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Journal of Chromatography A, 934 (2001) 75–85

JOURNAL OF CHROMATOGRAPHY A

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On-line capillary electrophoresis with mass spectrometry detection for the analysis of carbohydrates after derivatization with 8-aminonaphthalene-1,3,6-trisulfonic acid☆

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Received 11 June 2001; received in revised form 21 August 2001; accepted 5 September 2001

Abstract

Capillary electrophoresis (CE) with mass spectrometry (MS) detection is an ideal tool for analytical use, which combines a nano quantity assay with mass determination. Carbohydrate analysis has always been a challenge because of the inherent structural complexity and the lack of a chromophore, unless derivatization is used. Here we use the derivatization of carbohydrates with a fluorophore, 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS). This chromophore has two advantages, first, it facilitates UV and fluorescence detection and, second, it introduces negative charge to the analyte, which enhances zone electrophoretic separation. In this study, CE combined with negative ion electrospray MS (ESI-MS) was evaluated for the on-line analysis of ANTS labeled carbohydrates and cellulose fragments. The CE system was connected to the MS by a sheath-liquid electrospray arrangement. The ANTS reagent and Dextrin-15, which contains oligomers of maltose, were used as model samples for ESI-MS optimization in flow-injection–MS and CE–MS modes, respectively. Various sheath-liquid compositions regarding organic modifier (isopropanol, methanol, or acetonitrile) and electrolyte (acetic acid–formic acid, ammonium acetate, or triethylamine) were studied. The response as well as the analyte charge state distribution was found to be dependent on the composition and the orifice voltage. Low-pH conditions with isopropanol as organic modifier were sensitive, stable, and the most favorable for analysis.

Keywords: Derivatization, electrophoresis; Carbohydrates; Aminonaphthalenetrisulfonic acid

1. Introduction

Carbohydrates present a challenge for separation and analysis due to the structural complexity of the sample in terms of monomer composition, degree of branching and polymerisation. In many applications, only limited sample amounts are available. Moreover, carbohydrates lack a chromophore or a fluorophore to facilitate the sensitive detection by common spectroscopic techniques. After chromatographic separation, detection is usually restricted to refractive index, amperometry or light-scattering techniques unless precolumn derivatization is used.

The importance of oligosaccharides in biological systems as free or as glycoconjugate constituents is well recognized. Bound to proteins, they may in-

☆Presented in part at the 15th Montreux Symposium on liquid chromatography–mass spectrometry, Montreux, 1998.

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)01274-2

fluence the protein stability, structure and biological function [1,2]. Characterization may also be crucial for the control of manufacturing processes in industrial carbohydrate related applications, e.g. in quality control of glycosylated proteins produced by recombinant techniques, or of carbohydrate based excipients in pharmaceutical formulations.

Oligosaccharides may be profiled in a high-resolution separation system, and/or subjected to mass spectrometry. Capillary electrophoresis (CE) is a high-resolution technique, which has shown great potential in the analysis of carbohydrates [3–5]. Detection modes and labeling schemes for carbohydrates separated by CE have been thoroughly reviewed [3,6]. One approach to enhance CE separation and UV or laser-induced fluorescence (LIF) detection is derivatization with fluorescent dyes by reductive amination, as first introduced for CE by Novotny and co-workers [7,8]. Derivatization with a sulfonated label such as 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) or 7-aminonaphthalene-1,3-disulfonic acid (ANDS) introduces strongly acidic groups to the analytes which thereby remain negatively charged also at low pH. Fast CE separations of labeled oligosaccharides have thus been reported for ANTS [9,10] and ANDS [11]. CE–LIF profiling of N-linked oligosaccharides was demonstrated after derivatization with 8-aminopyrene-1,3,6-trisulfonate (APTS) [12].

Mass spectrometry (MS) has already been shown to be useful for molecular mass determination of oligosaccharide variants [13–17]. Native carbohydrates are usually detected as clusters of salt adducts and the sensitivity may be limited. Derivatization may offer several advantages such as less complicated spectra and increased response. For example, electrospray ionization (ESI) in the positive mode combined with tandem mass spectrometry (MS–MS) proved highly sensitive for the analysis of oligosaccharides after derivatization with 4-aminobenzoic acid 2-(diethylamine)ethyl ester [18] or with trimethyl(*p*-aminophenyl)ammonium chloride [19]. Kazmaier et al. [20] evaluated matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF)-MS and CE–UV for quantitation of oligosaccharides after derivatization with ANTS or with 4-aminobenzonitrile.

The combination of CE and MS provides the analyst with an additional dimension for molecular mass determination, for example, for identification of individual components in fingerprints from complex samples. CE–MS technology and applications have been reviewed [21–24]. An off-line method using a CE separation, combined with fraction collection of CE–UV peaks, and subsequent MALDI-TOF-MS analysis of APTS was reported [15]. APTS-derivatized mannooligosaccharides were characterized by on-line CE–ESI-MS [25]. Up to tetra-mer oligomers were detected as singly and doubly charged pseudomolecular ions in the negative electrospray mode. CE–ESI-MS analysis of native heparin, which is a complex mixture of sulfated oligosaccharides, was demonstrated by Duteil et al. [26], investigating both negative and positive mode MS. They observed adduct formation with cations emanating from the background electrolyte and the sheath-liquid, and concluded that negative MS ionization was preferred because it gave less complex spectra and provided the pseudomolecular peak. Phosphorylated carbohydrates from bacterial isolates have been analyzed by CE–MS using on-line isotachophoretic sample concentration (ITP–CE–MS), which provided a 10- to 50-fold sensitivity enhancement [27].

The on-line CE–ESI-MS using quadrupole ion trap (QIT) detection was recently reported for ANTS derivatized carbohydrates [28]. Singly and doubly charged pseudomolecular ions were detected in the negative ESI mode, for up to eight-mer oligosaccharide. However, peak distortion and signal instability was observed in the CE–MS total ion trace. A preliminary report [29] showed spectra of 3-(acetylamino)-6-aminoacridine derivatized N-linked glycans obtained from CE–ESI-MS analysis.

In this work, CE with on-line ESI single quadrupole MS, was performed in the negative ion mode. A tri-coaxial sheath-liquid arrangement was used as an interface to a single quadrupole mass analyzer. Dextrin-15, which contains oligomers of maltose, was used as a model sample for MS analysis after derivatization with ANTS. The diluted ANTS reagent itself was introduced in automated flow injection experiments for optimization of MS conditions, such as sheath-liquid composition and orifice voltage. The FIA arrangement allows a higher analysis throughput for optimization compared to CE–MS, and facilitates the study of a larger number of

experimental conditions. It is shown that the addition of acetic acid–formic acid (the background electrolyte, BGE, in the CE separation) to the sheath-liquid enhances the ionization. Such conditions provide a higher signal intensity as well as higher abundance of doubly and triply charged species, compared to a sheath-liquid containing a medium or high-pH electrolyte. CE–MS peak widths are smaller than in earlier reported data [28]. The total ion trace shows oligomers up to dodecamers, and was limited by the used scan range. Mass spectra of the separated components readily give molecular mass information of ANTS derivatives, showing the potential of CE–ESI-MS to become a viable tool for carbohydrate characterization.

2. Experimental

2.1. Electrolytes and solvents

Deionized water was produced in a purification system (ELGA, High Wycombe, UK). The volatile BGE, pH 2.3, was prepared by diluting 0.87 ml glacial acetic acid and 0.25 ml formic acid (98–100%, analytical grade, Merck, Darmstadt, Germany) to 100 ml with water. The 50 m*M* phosphate–35 m*M* triethylamine buffer, pH 2.5 was made by diluting 0.68 ml *ortho*-phosphoric acid (85%, analytical grade, Merck) and 0.97 ml triethylamine (analytical grade, Fluka, Buchs, Switzerland) with water to 200 ml. Methanol, isopropanol and acetonitrile of HPLC grade were from Rathburn (Walkerburn, UK). Ammonium acetate (analytical grade) was from Fluka.

2.2. CE–MS instrumentation

The CE–MS set-up is presented in Fig. 1. CE and flow injection experiments were made with a programmable injector for CE (Prince; Lauerlabs, Emmen, The Netherlands). Fused-silica capillaries (70–100 cm×50 μm I.D.×375 μm O.D.; Polymicro Technologies, Phoenix, AZ, USA), were coated in-house with polyacrylamide [30] or used as received. Custom-made polyvinyl alcohol (PVA) coated capillaries (100 cm×50 μm I.D.×360 μm O.D.) were obtained from Agilent Technologies (Waldbronn, Germany).

Samples were introduced by hydrodynamic injection at 100 mbar for 0.2–0.4 min for CE experiments. The CE conditions during CE–MS analysis were −30 kV, (effective electric field over capillary: −26.2 kV), with a hydrodynamic pressure of +10 mbar. For on-line CE–UV–MS, a Spectra 100 (Spectra-Physics, San Jose, CA, USA) UV detector was used at $\lambda = 223$ nm. The distance between the UV detector and the electrospray was typically 40 cm.

In flow injection experiments, sample plugs were typically injected hydrodynamically at 50 mbar for 0.2 min, and pushed by the CE BGE to the MS detector at a hydrodynamic pressure of 800 mbar.

MS detection was made in the negative ion mode with a PE Sciex API-1 single quadrupole instrument (Concord, Canada), equipped with a nebulizer as-

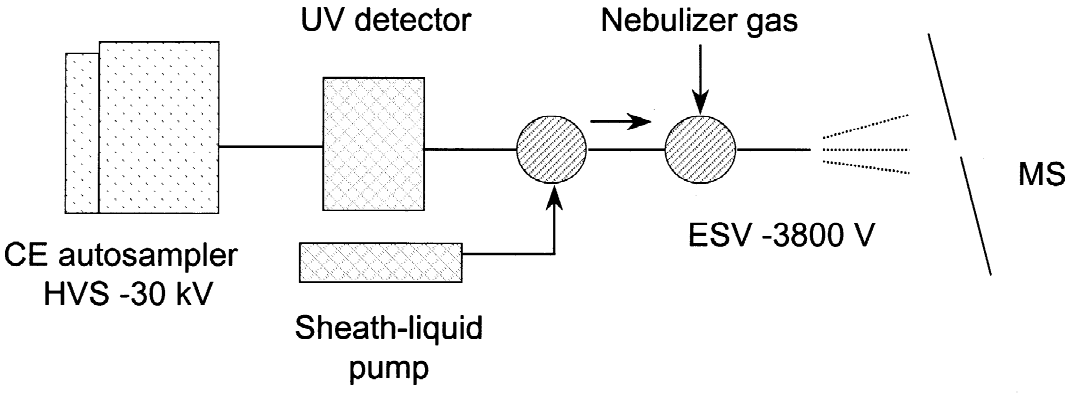

Fig. 1. Experimental set-up for CE–MS and flow injection–MS experiments.

sisted ESI interface and a laboratory-assembled sheath-liquid set-up [31]. A Harvard 22 syringe pump (Harvard Apparatus, Saint-Laurent, Canada) delivered the sheath-liquid at a flow-rate of 5 μl/min. The electrospray voltage was −3800 V, and the orifice voltage was varied from −50 to −75 V. Using the programmable CE injector, automated CE and flow injection experiments were combined with automated MS data acquisition by RAD (Routine data acquisition software, Sciex). Peak area evaluation was made with MACQUAN (Quantification Software, Sciex). The CE current and the electrospray current were monitored during CE–MS experiments for control of system stability.

The MS was set in the scanning mode with a mass range of m/z 250–800, dwell time 0.6 ms, step size 0.2 (cycle time 1.7 s/scan). Flow injection experiments were recorded by selected ion monitoring (SIM) of ions at m/z 382.2 and 190.6 (ANTS reagent singly and doubly charged, respectively), dwell time 350 ms (cycle time 0.9 s/scan).

2.3. Optimization of MS conditions by flow injection experiments

The ANTS reagent was used as a model compound for optimization of ESI-MS conditions for ANTS derivatized oligosaccharides. Plugs of a dilute ANTS solution (50 μ*M* in volatile BGE) were injected by the CE autosampler ($n=3$–6) in a flow injection system for each combination of experimental conditions. The sheath-liquid, delivered by a syringe pump, was changed manually.

The sheath-liquid for ESI contained organic modifier and electrolyte and various compositions were investigated. The studied organic modifiers were methanol, isopropanol, and acetonitrile, at concentrations of 50, 80 or 100% (v/v) vs. electrolyte. Three electrolytes were added: triethylamine, 2 m*M* (pH 10.6) or ammonium acetate, 2 m*M* (pH 6.9), or volatile BGE 0.5% in water (pH 3.5). Besides varying the sheath-liquid composition, the orifice voltage was set at −50, −65 or −75 V.

2.4. Derivatization of carbohydrates with ANTS

The derivatization by reductive amination of Dextrin-15 starch hydrolysate from maize (Fluka) was adapted from the method described by Jackson [32] according to the reaction scheme outlined in Fig. 2. Carbohydrate solution (10 μl, 40 mg/ml Dextrin 15 in water) was mixed with 2 ml ANTS (Molecular Probes, Eugene, OR, USA) solution (0.1 *M* in 25% acetic acid) and 10 μl $NaCNBH_3$ (95%, Aldrich, Gillingham, UK) solution [1 *M* in tetrahydrofuran (THF)–methanol, 10:1]. THF (analytical grade) was from Merck and methanol (HPLC grade) was from Fisons, Loughborough, UK. The reaction took place at 37°C or 75°C under shaking overnight. A 10-μl aliquot of the reaction mixture was diluted to 250 μl with water before injection onto the CE.

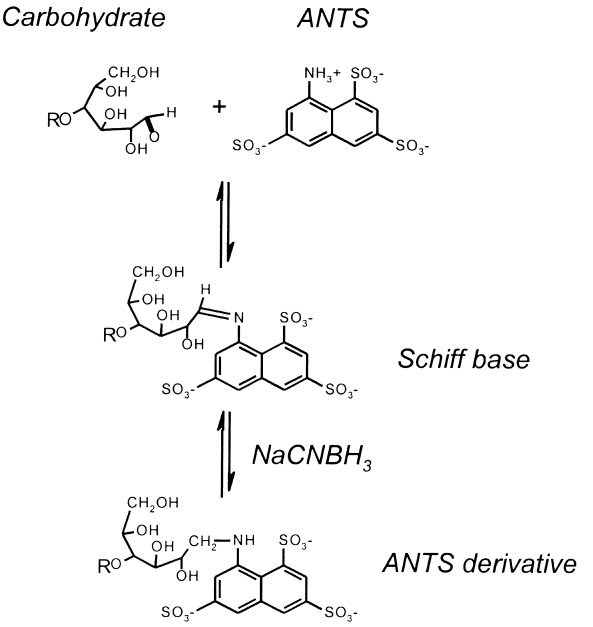

Fig. 2. Derivatization of carbohydrates with ANTS. Reductive amination scheme adapted from Jackson [32].

3. Results and discussion

3.1. Derivatization of carbohydrates with ANTS

The derivatization scheme is based on the reductive amination of the reducing carbonyl group of the oligosaccharide with the primary amino group of a fluorophore like ANTS [32]. The primary reaction product is a Schiff base, which is subsequently reduced to a secondary amine by sodium cyano-

borohydride (Fig. 2). The advantage of ANTS is two-fold: first, it provides the sensitivity for UV or laser-induced fluorescence detection of trace amounts and second, it leaves the derivatized analyte with a negative charge even at low pH. A sensitive CE–MS system would provide molecular mass information of components separated by CE.

3.2. On-line CE–MS analysis

ESI conditions were investigated using the CE–MS configuration with the sheath-liquid interface. The electrospray voltage was −3800 V throughout the study. A higher voltage did not improve the response considerably, however, corona discharge is more likely to occur in negative electrospray analysis at high field strength. The lowest nebulizer gas flow that gave a stable spray was selected because, in general, the electrical contact between the sheath-liquid and the capillary outlet in the electrospray tip was better at low gas flow-rates.

The most commonly used CE–MS interface is the sheath-liquid interface since it is considered to be easily and reproducibly constructed, compared to sheathless or liquid–junction interfaces [22]. Since robustness is a prerequisite for an automated, unattended analysis, the sheath-liquid electrospray configuration was selected in this study. By adding a sheath-liquid, the CE conditions can be more freely selected and optimized. Coated capillaries were used in order to suppress analyte adsorption to the fused-silica wall and minimize the electroosmotic flow (EOF). It is worth indicating that a reversed EOF flow is expected on bare fused-silica, as the sample is injected at the cathode side of the CE capillary. However, the EOF would also be low in untreated capillaries at the low pH used in this work.

The sheath-liquid flow-rate should be high enough to give a stable electrospray. On the other hand, dilution of analytes in the mixing volume at the CE capillary end should be minimized. A 5-μl/min flow-rate was used in our set-up. The position of the fused-silica at the spray needle is critical for a reliable operation as well. In our spray arrangement, the CE current stability was improved when the capillary tip was positioned ~0.5 mm from the end, inside the sheath-liquid capillary.

In CE–MS, the sheath-liquid provides counter ions in the zone electrophoretic separation. The electrolyte should be carefully selected so that moving ionic boundaries do not adversely affect the CE separation [33].

3.3. Optimization of ESI-MS conditions by flow injection analysis (FIA)

In order to increase experiment throughput, a flow injection approach was preferred. Sample zones were injected in triplicate by the CE injector for each set of conditions (see Experimental) and recorded by SIM MS. Peak integration was made by batch processing in the quantification software. The negative ionization of the ANTS reagent, which served as a model compound for derivatized carbohydrates, was investigated concerning sheath-liquid composition and orifice voltage.

The electrolyte component in the sheath-liquid was varied to provide different cationic counter ions to the negatively charged analyte, hydrogen, ammonium, and triethylammonium. The response was measured at three pH values — acidic, neutral and high. The pH of the electrolytes ranging pH 3.5–10.6 was measured before adding the organic solvent (see Experimental). Fig. 3 (left panel) presents the MS trace for the ANTS reagent, using the same MS response scale for the three different electrolytes. The doubly charged ion gave a higher response than the singly charged ion at low and neutral pH, while the singly charged ion showed the largest response using triethylamine (TEA, high pH) as sheath-liquid electrolyte. The response ratio between the doubly to singly charged species ($2^-/1^-$) increased in the order TEA < ammonium acetate (AmAc) < acetic acid–formic acid (BGE) (Table 1). This indicates that the charging process of the negatively charged ANTS reagent (removal of positively charged counter ions) in the electrospray is more efficient with the acetic acid–formic acid additive. In contrast, the co-addition of organic bases such as TEA and imidazole has been shown to improve MS sensitivity and to reduce cation adduction in negative ESI for oligonucleotides [34]. Moreover, triethylammonium ions are believed to dissociate and evaporate during the ESI process, leaving the nucleic acid molecules in the hydrogen form [35].

The abundance of the singly and doubly charged

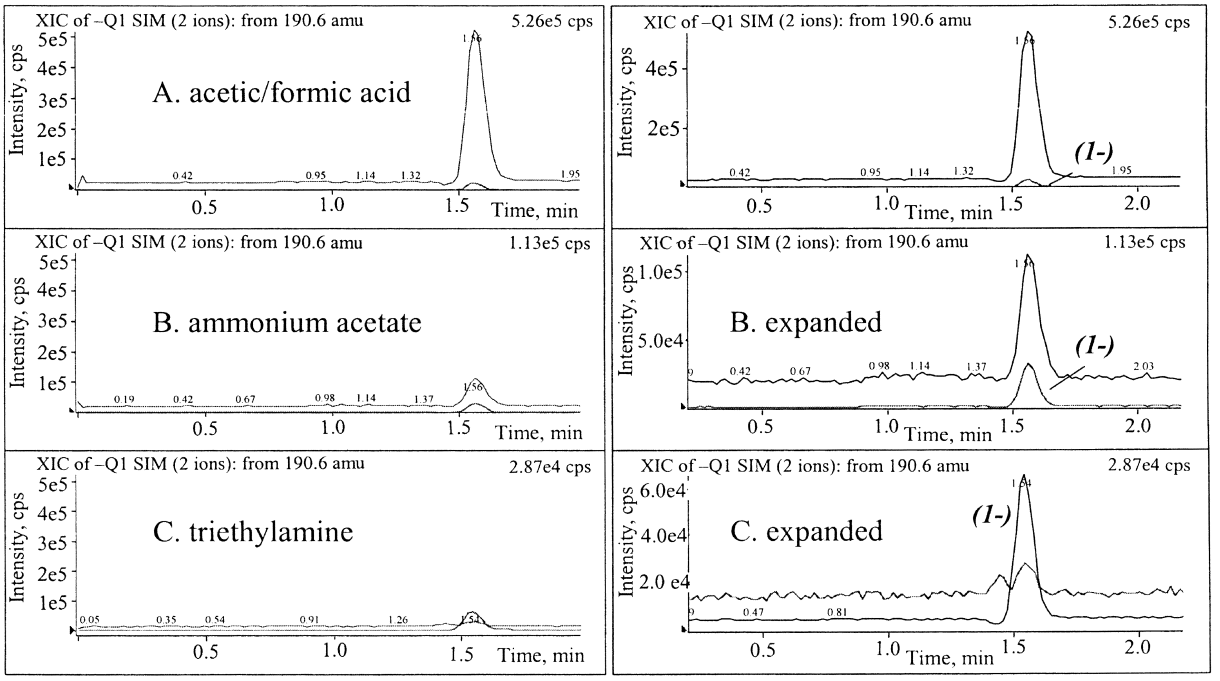

Fig. 3. Sheath-liquid composition investigated by flow injection of ANTS using different electrolyte additives. Sheath-liquid 50% isopropanol, 50% electrolyte additive: (A) 0.5% BGE (acetic acid–formic acid), (B) 2 m*M* ammonium acetate, and (C) 2 m*M* triethylamine. Conditions: flow injection of sample plugs, selected ion monitoring of ANTS in negative ion electrospray MS, m/z 190.6 (doubly charged) and m/z 382.2 (singly charged), orifice voltage −65 V, see text and Experimental for details.

ANTS reagent was evaluated for different sheath-liquid compositions, varying the orifice voltage. The orifice voltage controls the speed of ions when entering the vacuum region and therefore the degree of declustering and fragmentation. Fig. 4 shows the response ratio for doubly/singly charged ANTS reagent, measured at three different orifice voltages for various sheath-liquids. The response ratio $(2^-)/(1^-)$ drops rapidly with a higher orifice voltage (note the logarithmic scale), in all tested sheath-liquid systems. Again, a higher charge ratio is found at low pH (BGE as electrolyte), compared to AmAc and TEA.

Table 1
Ratio between the peak area response for doubly and singly charged ANTS reagent with different electrolyte additives in the sheath-liquid

Electrolyte	Orifice voltage (V)	Response ratio $(2^-/1^-)$
Acetic acid–formic acid	−50	59
Ammonium acetate	−50	6.4
Triethylamine	−50	1.4
Acetic acid–formic acid	−75	9.4
Ammonium acetate	−75	1.6
Triethylamine	−75	0.2

Organic modifier: 50% (v/v) isopropanol. Flow injection–MS with selected ion monitoring, see Experimental.

3.4. Role of organic modifier in sheath-liquid

The response was measured at 50, 80 and 100% organic modifier in the sheath-liquid. Methanol and isopropanol were investigated extensively, while only a few FIA experiments were made for MS response evaluation with acetonitrile. The reason is that acetonitrile causes swelling of the outer polyimide coating on the capillary, which makes the capillary more brittle and affects the CE capillary tip position in the spray assembly.

Fig. 5 shows results for 80% isopropanol (A) and 80% methanol (B) as organic modifiers, at low pH,

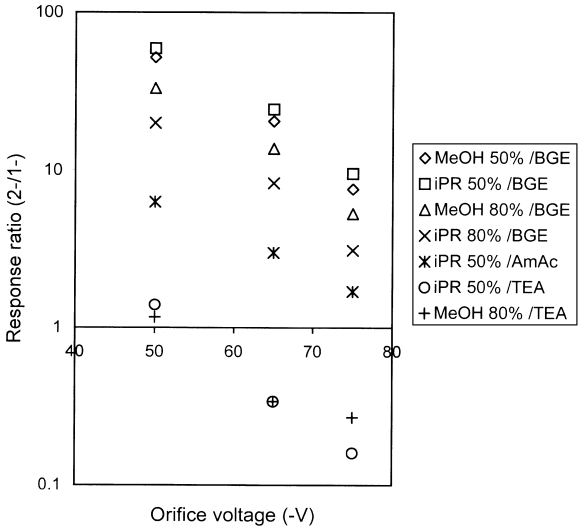

Fig. 4. Response ratio for doubly/singly charged ANTS at different orifice voltage and sheath-liquid composition. Peak area ratio (2−/1−, logarithmic scale) versus negative orifice voltage. Organic modifier: methanol (MeOH) or isopropanol (iPR) (50 or 80% v/v). Electrolyte: 0.5% BGE (acetic acid–formic acid), 2 m*M* ammonium acetate (AmAc), or 2 m*M* triethylamine (TEA) (50 or 20% v/v). Conditions: flow injection of sample plugs, selected ion monitoring of ANTS in negative ion electrospray MS, m/z 190.6 (doubly charged) and m/z 382.2 (singly charged).

and orifice voltage −65 V. The ANTS (2−) ion dominates over the singly charged ion. Isopropanol shows a 6-fold higher response than methanol in spite of the fact that the response for 80% isopropanol exceeded the linear range of the MS detector in this experiment. The advantage of isopropanol was most pronounced for the low-pH sheath-liquids. Furthermore, the response was higher at 80–100% organic modifier compared to 50% organic content, with a 2- to 5-fold response gain for isopropanol. Although attractive for the high sensitivity, a high organic content gave a less stable CE–MS system, see below.

3.5. CE separation and MS analysis

To confirm that the optimization made by flow injection experiments with the ANTS reagent is relevant for the on-line CE–MS analysis of derivatized oligosaccharides, various sheath-liquid electrolytes were examined by CE–MS. Unmanipulated spectra are shown in Fig. 6A–C. With TEA as additive at high pH, the derivatives were mainly detected as the singly charged molecular ion (Fig. 6A). The number of charges on the ANTS deriva-

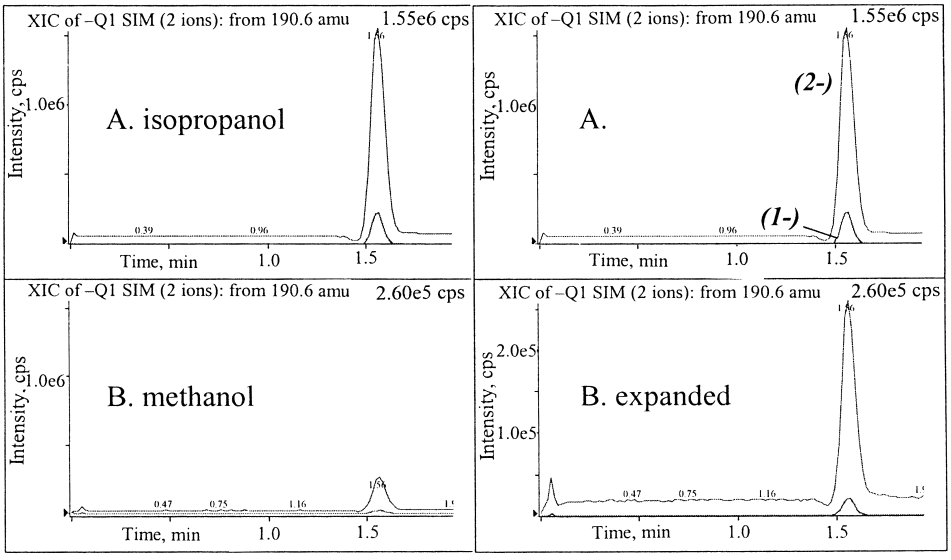

Fig. 5. Sheath-liquid composition investigated by flow injection of ANTS using different organic additives. Sheath-liquid electrolyte (20% v/v) 0.5% BGE acetic acid–formic acid, organic additive (80% v/v) (A) isopropanol, and (B) methanol. SIM of ANTS in negative ion electrospray MS, m/z 190.6 (doubly charged) and m/z 382.2 (singly charged), orifice voltage −65 V.

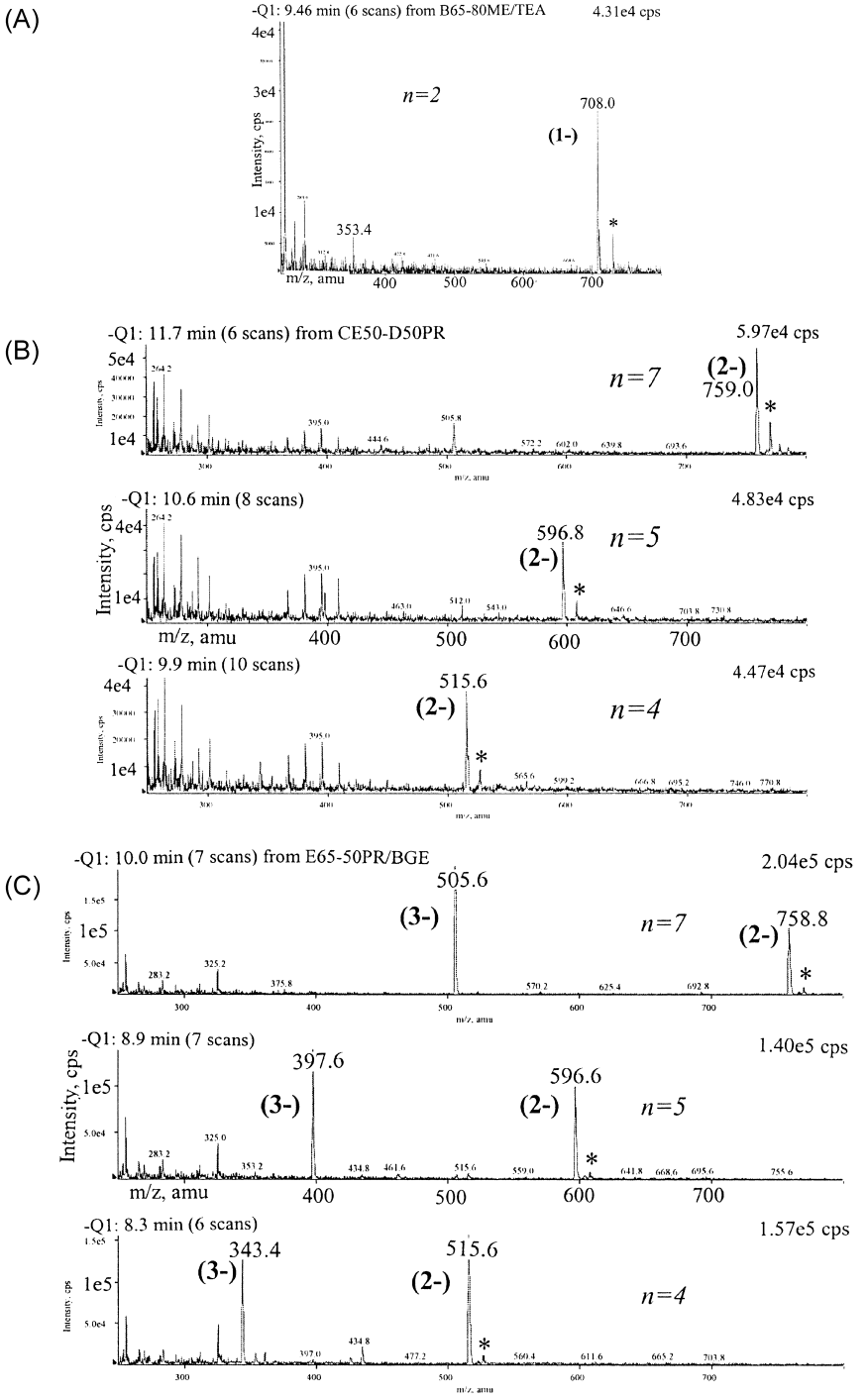

Fig. 6. Mass spectra from CE–MS separations of ANTS derivatized Dextrin-15 oligosaccharides using different sheath-liquid electrolytes. Unmanipulated data. The charge state is indicated for each molecular ion $(M-H)^-$, $(M-2H)^{2-}$ and $(M-3H)^{3-}$, and the number of glucose residues in the molecule is indicated in each spectrum. *, Sodium containing ion $(M+Na-2H)^-$ or $(M+Na-3H)^{2-}$. Electrolyte: (A) 2 m*M* triethylamine, (B) 2 m*M* ammonium acetate, (C) 0.5% BGE, acetic acid–formic acid. Organic additive: 80% (v/v) methanol in (A), 50% (v/v) isopropanol in (B) and (C). Orifice voltage: −65 V in (A) and (C), −50 V in (B). See Experimental for CE–MS details.

tives is higher at neutral pH with ammonium acetate where the doubly charged ion is abundant (Fig. 6B). A spectrum of yet higher quality with abundant doubly and triply charged ions is obtained at a low pH with acetic acid–formic acid as sheath-liquid electrolyte (Fig. 6C). Less intense sodium containing molecular ions are visible in all spectra in Fig. 6, the smallest relative intensity is found for acetic acid–formic acid. In summary, the low-pH conditions favor derivative ionization, in correspondence with the FIA experiments using the ANTS reagent as model compound. Interestingly, the same BGE and sheath-liquid system was successfully used for the CE–MS analysis of peptides, including the isotachophoretic focusing of large-volume injections [36].

Extrapolating from FIA experimental results, an improved response was expected with high (80–100%) organic content in the sheath-liquid. However, the CE current was unstable at high organic content. As a high stability is crucial for reliable automated operation of CE–MS, 50% organic content was preferred for CE–MS. It was found that the positioning of the fused-silica capillary for 50% organic content was not optimal for the more volatile 80–100% organic content conditions. The best results were obtained when the CE capillary tip was pulled further inside the sheath-liquid capillary. A possible explanation is that a longer void at the capillary end more easily retains the volatile sheath-liquid, thereby improving the electrical contact between the CE electrolyte and the sheath-liquid.

Fig. 7 describes an electropherogram of a mixture of ANTS-derivatized oligosaccharides from maize, Dextrin 15, at optimized CE–MS conditions. Experiments were carried out using a sheath-liquid consisting of the volatile CE BGE (acetic acid–formic acid) and isopropanol. In the mass range used (m/z 250–800), the largest observed oligomer derivative is a glucose homo-dodecamer with a derivative molecular mass of 2329.3, at 12.1 min migration time. This oligomer is detected as the triply charged ion at m/z 775.8 (calculated m/z 775.4). It is expected that higher oligomers can be ionized and detected in a higher m/z range. The oligomers are readily separated from each other, however, peak-broadening is observed in the total ion current (TIC) trace because of incomplete separation of other components, see peaks for oligomers $n=6$, 11 and 12. The measured number of theoretical plates for the ANTS derivative of the heptamer is $n=21\,500$. The peak width is about 17 s (at 4 σ) and 10 scans are acquired over the peak. Peak widths are smaller than those earlier reported for on-line CE–MS of ANTS derivatives, where spectra were collected for 0.5–1.3 min over the peak [28].

In CE–MS operation, volatile BGEs are generally preferred, since contamination of the MS interface

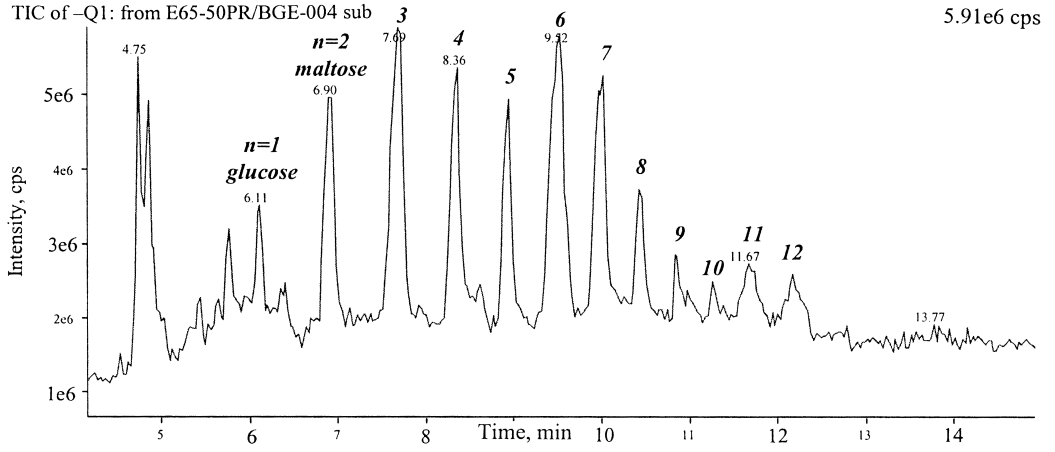

Fig. 7. Total ion current trace from a CE–MS separation of ANTS-derivatized oligosaccharides from Dextrin 15 using a low-pH electrolyte in the sheath-liquid. $n=1$–12 denote the degree of polymerization of the oligosaccharide analogues. Background subtracted unsmoothed data. Sheath-liquid: isopropanol–0.5% BGE acetic acid–formic acid (1:1, v/v) at 5 μl/min. Negative ion electrospray MS at −3800 V, MS scan m/z 250–800, step size 0.2, 1.7 s/scan. CE conditions: −26.2 V over the capillary, polyacrylamide coated capillary, 70 cm.

may adversely affect the MS response. Moreover, BGE components compete with the analytes in the ionization process and therefore may suppress the analyte response. Nevertheless, an attempt to use phosphate as BGE in CE–MS was made, since high separation efficiency in CE–UV has been reported for ANTS derivatives using phosphate–triethylamine pH 2.5 as BGE [9]. In our experiment, the sheath-liquid was isopropanol (50%), and 2 m*M* TEA was added to provide counter ions for the CE separation. However, no derivatives were detected in the total ion or in the extracted ion electropherograms, because of the elevated background and ion suppression from phosphoric acid [$(H_3PO_4)_n\ H_2PO_4^-$] (data not shown).

A general observation in the CE–MS study in the scanning mode was that both the analyte response and the background levels affect the peak visibility in the TIC trace. The background level depends on sheath-liquid composition, and it was lower at 80–100% organic content. As usually observed, the electrospray background level decreased at higher orifice voltage. For example, no carbohydrate peaks were discernible in the CE–MS total ion current electropherogram at orifice voltage −50 V (compare with the CE–MS separation at orifice voltage −65 V, Fig. 7). However, extraction of the ion trace for the analytes from the spectral data showed that the analyte response was as high as at −65 V. The limiting factor for detection of unknown components in such CE–MS systems is therefore the MS background level, rather than the ionization efficiency in the electrospray process. Thus, the implementation of mathematical methods for extraction of relevant data from a spectral background in ESI-MS [37,38] would, in practice, improve CE–MS detection limits.

4. Conclusions and perspectives

This paper demonstrates the on-line application of CE–MS to the peak characterization in the CE analysis of complex carbohydrates, such as the ANTS derivatized maize starch oligosaccharides. The most appealing configuration was a low-pH system with a volatile CE electrolyte and a sheath-liquid with 50% isopropanol as organic modifier. Adjustments to optimize the electrospray assembly, position, and conditions resulted in a robust CE–MS system that allowed unattended operation. High sensitivity detection was achieved in the ESI-MS negative ion mode by careful selection of sheath-liquid composition. The conditions were optimized by flow injection experiments. The response and the charge state distribution of the derivatives were highly affected by the sheath-liquid composition. A shift to higher charge states was observed for the reagent and ANTS derivatized maltodextrins at low pH. In addition, lower orifice voltage favored a higher degree of charging.

The high quality CE–MS spectra provide on-line molecular mass information of the carbohydrate derivatives. The separated components can potentially be further characterized by MS–MS. For optimum MS analysis of faster CE separations (few seconds' peak width) and/or a large mass range, a TOF mass analyzer would be preferred.

Acknowledgements

The laboratory-assembled CE–MS interface was built with the skillful support of Olle Jacobson. The authors gratefully acknowledge Dr. Aharon S. Cohen for valuable comments on the manuscript.

References

[1] P. Van den Steen, P.M. Rudd, R.A. Dwek, G. Opdenakker, Crit. Rev. Biochem. Mol. Biol. 33 (1998) 151.
[2] B. Imperiali, S.E. O'Connor, Curr. Opin. Chem. Biol. 3 (1999) 643.
[3] M.V. Novotny, J. Sudor, Electrophoresis 14 (1993) 373.
[4] M. Stefansson, M. Novotny, Anal. Chem. 66 (1994) 1134.
[5] Z. El Rassi, Electrophoresis 20 (1999) 3134.
[6] A. Paulus, A. Klockow, J. Chromatogr. A 720 (1996) 353.
[7] J. Liu, O. Shirota, D. Wiesler, M. Novotny, Proc. Natl. Acad. Sci. USA 88 (1991) 2302.
[8] M. Stefansson, M. Novotny, Carbohydr. Res. 258 (1994) 1.
[9] C. Chiesa, Cs. Horvath, J. Chromatogr. 645 (1993) 337.
[10] A. Klockow, R. Amado, H.M. Widmer, A. Paulus, J. Chromatogr. A 716 (1995) 241.
[11] I. Rethfeld, G. Blaschke, J. Chromatogr. B 700 (1997) 249.
[12] F.T. Chen, R.A. Evangelista, Electrophoresis 19 (1998) 2639.
[13] A.L. Burlingame, R.K. Boyd, S.J. Gaskell, Anal. Chem. 66 (1994) 634R.

[14] D.I. Papac, A. Wong, A.J.S. Jones, Anal. Chem. 68 (1996) 3215.
[15] H. Suzuki, O. Muller, A. Guttman, B.L. Karger, Anal. Chem. 69 (1997) 4554.
[16] B. Stahl, M. Steup, M. Karas, F. Hillenkamp, Anal. Chem. 63 (1991) 1463.
[17] A.L. Burlingame, Curr. Opin. Biotechnol. 7 (1996) 4.
[18] W.J. Mo, H. Sakamoto, A. Nishikawa, N. Kagi, J.I. Langridge, Y. Shimonishi, T. Takao, Anal. Chem. 71 (1999) 4100.
[19] M. Okamoto, K. Takahashi, T. Doi, Rapid Commun. Mass Spectrom. 9 (1995) 641.
[20] T. Kazmaier, S. Roth, J. Zapp, M. Harding, R. Kuhn, Fresenius J. Anal. Chem. 361 (1998) 473.
[21] J. Cai, J. Henion, J. Chromatogr. A 703 (1995) 667.
[22] J. Ding, P. Vouros, Anal. Chem. 71 (1999) 378A.
[23] G.K. Poon, in: R.B. Cole (Ed.), Electrospray Ionization Mass Spectrometry, Wiley, New York, 1997, p. 499.
[24] J.C. Severs, R.D. Smith, in: R.B. Cole (Ed.), Electrospray Ionization Mass Spectrometry, Wiley, New York, 1997, p. 343.
[25] B. Monsarrat, T. Brando, P. Condouret, J. Nigou, G. Puzo, Glycobiology 9 (1999) 335.
[26] S. Duteil, P. Gareil, S. Girault, A. Mallet, C. Feve, L. Siret, Rapid Commun. Mass Spectrom. 13 (1999) 1889.
[27] S. Auriola, P. Thibault, I. Sadovskaya, E. Altman, Electrophoresis 19 (1998) 2665.
[28] F.Y. Che, J.F. Song, R. Zeng, K.Y. Wang, Q.C. Xia, J. Chromatogr. A 858 (1999) 229.
[29] J. Charlwood, H. Birrell, A. Gribble, V. Burdes, D. Tolson, P. Camilleri, Anal. Chem. 72 (2000) 1453.
[30] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[31] R.D. Smith, J.A. Olivares, N.T. Nguyen, H.R. Udseth, Anal. Chem. 60 (1988) 436.
[32] P. Jackson, Biochem. J. 270 (1990) 705.
[33] F. Foret, T.J. Thompson, P. Vouros, B.L. Karger, P. Gebauer, P. Bocek, Anal. Chem. 66 (1994) 4450.
[34] M. Greig, R.H. Griffey, Rapid Commun. Mass Spectrom. 9 (1995) 97.
[35] C.G. Huber, M.R. Buchmeiser, Anal. Chem. 70 (1998) 5288.
[36] M. Larsson, E.S.M. Lutz, Electrophoresis 21 (2000) 2859.
[37] C.M. Fleming, B.R. Kowalski, A. Apffel, W.S. Hancock, J. Chromatogr. A 849 (1999) 71.
[38] J. Vogels, E. Verheij, R. Vreeken, A. Tas, in: 15th Montreux Symposium on Liquid Chromatography/Mass Spectrometry, Montreux, Switzerland, 1998, p. Poster 110.