Determination of sugars by capillary electrophoresis with electrochemical detection using cuprous oxide modified electrodes

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Abstract

The separation by capillary electrophoresis and off-column amperometric detection of underivatized sugars has been studied. A palladium-metal union was used as grounded cathode to decouple the high separation voltage before detection. With a second piece of capillary the field decoupler was connected to a home-made T-shaped detection cell. Cuprous oxide modified microelectrodes were prepared and evaluated. For the detection of sugars they were operated at a constant potential of $+0.60$ V. The peak-height reproducibility with these electrodes over a period of 18 h (25 injections) was within 7% absolutely and within 4% when one of the analytes was regarded as internal standard.

To preserve the flat electroosmotic flow profile in the separation capillary, pressure compensation was applied. The contribution of various sources to the system zone variance was studied. Under optimized conditions plate numbers of over 100 000 were obtained.

For the baseline separation of a mixture of sugars 0.10 mol l $^{-1}$ sodium hydroxide solutions had to be used as background electrolyte. Detection limits of 1–2 μmol l $^{-1}$ were found for various carbohydrates.

1. Introduction

Since the inception of capillary electrophoresis (CE) [1] and the clear demonstration of its high separation power by Jorgenson and Lukacs [2], CE has gained popularity over the past decade with an increasing number of publications each year in the field. CE is becoming an alternative separation technique for liquid chromatography on one hand and conventional electrophoresis on the other. Achievements and trends in CE have been reviewed extensively [3–11].

The monitoring of the CE separation process is usually carried out with on-column UV absorption spectrometry or (laser-induced) fluorometry. However, several research groups work on the development of amperometric detectors for CE [12,13]. This interest can be explained by the fact that the sensitivity of amperometric detection is not hampered by the small volumes of the sample zones as encountered in CE, in contrast to most spectrometric techniques. With amperometry, detection limits of 10^{-19} mole have already been reported [14].

A prerequisite to perform amperometric detection in CE is to isolate the electrophoretic

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current generated by the high-voltage source used for the separation from the electrochemical detection current. To achieve this, there are basically two approaches: the end-column mode and the off-column mode. In the end-column mode [15,16], the working electrode is placed in the buffer vial with the grounded electrode of the high-voltage source, at a short distance from the capillary end. A compromise must be found between minimum interference of the electric field of the separation on the detection and an acceptable mass sensitivity. Such a set-up is suitable for capillaries with an inside diameter of 25 μm or less, when the electrophoretic currents are small and the detection potential is not biased significantly. Detection limits for end-column amperometric detection have been reported in the range 10–100 amol; due to the extremely low sample volumes in the narrow capillaries, the detection limits in concentration units are less impressive (approximately 10^{-6} mol l^{-1}).

The limitation of the inner capillary diameter is not imposed in the off-column mode [14,17–21]. Here, the separation field is decoupled before the detector through a gap in the capillary system. Electrical contact with the grounded electrode of the high-voltage source, placed in an external electrolyte solution, is made through a sleeve over the gap, made of a porous or semipermeable material. The solution is propelled by the electroosmosis in the separation capillary via a small piece of coupling capillary towards the detector.

A new type of field decoupler for off-column amperometric detection has been developed recently in our laboratory [22]. A union of palladium metal between the separation and the coupling capillary is used as the grounded cathode of the high-voltage source. The hydrogen generated at the inner wall of the union, which constitutes a part of the capillary system, can dissipate through the palladium metal. The main advantages of this decoupler over the previously used types are its robustness and experimental convenience. It was also shown that to preserve the flat electroosmotic flow profile in the separation capillary, pressure compensation can be applied [23]. In this method a pressure is applied on the front end of the separation capillary, balancing the backpressure of the coupling capillary.

CE has been applied for the determination of a variety of compounds and in most cases a separation efficiency superior to other (chromatographic) methods has been demonstrated. Recently, there is an increasing interest to apply CE methods for the analysis of sugars. For the separation of the many closely related mono- and oligosaccharides the efficiency of CE would be of much value. However, due to their lack of strong chromophore or fluorophore, carbohydrates can not be detected directly with optical methods. To avoid sometimes complicated precolumn derivatization procedures [24–26], on-column complexation with borate and detection at 195 nm [27] or indirect fluorescence [28] or UV detection [29–30] has to be applied. These methods suffer from poor selectivity and high concentration detection limits, which are in the order of 10^{-4} mol l^{-1}.

In liquid chromatography pulsed amperometric detection (PAD) with gold or platinum electrodes [31] has become a routine method for the determination of sugars. Recently it has been shown that PAD can also be applied in CE for the detection of sugars [32,33]. A problem here may be that the optimal cycle time in PAD (approximately 1 s) is rather long for the fast separations obtained in CE. Alternatively, constant-potential detection with copper electrodes has been applied [34,35]. The high pH necessary for the separation is also essential for sensitive detection in both cases.

In previous work it has been found that cuprous oxide is an effective electrode modifier for the detection of sugars in flow injection analysis [36,37], giving a high sensitivity and selectivity. We used conductive carbon cement (CCC) as matrix for the modified electrodes, since it provides a better versatility and mechanical stability for use in flowing solutions, compared to for instance carbon paste [38]. In the work presented here, we have studied the possibility to use cuprous oxide modified microelectrodes in a CE system. The electrochemical characteristics of the modified microelec-

trodes have been studied and compared with those of normal-sized electrodes [36,37]. Off-line detection utilizing a palladium decoupler was applied. The importance of pressure compensation on the separation efficiency has been studied. The performance of the system has been assessed using standard mixtures of carbohydrates.

2. Experimental

2.1. Apparatus

The experimental set-up is depicted in Fig. 1. A Prince programmable injector for capillary electrophoresis, including a high-voltage supply, was obtained from Lauer Labs (Emmen, Netherlands). Samples were injected hydrodynamically at a pressure of 20 mbar for 12 s; the volume of sample loaded was approximately 15 nl. The forced-air thermostat was set at 28°C. Fused-silica capillaries were provided by Polymicro Technologies (Phoenix, AZ, USA). The internal diameter of the separation capillary was 75 μm and that of the coupling capillary 50 μm. Unless stated otherwise, their lengths were 100 and 8 cm, respectively. New separation capillaries were etched with 1 mol l^{-1} NaOH for 1 h before use. The field decoupler was made from a palladium cylinder [22,23]. The T-shaped electrochemical

microcell was a modification of the prototype previously described [39], with a three-electrode instead of the two-electrode system. A silver tube functioned as outlet and as in situ Ag/AgCl reference electrode, with the addition of 1 mmol l^{-1} NaCl to the background electrolyte to stabilize the potential. A stainless steel tube was used as the auxiliary electrode. PEEK tubes of 6 cm length (1/16 inch O.D., 0.5 mm I.D.) were used to construct Cu$_2$O modified carbon working electrodes. The detector cell was located inside a Faraday cage. A Hewlett-Packard (Waldbronn, Germany) 1049A programmable electrochemical detector was used. The signal was registered with a HP 3394A integrator and a strip-chart recorder.

2.2. Chemicals and solutions

Carbohydrates, obtained from standard suppliers, were analytical grade and used as received. Concentrated stock solutions (10^{-2} mol l^{-1}) were kept in the refrigerator (4°C) and diluted to the desired concentration daily. The background electrolytes, NaOH solutions containing 1 mmol l^{-1} NaCl, were degassed with helium before use. Subboiled demineralized water was used to prepare solutions.

2.3. Working electrode preparation

Cuprous oxide (20% w/w) modified carbon paste [36] and conductive carbon cement [37] were prepared as described previously. To fabricate electrodes suited for the microcell, PEEK tubes were cut into 4-cm long pieces. To make electric contact, a bundle of copper wires was inserted into the PEEK tube and sealed at one end of the tube using fast-drying glue, leaving a chamber of approximately 1 mm depth at the other end of the tube. Thick Cu$_2$O/CCC paste was pressed into this chamber and allowed to dry at room temperature for one week. The conductivity of the electrodes was examined with a multimeter, and only those with a resistance of less than 200 Ω were used. Electrodes were polished using grade 2/0 dry emery paper, a 3-μm imperial micro finishing film sheet and
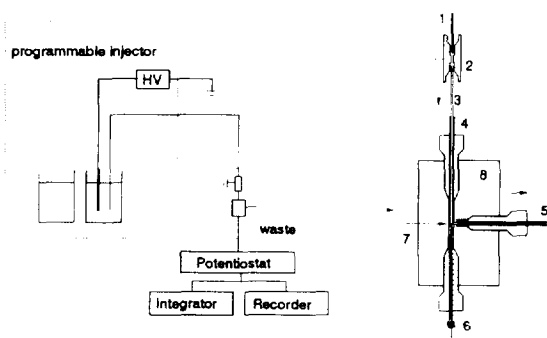

Fig. 1. Scheme of the experimental set-up: 1 = separation capillary; 2 = Pd-decoupler; 3 = coupling capillary; 4 = counter electrode; 5 = reference electrode and outlet; 6 = working electrode; 7 = detection chamber; 8 = T-shaped microcell.

0.05-μm alumina particles on a Buehler pad, successively. The electrode surface was rinsed with water before use.

3. Results and discussion

3.1. Electrochemistry

The miniaturized Cu_2O modified CCC electrodes were tested using cyclic voltammetry (CV) in quiescent solution. In a deaerated blank 0.1 mol-l^{-1} NaOH solution a broad anodic peak at + 0.15 V appeared only in the first scan. This peak may be attributed to the oxidation of the surface layer of the cuprous oxide modifier. In subsequent scans no anodic peaks were observed in the potential range up to + 0.6 V. After the addition of different sugars to the solution, anodic peaks were observed with peak potentials between + 0.55 and + 0.60 V. An example is shown in Fig. 2. Since these peaks did not appear with unmodified electrodes, they can be attributed to the electrocatalytic oxidation of the carbohydrates tested. These phenomena are in agreement with the findings with large-area (3 mm diameter) electrodes [36,37].
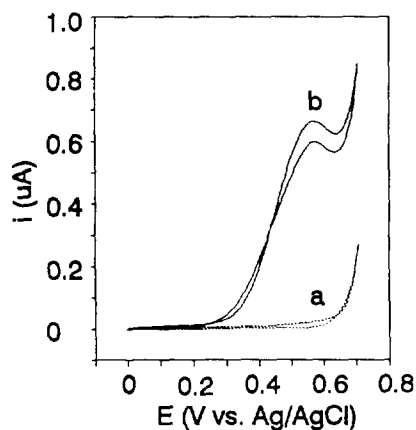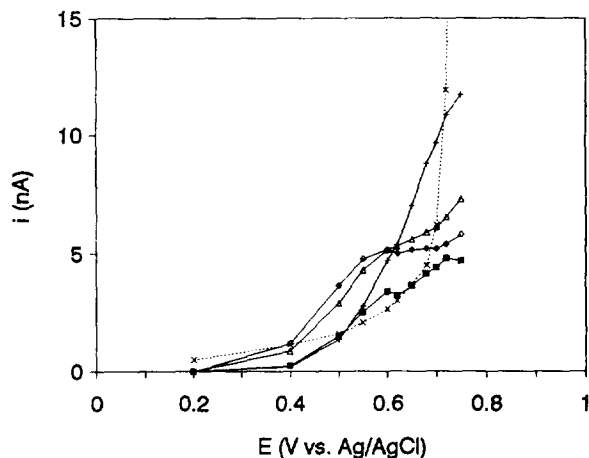
Fig. 3. Hydrodynamic voltammograms with a Cu_2O/CCC electrode in CE. Samples: (■) glycerol; (+) raffinose; (◇) fucose; (△) mannose; (×) background. Conditions: L_1 = 100 cm; L_2 = 8 cm; 0.05 mol l^{-1} NaOH; 14 kV high voltage; 20 mbar compensation pressure.

The optimal detection potential for CE was determined by measuring hydrodynamic voltammograms for several sugars in a background electrolyte of 0.05 mol l^{-1} NaOH (Fig. 3). For most sugars a limiting current is obtained at + 0.60 V, consistent with the CV experiments. Although the signal for raffinose still increased up to + 0.75 V, with increasing the applied potential the background current became higher and poorer signal-to-noise ratios were obtained. Therefore, further measurements were carried out with an applied potential of + 0.60 V. From the peak areas obtained it was calculated (taking 2 eq. mol^{-1} for the number of electrons involved in the oxidation process) that the coulometric efficiency of the microcell ranged from 19% for raffinose to 32% for mannose.

The stability of the modified conductive carbon cement electrode was examined and compared to an electrode with a carbon paste matrix. A standard mixture of four sugars was injected repeatedly during a 18-h period and peak heights were measured (see Fig. 4). The signals obtained with the carbon paste electrode kept decreasing. It is clear that even with the low liquid velocities
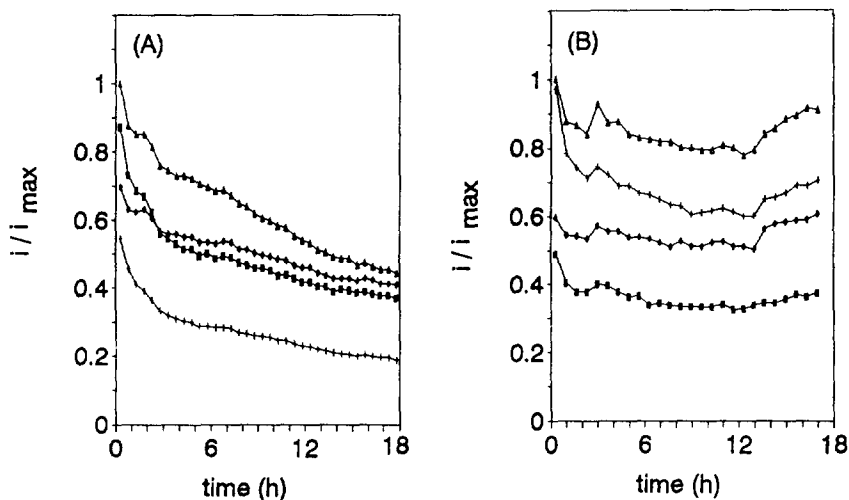
Fig. 2. Cyclic voltammetric responses for a Cu_2O modified CCC electrode in deaerated 0.1 mol l^{-1} NaOH: a = blank; b = with 5 mmol l^{-1} glucose. Scan rate 20 mV s^{-1}.

Fig. 4. Stability of Cu$_2$O modified (A) carbon paste and (B) conductive carbon cement electrodes at + 0.60 V. Conditions as in Fig. 3.

in CE, the leaching of modifier from carbon paste was still problematic. The signals with the CCC electrode became reasonably stable after a short time. The relative standard deviation of the

peak heights after the first hour was from 5.3 to 6.2% for the different compounds ($n = 25$). When the mannose peak was regarded as an internal standard peak, the relative standard
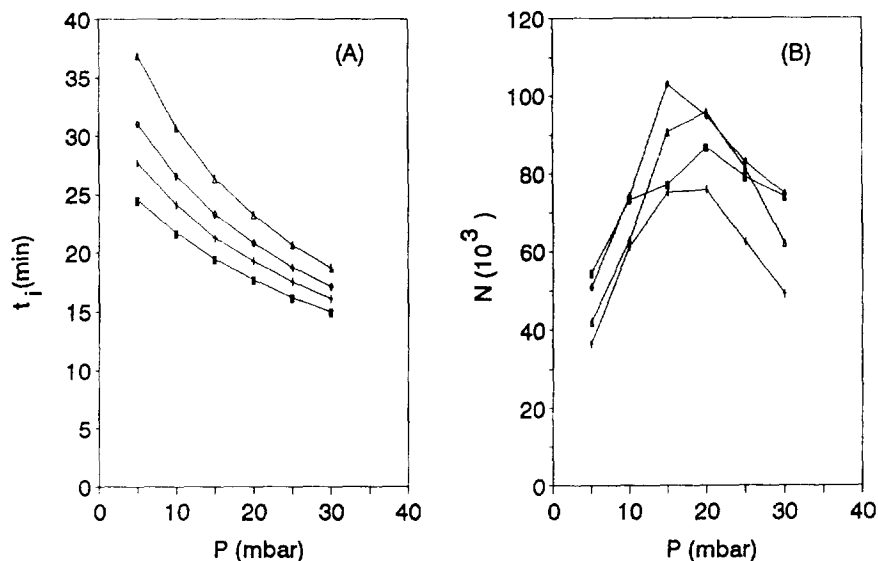

Fig. 5. Influence of the applied pressure on the apparent migration times (A) and the observed plate numbers (B). Sample components: (■) glycerol; (+) raffinose; (◇) fucose; (△) mannose. Conditions as in Fig. 3.

deviation for the other peaks was reduced to 2.2–3.5%.

3.2. Pressure compensation optimization

With off-column detection in capillary electrophoresis the flow resistance of the coupling capillary induces a back-pressure against electroosmotic flow in the separation capillary, distorting the uniform flow profile and causing extra zone broadening. The essence of pressure compensation is to provide a suitable driving force on the inlet of the separation capillary, driving the liquid through the coupling capillary with a flow-rate equal to the electroosmotic flow in the separation capillary ($F_2 = F_{eo}$). To obtain a flat-plug flow profile in the separation capillary, this compensation pressure P_{cmp} should be:

$$P_{cmp} = 32\eta L_2 \mu_{eo} E \frac{d_1^2}{d_2^4} \quad (1)$$

where η is the solution viscosity, L_2 the length of the coupling capillary, μ_{eo} the electroosmotic mobility, E the electric field strength and d_1 and d_2 are the internal diameter of the separation and the coupling capillary, respectively.

Fig. 5 shows the influence of the applied pressure on apparent migration times and separation efficiencies for four sugars. Deviations from the optimal P_{cmp} resulted in decrease of the separation efficiency, as discussed above. The optimal pressure was found to increase proportional to the coupling capillary length L_2 (varied from 7 to 20 cm) and the field strength E (from 100 to 160 V cm^{-1}), as predicted by Eq. 1. While a compensating pressure of 16 mbar was calculated for the system as used in further experiments (with a 7-cm coupling capillary and a field strength of 140 V cm^{-1}), an optimum between 15 and 20 mbar was found experimentally for the different sugars. No obvious influence of L_2 or E on the maximally obtainable plate numbers was found; these were always in the order of 90 000 to 110 000 when the compensating pressure was optimized. Apparently the dead volume of the decoupler played a major role in the zone broadening under optimized conditions. From

the difference between calculated and experimental values for the plate numbers, this dead volume was estimated as 10–20 nl.

3.3. Separation conditions

Since the pK_a values of most mono- and oligosaccharides are in the order of 12.5, concentrated solutions of sodium hydroxide have to be used as background electrolyte for the electrophoretic separation. Concentrations of 0.01–0.02 mol l^{-1}, as have been suggested in the literature [28,29], are insufficient to obtain a satisfactory separation. In accordance to the findings of Colón et al. [34], we found that even with a 0.05 mol l^{-1} sodium hydroxide solution the resolution between zones of sugars is low. In Fig. 6, separations of a standard mixture of sugars with 0.05 and 0.10 mol l^{-1} NaOH as background electrolyte are compared.

The apparent migration time t_i of solute i consists of the migration time $t_{1,i}$ in the separation capillary and the hold-up time t_2 in the coupling capillary. With the appropriate pressure compensation, these are given by:

$$t_{1,i} = \frac{L_1}{(\mu_{eo} \pm \mu_i)E} \quad (2)$$

$$t_2 = \frac{L_2 d_2^2}{\mu_{eo} E d_1^2} \quad (3)$$

The electroosmotic mobility μ_{eo} and effective electrophoretic mobilities of solutes μ_i can be calculated from the experimentally available apparent migration times of the osmotic flow marker (t_{eo}) and the solutes (t_i) with:

$$\mu_{eo} = \frac{L_1 + (d_2/d_1)^2 L_2}{t_{eo} E} \quad (4)$$

$$\mu_i = -\frac{(t_i - t_{eo})[L_1 + (d_2/d_1)^2 L_2]^2}{E t_{eo}[t_i L_1 + (t_i - t_{eo})(d_2/d_1)^2 L_2]} \quad (5)$$

In Table 1 the mobilities of the sugars are given, calculated with Eqs. 4 and 5 from their apparent migration times. The increased ionization of the sugars in 0.10 mol l^{-1} NaOH results in an improved resolution. However, the analysis time
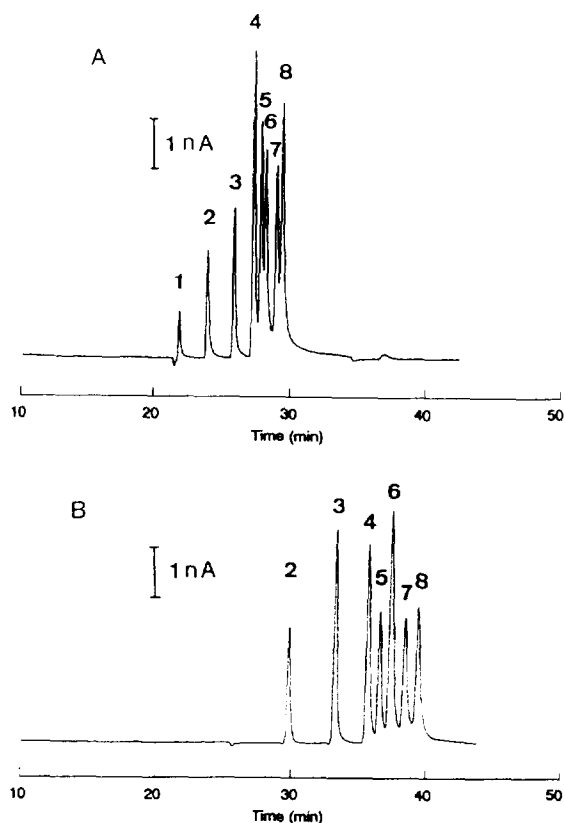
Fig. 6. Electropherograms obtained with (A) 0.05 mol l^{-1} and (B) 0.1 mol l^{-1} NaOH electrolyte at 12 kV high voltage with optimal compensation pressure. Peaks: 1 = glycerol; 2 = raffinose; 3 = fucose; 4 = galactose; 5 = mannose; 6 = arabinose; 7 = xylose; 8 = glucose (20–240 μmol l^{-1}).

Table 1
Effective electroosmotic and electrophoretic mobilities[a]

Peak	μ_i (10^{-8} m^2 V^{-1} s^{-1})	
NaOH concentration (mol l^{-1})	0.05	0.10
Electroosmosis	+7.06	+6.71
Glycerol	−0.09	n.m[b]
Raffinose	−0.68	−0.79
Fucose	−1.15	−1.32
Galactose	−1.45	−1.65
Mannose	−1.56	−1.74
Arabinose	−1.62	−1.83
Xylose	−1.77	−1.95
Glucose	−1.84	−2.04
Galacturonic acid	−3.51	−3.90

[a] For conditions see Fig. 6.
[b] Not measured.

is increased with the 0.10 mol l^{-1} background electrolyte, partly also by a decrease of the electroosmotic mobility. A relatively low electric field (120 V cm^{-1}) had to be used to limit the zone broadening by the Joule heating with this high-conductivity electrolyte. The electrophoretic current was 124 μA under these conditions.

The sensitivities, linear range and limits of detection (LOD) for the different sugars are listed in Table 2. Detection limits of 20–30 fmol or 1–2 μmol l^{-1} are found based on a signal-to-noise ratio of 3.

The concentration detection limits are at least two orders of magnitude lower than those obtained in CE by indirect UV detection [29–30] or

borate complexation [27]. In comparison to end-column electrochemical detection, an advantage in concentration limits is generated by the larger capillary diameters allowed. Lu and Cassidy [33] found detection limits for sugars, using PAD, of approximately 1 fmol. However, with the 10-μm capillaries they used, this corresponds to sample concentrations of 2–3 μmol l^{-1}. With a copper wire electrode in the end-column mode, Colón et al. [34] found detection limits of less than 50 fmol. Together with the sample volume disadvantage in the 50-μm capillaries used, the concentration limits were almost one order of magnitude higher than in our method.

4. Conclusion

It may be stated that we have shown the feasibility of off-column amperometric detection of sugars in CE using chemically modified electrodes. Our results with respect to separation efficiency and limits of detection are similar to or slightly better than what has been achieved with end-column detection. However, with our system, capillaries and electrodes can be installed and changed easily without the use of a microscope or micromanipulator, so that the detection set-up might be suited for routine analysis.

Table 2
Linearity and limits of detection[a]

Compound	Sensitivity (nA pmol^{-1})	Injected amount (pmol)	R	LOD (μmol l^{-1})
Raffinose	1.92 ± 0.05	0.37–3.7	0.997	1.9
Fucose	3.17 ± 0.03	0.21–2.1	0.9996	1.4
Mannose	3.44 ± 0.04	0.31–3.1	0.9995	1.3

[a] For conditions see Fig. 6B.

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