. The formation of traces of additional glucose ethers (terminal constituents) is possible.

Glucose ether	$M_{Glc}{}^a$	M_{Glc} migration zones, hydrolysate of MC[b]
Unsubstituted glucose	1.00	1.00
2-O-Methylglucose	0.23	0.21
3-O-Methylglucose	0.80	0.86
6-O-Methylglucose	0.80	0.86
2,3-Di-O-methylglucose	0.12	0.14
2,6-Di-O-methylglucose	—	—
3,6-Di-O-methylglucose	—	—
2,3,6-Tri-O-methylglucose and incompletely hydrolysed MC	0.00	0.00
No reference mobility	—	0.58
No reference mobility	—	1.07

^{a} Ref. 1.

^{b} According to the electrophoretic mobilities, M_{Glc}, assigned to the glucose ethers.

According to these reference mobilities, the zones of the electrophorograms obtained on the cooling plate were assigned to the different glucose ethers (Table II); 2,6- and 3,6-di-O-methyl-D-glucose could not be assigned because of lack of corresponding reference mobilities. Throughout the whole migration area, further diffuse zones are visible. These may be explained by additional degradation products caused by incomplete acid hydrolysis of the analysed MC.

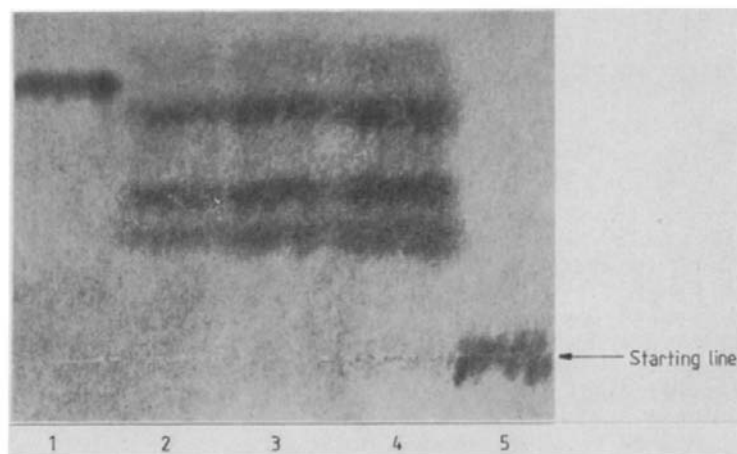HPMC is an MC that has been additionally reacted with small amounts of

Fig. 4. Suspended-strip electrophoresis of a hydrolysate of gellan. 0.05 M borax (pH 9.2), 6 V/cm, 90 min, detection with 1,3-naphthalenediol reagent. 1 = Sodium pectate (mobility marker), 10 μl; 2, 3 and 4 = hydrolysate gellan, 5, 10 and 15 μl, respectively; 5 = MC (stationary marker), 10 μl.

propylene oxide. The HPMC used in this work contained 27–30% methoxy and 4–7.5% hydroxypropyl groups[22]. After electrophoresis, the HPMC hydrolysate shows the same electrophoretic pattern as MC. This can be explained by the same content of methoxy groups in MC and HPMC. The low degree of hydroxypropyl substitution has no influence on the electrophoretic pattern.

As an example of the electrophoresis of polysaccharide hydrolysates, the suspended-strip electrophoresis of a gellan hydrolysate is shown in Fig. 4. Gellan is a polysaccharide containing rhamnose, glucose and glucuronic acid in a molar ratio of 1:2:1[23]. On the basis of results obtained by electrophoresis of free sugars (Table I), three of the four migration zones were assigned to rhamnose, glucose and glucuronic acid. The unidentified migration zone ($M_{Pect.}$ 0.62) shows the mobility of mannose. This sugar can be excluded because it is not a constituent of gellan. This was confirmed by GC analysis, where no mannose could be detected[24]. Miles et al.[23] described the high stability of the glucuronic acid–(1,4)-glucose linkage. It is assumed that the unidentified zone represent an aldobiuronic acid (glucuronosylglucose) obtained by incomplete hydrolysis of gellan. This would also explain the low glucose:rhamnose ratio of 1.43:1 as determined by GC[24]. Similarly, other polysaccharides containing uronic acids could also, on incomplete hydrolysis, form acid resistant fragments such as aldobiuronic acids.

This investigation has clearly shown that electrophoresis on silylated glass-fibre paper could be used as a method of choice for a rapid characterization of polysaccharides. The advantages of electrophoresis over other separation and identification techniques are that electrophoresis needs only modest instrumentation, the silylation of glass-fibre paper is simple and several strips can be prepared at the same time. The electrophoretic method needs no derivatization procedure and allows the detection of most of the degradation products. Especially interesting is the fact that high- and low-molecular-weight carbohydrates can be separated in the same run. Electrophoresis may also be helpful for the confirmation of gas chromatograms, for example, by revealing sugar constituents or higher molecular weight fragments not detectable by GC. Finally, electrophoresis is an efficient alternative and complement to TLC methods[21,25] for the screening of polysaccharide hydrolysates. Sugar constituents difficult to separate by TLC often are easily separated by electrophoresis in a short time.

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