Published in final edited form as:

Anal Chem. 2011 July 1; 83(13): 5329–5336. doi:10.1021/ac2007587.

Rapid High Resolution Characterization of Functionally Important Monoclonal Antibody *N*-Glycans by Capillary Electrophoresis

Zoltan Szabo, András Guttman, Jonathan Bones, and Barry L. Karger*

Barnett Institute, Northeastern University, Boston, Massachusetts 02115

Abstract

Characterization of the *N*-glycosylation present in the Fc region of therapeutic monoclonal antibodies requires rapid, high resolution separation methods to guarantee product safety and efficacy during all stages of process development. Determination of fucosylated oligosaccharides is particularly important during clone selection, product characterization and lot release as fucose has been shown to adversely affect the ability of mAbs to induce antibody dependent cellular cytotoxicity (ADCC). Here, we apply a general capillary electrophoresis optimization strategy to separate functionally relevant fucosylated and afucosylated glycans on monoclonal antibody products in the presence of several high mannose oligosaccharides. The *N*-glycans chosen represent those most commonly reported on CHO cell derived therapeutic antibodies. A rapid (<7 min) high resolution separation of twelve commonly reported and functionally important IgG glycans was developed by systematically evaluating the effects of selectivity (boric acid) and efficiency (linear polyacrylamide) enhancing additives. The approach can be used to rapidly optimize capillary electrophoresis separation of other glycan mixtures. Following optimization, the method was applied to overnight sample processing for automated 96 well plate-based glycosylation analyses of two non-proprietary therapeutic monoclonal antibodies, demonstrating ruggedness and suitability for high-throughput process and product monitoring applications.

INTRODUCTION

Monoclonal antibody-based therapeutics represent a major product development area within the biotechnology industry. The market share for these products was over \$20 B in 2006 and is predicted to be greater than \$50 B in 2011^{1, 2}. In addition, the emergence of biosimilar products into the mAb market is on the horizon as patent protection for many of the current innovative blockbuster monoclonal therapeutics will expire in the next few years^{2, 3}.

The biological effectiveness of mAbs, such as their ability to invoke antibody dependent cellular cytotoxicity (ADCC), is dependent on their receptor binding characteristics⁴. For ADCC, the efficacy of natural killer (NK) cell recruitment is determined by the affinity of cell surface Fc receptors, in particular FcγRIIIa, to the Fc region of the IgG molecule. The presence or absence of a fucose residue on the reducing terminal core GlcNAc residue of the oligosaccharides at the conserved Asn297 site of the C_H2 domain has been demonstrated to significantly affect the affinity of the antibody to this receptor^{5, 6}. Recent reports indicated that the absence of a fucose residue at the innermost GlcNAc of the reducing end results in increased ADCC activity⁵. Monoclonal antibodies produced in widely used Chinese Hamster Ovary (CHO) cells can be greater than 90% core fucosylated; however, cell lines can be engineered to yield non-fucosylated glycoforms⁷. Selection of the cell line producing

*To whom correspondence should be addressed. b.karger@neu.edu.

antibodies with the desired glycosylation during clonal selection and quality by design optimization generates a requirement for rapid, highly efficient, robust and automated bioanalytical methods for *N*-glycan characterization. Of particular interest is the separation and quantitation of the agalactosyl (G0), monogalactosyl (with the positional isomers of G1 and G1') and digalactosyl (G2) complex biantennary oligosaccharides along with their fucosylated and afucosylated forms (see structures in Scheme 1). Details regarding the presence and relative amounts of these glycans are required by regulatory agencies during product filing to guarantee product safety and efficacy, even if the intended biological activity does not require ADCC function ⁸.

The structural complexity and diversity of oligosaccharides, especially when positional and linkage isomers are considered, makes glycan analysis particularly challenging. At present, the three major strategies for monoclonal antibody glycosylation analysis are: 1) intact top down characterization of the antibody with or without reduction, 2) characterization at the glycopeptide level and, 3) characterization of released oligosaccharides ⁹. Here, we focus on the characterization of released sugars using capillary electrophoresis (CE) with laser induced fluorescent (LIF) detection. CE-LIF is an automated approach to electric field mediated separation of oligosaccharides, which offers high separation efficiency (including positional and linkage isomers) and rapid separation times ¹⁰.

Polyhydroxy compounds, such as sugars, form complexes with tetrahydroxy borate ions above pH 8 ¹¹. At basic pH, boric acid acts as a Lewis acid and accepts a hydroxyl ion to form $B(OH)_4^-$, leading to the formation of stable five and six-membered ring structures with cis-1,2 or cis-1,3 diols, either as mono-borate or di-borate complexes ¹². The recognition of these small steric differences between closely related isomers makes CE a powerful method for the separation of oligosaccharides. However, attaining high selectivity *via* the addition of boric acid is not always sufficient to separate closely related species in a rapid manner. In such instances, the use of efficiency increasing additives (such as viscosity modifiers) may also be required.

Peak efficiency in capillary electrophoresis can be characterized by the number of theoretical plates (*N*) using the following equation ¹³:

$$N = \frac{\mu E l}{2 D}$$

equation 1

where μ is the electrophoretic mobility, *E* is the applied electric field strength, *l* is the effective capillary length and *D* is the diffusion coefficient. According to equation 1, a decrease in the diffusion coefficient increases separation efficiency. The use of borate-based buffer systems limits the number of possible viscosity modifying additives in capillary electrophoresis as polyols complex with borate and therefore, cannot be used. Other linear polymers such as polyacrylamide or polyethylene oxides are, however, good alternatives. Boric acid containing buffers with long chain linear polyacrylamide additives were successfully used to separate closely related IgG glycans ¹⁴. The long separation time required, >25 min, did not facilitate high throughput applications, (30 min per analysis would require 2 days to complete the measurement of 100 samples). Therefore, a need currently exists for a strategy to optimize capillary electrophoresis analysis of glycans wherein sample throughput can be substantially increased while maintaining the information content.

In this paper, we introduce an optimization strategy by evaluating the effect of selectivity and efficiency enhancing buffer additives on the separation of functionally important oligosaccharides commonly found in the CH₂ domain of the Fc regions of IgG. The goal

was to obtain fast analysis applicable for large numbers of samples in reasonable time, i.e., < 10 min from run to run to enable automated overnight processing of 96 samples. The optimized method developed in this work resulted in rapid (7 min) separation of all fucosylated and afucosylated positional isomers of the biantennary complex IgG sugars as well as several high mannose oligosaccharides (structures shown in Scheme 1). The platform has been applied to the reproducible analysis of the glycosylation present on two monoclonal antibodies. The approach is general and readily applicable for the optimization of glycosylation analysis of other glycoproteins.

MATERIALS AND METHODS

Chemicals

Acetic acid (glacial), boric acid, ammonium hydroxide solution (25 %), citric acid, and sodium cyanoborohydride (1 M solution in tetrahydrofuran) were obtained from Sigma-Aldrich (St. Louis, MO). 8-aminopyrene-1,3,6-trisulfonic-acid (APTS) was the kind gift of Beckman Coulter Inc. (Brea, CA). Oligomannose 5, 6 and 9, the disialo-biantennary glycan (A2-F) and all fucosylated and afucosylated asialo-galactosylated-biantennary (G2 and G2-F), asialo-monogalactosylated-biantennary (G1/1' and G1/1'-F), asialo-agalacto-biantennary (G0 and G0-F) structures and the GlycoPrep deglycosylation kit were kindly provided by Prozyme (Hayward, CA) (see structures in Scheme 1). Polyacrylamide (MW: 10,000) was purchased from Polysciences, Inc. (Warrington, PA) and acetonitrile was from Thermo Fisher Scientific (Fair Lawn, NJ). Model therapeutic monoclonal antibodies (herein referred to as antibody A and antibody B) for the high throughput experiments were kindly provided by Beckman Coulter.

APTS labeling and CE-LIF analysis

Oligosaccharide standards were labeled according to our recently published protocol ¹⁵. Briefly, solutions containing 5 µg of individual glycans were evaporated to dryness in a centrifugal vacuum evaporator (Centrivap, Labconco, Kansas City, MO), followed by the addition of 2 µL of APTS solution (50 mM APTS in 1.2 M citric acid) and 2 µL of NaBH₃CN (1 M in THF), with subsequent incubation at 55°C for 60 minutes. The reaction was stopped by the addition of 50 µL of HPLC grade water (Mallinckrodt Baker, Inc., Phillipsburg, NJ). To remove the large excess of unreacted APTS after the labeling reaction, 450 µL acetonitrile was added to the reaction mixture, followed by the addition of 5 µL of concentrated (25 %) ammonia solution. The solution was passed over 5 µL bed volume normal-phase resin filled pipet tips to remove unconjugated labeling dye from the reaction mixture using a semi-automated 12-channel pipettor and the purification process was controlled by the PhyTip operating software (PhyNexus, San Jose, CA). After glycan capture, the tips were washed four times with 200 µL of 95 % acetonitrile. Captured glycans were eluted with 50 µL of HPLC water and further diluted 100 times with HPLC water prior to CE-LIF analysis.

All capillary electrophoretic experiments were conducted using a PA 800 *Plus* capillary electrophoresis system (Beckman Coulter) equipped with laser-induced fluorescence detection (488 nm excitation/520 nm emission). 50 µm i.d. bilayer coated capillaries (with a hydrophilic layer of polyacrylamide ¹⁶, Beckman Coulter) were used for CE analysis. All samples were pressure injected (0.5 psi, 10sec) and then separated by applying 500 or 600 V/cm. For 96 well-plate overnight operation, the system was programmed following the manufacturer's instructions.

Background electrolyte viscosity measurement

The viscosity of the background electrolyte was measured following the method of Busnel et al.¹⁶. Briefly, a 20 μm i.d. fused silica capillary with 60 cm total (50 cm effective) length was filled with the background electrolyte. A short plug of APTS solution was then pressure injected (1 psi for 10 s), and the sample zone was mobilized toward the detector by applying 4 psi pressure from the inlet side under tight temperature control. The viscosity (η) was calculated from the mobilization time:

$$\eta = d_c^2 \Delta P t / (32 L^2) \quad \text{equation 2}$$

where, d_c is the internal diameter of the capillary, ΔP the mobilization pressure, t the mobilization time, and L the total capillary length.

RESULTS AND DISCUSSION

An optimization of additives to the CE-LIF separation buffer for the modulation of selectivity and efficiency was performed, leading to the baseline separation of all *N*-glycans in our mAb IgG test mixture (see Scheme 1, structures 1–12). The evaluation test mixture comprised oligosaccharides commonly found on mAb molecules produced in CHO cells, including sialylated (A2-F) and neutral biantennary complex (G0, G0-F, G1, G1-F, G1', G1'-F, G2 and G2-F) as well as oligomannose (Man5, Man6, Man9) structures. The A2-F glycan was added to the test mixture as a sialylated reference structure. Using a relatively long capillary (50 cm effective length, 60 cm total length), boric acid addition was first investigated to increase the selectivity of the glycan separations. Considering previous reports favoring short chain polymeric additives¹⁷ and linear polyacrylamide (LPA) based buffer systems¹⁴, a short chain LPA polymer was selected to enhance separation efficiency after optimizing selectivity. The use of a short chain polymeric additive is useful to increase viscosity for improving plate number while not greatly affecting separation speed or column washing time. Finally, after optimization, the column length was shortened to an appropriate length, while maintaining the voltage drop (increased field strength) to obtain baseline separation of all the peaks in the shortest time.

Selectivity and efficiency optimization

For the boric acid - mediated selectivity enhancement experiments, the pH of the background electrolyte was examined between 7 and 10 (in unit pH intervals) and set at 9.0 to ensure strong complexation (data not shown)¹⁸. At this pH, the use of a bilayer coated capillary was crucial to eliminate electroosmotic flow (EOF), to yield higher efficiency and improved migration time reproducibility, relative to capillary with EOF flow. The effect of boric acid concentration (ranging from 5 mM to 50 mM) on the separation of the oligosaccharides commonly found on mAbs of CHO origin is shown in Figure 1. Complexation between the glycans and the B(OH)_4^- is clearly demonstrated by the altered migration times and migration order changes with increasing boric acid concentration. Using only 5 mM boric acid in the background electrolyte resulted in an incomplete separation of the standard glycans, numbered 1 to 12 in Scheme 1. Increasing the additive concentration to 25 mM significantly improved the separation, presumably due to the higher degree of oligosaccharide-borate complex formation. For example, while G2-F and G1 (biantennary structures with 10 sugar units) were not separated with the 5 mM boric acid level, they were baseline separated at 25 mM. Interestingly, at 50 mM (see Fig 1), G2-F moved to overlap with G0. The differences in mobility alterations for the high mannose structures (Man series) and the biantennary complex sugars (G series) as a function of additive concentration, indicates structure dependent complexation, as would be expected¹². Based

on the data shown in Figure 1, the optimum separation of the IgG *N*-glycan test mixture components was obtained at 25 mM boric acid containing 7.5 mM ammonium acetate buffer (pH 9.0).

To obtain complete separation of the test mixture components, the effect of linear polyacrylamide (Mw 10,000) as an efficiency modulating additive was next studied in the range of 0.5% to 2.5% in 25 mM borate containing 7.5 mM ammonium acetate buffer (pH 9) background electrolyte (Figure 2). The general increase in migration times was attributed to the increased viscosity of the higher concentration LPA containing buffers ($\eta=1.05 - 1.41$ cp). While the efficiency of the separated peaks increased with elevated LPA concentration (Table 1), a small change was observed in the relative migration time of the sample components, probably due to minor interaction of the LPA with the glycans. Therefore, for best separation performance, the amount of polymeric additive in the buffer system was carefully optimized and found to be 1.5 % LPA (Mw 10,000) (Figure 2, middle trace).

The optimization strategy for glycan analysis commenced with the use of a relatively long capillary column for maximum efficiency (50 cm effective length). The next focus of the optimization was to reduce the analysis time while maintaining the separation performance. Thus, shorter (30 and 40 cm) effective length capillaries were evaluated, which allowed shorter migration distance and at the same time, given constant inlet voltage (30,000 V), an increase of the applied electric field strength. The latter facilitated even faster analysis, and by decreasing the time for diffusion also led to increased column efficiency. The traces in Figure 3 show that 50 and 40 cm effective column lengths both provided full separation of all test mixture components, but the latter one was accomplished in only 7 minutes. While the use of 30 cm effective column length further decreased the separation time, it did not result in full separation of all the test mixture glycans. Therefore, 40 cm effective separation distance was used in the following high throughput experiments with 600 V/cm applied electric field strength.

High throughput operation under optimal separation conditions

Using the optimal separation conditions of 25 mM borate and 1.5 % LPA (Mw 10,000) with 7.5 mM ammonium acetate (pH 9.0) as background electrolyte, 40 cm effective separation length and 600 V/cm applied electric field strength at 25°C, baseline resolution of all test mixture components for CHO cell derived therapeutic antibodies was obtained in less than 7 min. This is as approximately two times faster than the benchmark separation with commercially available kits, which, in addition, cannot separate the fucosylated and afucosylated IgG glycans; and four times faster than was recently reported by using other formulations¹⁴. Considering buffer rinse, injection, vial positioning and separation times, (~1.4 min rinse, injection and positioning time from sample to sample or a total of 8.4 min.), approximately 13.5 hours would be required to process 96 samples from a well plate in an automated fashion, i.e., overnight processing.

Figure 4 shows representative traces of the glycosylation patterns from two non-proprietary therapeutic antibody samples (antibody A and B) generated using the high throughput 96 well operation mode. The two samples were injected in blocks of 6 (antibody A: injections 1–6, antibody B: injections 7–12 and so forth), and the traces in Figure 4 represent the last runs of each block (6, 12 and so forth). Excellent migration time reproducibility, RSD=0.16% (N=96, for the four major peaks of G0, G1, G1' and G2) and RSD=1.21% (N=96, for the afucosylated counterparts of G0-F, G1-F, G1'-F and G2-F), was obtained even without the use of migration time standards. The relative peak area reproducibility was RSD=3.5% (N=96, for the four major peaks of G0, G1, G1' and G2) and RSD=0.82% (N=96, for the afucosylated counterparts of G0-F, G1-F, G1'-F and G2-F). Our reproducibility data suggests good coating stability at 25°C, well beyond 100 runs at pH 9.0

using the bilayer coated capillary. The insets show the enlarged separation windows of the peaks from the left and right panels at the beginning (mAB A: run # 6 and mAB B: run # 12) and at the end (mAB A: run # 90 and mAB B: 96) of each block, revealing several minor oligosaccharides (peaks a, b and c) in addition to those present in our 12 glycan test mixture (numbers and letters correspond to structures in Scheme 1). The peaks corresponding to the minor components were annotated based on our in-house built GU value database as disialo-biantennary-core fucosylated (peak a), monosialo-biantennary-core fucosylated (peak b) and oligomannose 7 (peak c) glycans (see corresponding structures in Scheme 1). The GU database structural annotation can be confirmed by exoglycosidase digestion based carbohydrate sequencing or by mass spectrometry. All peaks above 0.5 % of the total peak area were annotated except the two labeled by the asterisks. Identification of these components can be obtained by means of other methods, such as CE-MS and/or exoglycosidase array based tests.

Effect of separation temperature

Further reduction of analysis time was possible by increasing the separation temperature. As shown in Figure 5, baseline separation of all the sample components was obtained in less than 5 minutes at 35°C in 25 mM boric acid containing 7.5 mM ammonium acetate buffer (pH 9) and 1.5% LPA. However, we chose to use 25°C as the higher separation temperature could possibly influence the lifetime of the capillary coating, which is clearly of importance in high throughput applications where large sample numbers are to be run. With the advent of temperature stable capillary inner coatings, we envision routine use of higher analysis temperatures to obtain even more rapid and highly efficient separation of APTS labeled glycans.

CONCLUSIONS

We have described an optimization strategy for rapid and high resolution glycan analysis by capillary electrophoresis using oligosaccharides commonly found as major components on monoclonal antibody molecules of CHO derived origin. Boric acid and linear polyacrylamide were independently evaluated as selectivity and efficiency modulation additives for the separation of the IgG *N*-glycan reference panel.

The data demonstrate that boric acid in the background electrolyte plays a crucial role in separation selectivity, while linear polyacrylamide was important in improving separation efficiency. The optimized separation parameters with 25 mM boric acid, 1.5 % linear polyacrylamide (Mw 10,000) at pH 9.0 using 40 cm effective separation distance resulted in full separation of all selected components of CHO derived mAb glycosylation in less than 7 minutes at 25°C. Analysis of other neutral or highly sialylated glycans might require different separation conditions, thus the optimization strategy should be applied accordingly on a case by case basis.

The optimal separation conditions were applied to demonstrate high throughput glycosylation profiling of two non-proprietary monoclonal antibody therapeutics in 96 well-plate based automated overnight operation format. The sample preparation including glycan release, derivatization and purification took approximately 60 minutes, thus if only a couple of samples to be analyzed, the entire process could be done in approximately 90 minutes. We believe that this can be a general strategy for glycan separation optimization by capillary electrophoresis. We are currently fine tuning background electrolyte additive types and concentrations to accommodate fully automated analysis platforms for high throughput studies for other glycan structures.

Acknowledgments

This research was supported by NIH GM 15847. The authors also thank Beckman Coulter, Prozyme and PhyNexus for their generous gifts of products to support this study. Contribution No.989 from the Barnett Institute.

Abbreviations

LPA	linear polyacrylamide
APTS	8-aminopyrene-1,3,6-trisulfonic-acid
BGE	background electrolyte
IgG	immunoglobulin G

References

1. Maggon K. *Curr Med Chem.* 2007; 14:1978–1987. [PubMed: 17691940]
2. Walsh G. *Nat Biotechnol.* 2010; 28:917–924. [PubMed: 20829826]
3. Reichert JM, Valge-Archer VE. *Nat Rev Drug Discov.* 2007; 6:349–356. [PubMed: 17431406]
4. Roitt, I.; Brostoff, J.; Male, D. *Immunology.* Harcourt Publishers Limited; Edinburgh, UK: 2001.
5. Jefferis R. *Nat Rev Drug Discov.* 2009; 8:226–234. [PubMed: 19247305]
6. Ferrara C, Stuart F, Sonderrmann P, Brunker P, Umana P. *J Biol Chem.* 2006; 281:5032–5036. [PubMed: 16330541]
7. Raju TS, Briggs JB, Borge SM, Jones AJ. *Glycobiology.* 2000; 10:477–486. [PubMed: 10764836]
8. EMEA. Guideline on development, production, characterisation and specifications for monoclonal antibodies and related products. 2009.
9. Townsend, RR.; Hotchkiss, AT. *Techniques in Glycobiology.* Marcel Dekker; New York, NY: 1997.
10. Guttman A. *Nature (London).* 1996; 380:461–462. [PubMed: 8602248]
11. Boeseken J. *Adv Carbohydr Chem.* 1949; 4:189–210.
12. Paulus, A.; Klockow-Beck, A. *Analysis of carbohydrates by capillary electrophoresis.* Vieweg; Wiesbaden: 1999.
13. Giddings CJ. *J Chromatogr.* 1989; 480:21–26. [PubMed: 2592480]
14. Rampal, S.; Salas-Solano, O.; Lies, M. *MSB* 2009. Prague; 2009.
15. Szabo Z, Guttman A, Rejtar T, Karger BL. *Electrophoresis.* 2010; 31:1389–1395. [PubMed: 20309892]
16. Busnel JM, Schoenmaker B, Ramautar R, Carrasco-Pancorbo A, Ratnayake C, Feitelson JS, Chapman JD, Deelder AM, Mayboroda OA. *Anal Chem.* 2010; 82:9476–9483. [PubMed: 21028888]
17. Kamoda S, Ishikawa R, Kakehi K. *J Chromatogr A.* 2006; 1133:332–339. [PubMed: 16945378]
18. Hoffstetter-Kuhn S, Paulus A, Gassmann E, Widmer HM. *Anal Chem.* 1991; 63:1541–1547.
19. Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA, Rudd PM. *Proteomics.* 2009; 9:3796–3801. [PubMed: 19670245]

Figure 1.

The effect of boric acid additive on the separation of a test mixture of oligosaccharides commonly found on CHO cell derived therapeutic antibodies. The numbers above the peaks correspond to the structures in Scheme 1.

Conditions: Capillary: bilayer coated (total length: 60 cm, effective length: 50 cm, 50 μ m i.d.), Applied electric field: 500 V/cm. Injection: 10 sec/0.5 psi. Temperature: 25°C.

Running buffer: 7.5 mM ammonium acetate buffer (pH 9) with the boric acid concentrations shown above the traces.

Figure 2.

The effect of linear polyacrylamide (LPA, Mw: 10,000) additive on the separation of a test mixture of standard oligosaccharides of CHO cell derived therapeutic antibodies. The numbers above the peaks correspond to the structures in Scheme 1.

Conditions: Capillary: bilayer coated (total length: 60 cm, effective length: 50 cm, 50 μ m i.d.), Applied electric field: 500 V/cm. Injection: 10 sec/0.5 psi. Temperature: 25°C.

Running buffer: 7.5 mM ammonium acetate buffer (pH 9), 25 mM boric acid with LPA concentrations shown above the traces.

Figure 3.

The effect of effective separation distance on the separation of a test mixture of standard oligosaccharides of CHO cell derived therapeutic antibodies. The numbers above the peaks correspond to the structures in Scheme 1.

Conditions: Capillary: bilayer coated (50 μm i.d.). Upper trace: total length: 60 cm, effective length: 50 cm; Middle trace: total length: 50 cm, effective length: 40 cm; Lower trace: total length: 40 cm, effective length: 30 cm; Applied voltage: 30,000 V.

Injection: 10 sec/0.5 psi. Temperature: 25°C. Running buffer: 7.5 mM ammonium acetate buffer (pH 9), 25 mM boric acid and 1.5% LPA (Mw 10,000).

Figure 4.

96 well plate operation of glycosylation pattern analysis of two non-proprietary monoclonal antibody therapeutics (A and B). The insets display the enlarged separation window showing all separated minor components for traces 6, 12, 90 and 96. The numbers/letters above the peaks correspond to the structures in Scheme 1. The peaks labeled by the asterisks are unknown. Conditions: 25 mM boric acid and 1.5% LPA (Mw 10,000) in 7.5 mM ammonium acetate buffer (pH 9); Temperature: 25°C, Capillary: bilayer coated (total length: 50 cm, effective length: 40 cm, 50 μ m i.d.), Applied electric field: 600 V/cm. Injection: 10 sec/0.5 psi.

Figure 5.

Rapid separation of the glycan test mixture at 35°C. The numbers above the peaks correspond to the structures in Scheme 1.

Conditions: 25 mM boric acid and 1.5% LPA (Mw 10,000) in 7.5 mM ammonium acetate buffer (pH 9); Temperature: 35°C, Capillary: bilayer coated (total length: 50 cm, effective length: 40 or 10 cm, 50 μ m i.d.), Applied electric field: 600 V/cm. Injection: 10 sec/0.5 psi.

Scheme 1.

Structures of the oligosaccharide standards used in the study. The inset shows the symbol and linkage information adapted from ¹⁹.

Table 1

Theoretical plate numbers (N) of selected peaks in Figure 2. LPA: linear polyacrylamide (Mw 10,000), see structures in Scheme 1.

LPA (%)	0	0.5	1.5	2.5
Man6	109,000	120,000	165,000	229,000
G2-F	135,000	149,000	197,000	299,000
G2	178,000	196,000	241,000	260,000