Anal Bioanal Chem (2005) 381: 1401–1407
DOI 10.1007/s00216-005-3107-6

ORIGINAL PAPER

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# Quantitative determination of cationic modified polysaccharides on hair using LC–MS and LC–MS–MS

Received: 4 August 2004 / Revised: 12 January 2005 / Accepted: 16 January 2005 / Published online: 22 March 2005
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**Abstract** Cationic polysaccharides containing *N*-hydroxypropyl-*N*,*N*,*N*-trimethylammonium substituents are widely used as conditioning agents for hair-care products. A sensitive method has been developed for the quantitation of these polymers. After acidic extraction from hair the polysaccharides are hydrolyzed using trifluoroacetic acid. The cationic monoglycosides are determined using liquid chromatography–tandem mass spectrometry (LC–MS–MS). The developed method is independent of hair treatment. Even hair cut from test persons after customary hair wash can be analyzed. After treatment of natural and bleached hair tresses using a real-life treatment procedure 180 µg and 300 µg of polymer per gram hair were quantified, respectively. Additionally the fragmentation mechanism of the cationic *N*-hydroxypropyl-*N*,*N*,*N*-trimethylammonium group during electrospray ionization was investigated. A mass loss of 60 Da in combination with loss of a single charge is observed and associated with cleavage of trimethylamine and a proton. It is assumed that this process is promoted by the anionic counter-ion which might be hydroxide in an aqueous environment.

**Keywords** Cationic polysaccharides · Conditioning polymers · Hair · Quaternary ammonium · Cone-voltage fragmentation

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## Introduction

Cationic polymers based on natural polysaccharides are widely used as conditioning agents in cosmetic hair care products [1]. Major agents are Polyquaternium-10 and Guar hydroxypropyltrimonium chloride [2].

Polyquaternium-10 is synthesized from hydroxyethylcellulose by derivatization with *N*-(2,3-epoxypropyl)-*N*,*N*,*N*-trimethylammonium chloride [3]. The simplified generic chemical structures are shown in Fig. 1. Wilke and Mischnick showed that derivatization occurs not only at the sterically easily accessible hydroxyl group on the carbon at the 6-position but also mainly at the 2 and 3-positions [3, 4].

Guar hydroxypropyltrimonium chloride is based on guar gum, a natural polymer with a mannose backbone and several galactose side chains. The ratio between mannose and galactose was determined to be 2 [5]. Derivatization with *N*-(2,3-epoxypropyl)-*N*,*N*,*N*-trimethylammonium chloride gives the cationic polymer [6].

With an average degree of substitution of 0.7 or less, both polymers consist of underivatized and monocationized monomers only [4]. Because hair is composed mainly of keratin, the variety of natural amino acids results in a multitude of different functional groups. The additional carboxyl groups of aspartic acid and glutamic acid and the amino groups of arginine are responsible for the amphoteric character of the hair, which has an isoelectric point of pH 3.67. This means that in a neutral environment hair is charged negatively and cationic polymers can bind strongly to it by formation of ionic bonds. These bonds are strong enough to hold the polymer on the hair surface even when it is rinsed thoroughly. This behavior is named substantivity. The polymer forms a film resulting in a smoothing of the hair surface [7]. Less abrasion of the cuticle occurs and the hair is less vulnerable to splitting [8]. Furthermore, the polymer film reduces the friction at the hair surface resulting in a decrease of combing forces and hence

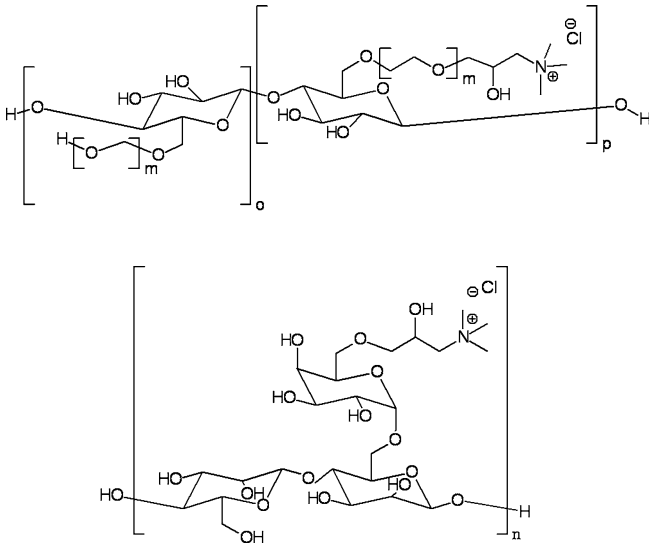


**Fig. 1** Simplified generic chemical structure of Polyquaternium-10 (*top*) and Guar hydroxypropyltrimonium chloride (*bottom*)

improvement of combability [9]. In addition body, stability, texture and curl retention of the treated hair are improved [2, 9].

Evidence for these effects can be obtained with test methods comparing hair tresses treated with formulations containing cationic polymers with others treated with a placebo formulation. Furthermore, several physical properties, for example combing force [10], zeta potential [11], and gloss [2] can be determined to obtain information about hair status. To characterize the efficacy of the whole cosmetic formulation, sensory tests performed by experts on proband hairs are of particular importance [12].

Each of these test methods enables determination of the efficacy of the cosmetic formulation as a whole. To obtain fundamental insights into the underlying mechanisms test results specifically for cationic polymers are needed. This requires quantitation of the cationic polymers remaining on hair after treatment.

In the past, several methods have been established for quantitation of the polymers on hair. A differential method comparing the polymer content of a solution before and after immersion of a hair tress has frequently been used. Quantitation was performed using surfactant titration or polyelectrolyte titration [11, 13, 14]. With this method the treatment conditions are very different from those of real-life usage. Although information about interactions of the polymer with the hair surface can be obtained, no conclusions relating to normal usage of the cosmetic formulation can be drawn from these findings.

X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), provides deep insight into the surface solidity ratio of hair [15, 16]. Because information on the thickness of the polymer layer is not accessible with this method, quantitation is not possible.

Occasionally fluorescence spectroscopy is used for the quantitative determination of cationic polymers on hair [17, 18]. Because of the absence of fluorescent groups in the polymer, it has to be derivatized. This may result in a different substantivity of the polymer, impeding characterization of real-life performance.

The method described in this work consists of an extraction step to dissolve the polymer and a hydrolysis step yielding monomers which can be quantified using liquid chromatography–mass spectrometry(LC–MS). The big advantage of this method is that it is independent of the type of hair treatment, thus enabling investigations close to reality.

## Experimental procedures

### Apparatus and reagents

Chromatographic separation was performed on an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) using a micro bore column (60 mm×2 mm) packed with a GromSil Diol stationary phase (Grom Analytik & HPLC, Herrenberg, Germany). Detection was carried out with a Micromass Quattro Micro triple quadrupole mass spectrometer (Micromass, Manchester, UK) and a Bruker Esquire-LC ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany).

Methanol, *tert*-butyl methyl ether, trifluoroacetic acid, and Triton X-100 were purchased from Merck (Darmstadt, Germany) in p.a. grade. Water was taken from a Milli-Q Gradient A 10 system (Millipore, Schwalbach, Germany). Cationic hydroxyethylcellulose (Polyquaternium-10) with an average degree of substitution of 0.1 was kindly supplied by Amerchol (Vilvoorde, Belgium). Guar hydroxypropyltrimonium chloride with an average degree of substitution of 0.1 was provided by Rhodia (Frankfurt, Germany). Cationic oligosaccharides with a chain length of seven monomers and an average degree of substitution of 0.5 were supplied by Cyclolab (Budapest, Hungary). Undamaged and bleached hair tresses were purchased from Kerling International (Backnang, Germany).

### Hair sampling

Commercial hair tresses (width 2 cm; length 23 cm; weight 2 g) were used for systematic investigations. The tresses were treated with a shampoo formulation containing 0.3% Polyquaternium-10 and 0.1% Guar hydroxypropyltrimonium chloride using a standardized washing procedure consisting of a rinsing step with pure water to condition the hair followed by shampooing with the cosmetic formulation. After this treatment the hair was rinsed thoroughly and then dried in a conditioning chamber at defined air humidity. All relevant conditions, for example rinsing time, water temperature and shampoo load were held constant.

For determination of the reproducibility of this treatment procedure 50 hair tresses were treated consecutively and then the cationic polymers were quantified. In addition to statistical fluctuation of the treatment, the inhomogeneity of the hair material was taken into account, resulting in an overall error for the tested hair tresses. It was shown that this experimental error of 10% (relative standard deviation, $n = 5$) agreed with the value for the inhomogeneity of hair material found by Busch [19].

## Extraction

In analytical determination of drug abuse it is common to quantify substances in hair by acidic or alkaline degradation of the hair matrix [20]. Because of the high matrix load of the hydrolysate a time-consuming purification step is necessary. In contrast, extraction from the hair surface significantly reduces the matrix load without reducing the amount of analyte, because the polymer does not diffuse into the hair but remains mainly at the surface [21]. Reducing the pH below the isoelectric point of keratin results in breaking of the ionic bonds. Now cationic polymers behave like neutral polymers and can be separated easily from hair [13]. The acid used must also act as the hydrolysis agent. It should be strong enough to break glycosidic bonds but weak enough to leave intact the ether bonds between the glycoside and the cationic group. This is possible with trifluoroacetic acid [22, 23]. To improve the extraction step a surfactant dilution containing 1% Triton X-100 was used; this non-ionic detergent is often employed to promote extraction processes [24]. Unfortunately, addition of hard bases like trifluoroacetate to a dilution containing surfactants based on polyethylene glycol like Triton X-100 results in a lowering of the cloud point of the surfactant [25]. At an acid concentration above 0.5 mol $L^{-1}$ the cloud point is reached at ambient temperature, and phase separation can be observed. To achieve maximum acidity without phase separation, extraction was performed with a solution of 1% Triton X-100 containing 0.5 mol $L^{-1}$ trifluoroacetic acid. The determined recovery ratio of 92% showed that ultrasonic extraction for 40 min is exhaustive.

## Hydrolysis and chromatography

In accordance with the extraction procedure the acid concentration is adjusted to 2 mol $L^{-1}$ and hydrolysis is performed at 100°C for 20 h. These optimized conditions gave a maximum yield of monomers, whereas with shorter hydrolysis times dimers will be present and with longer times the glycosides will be slowly dehydrated (see Fig. 2).

After decomposition of the polymer, the surfactant-rich phase of the hydrolysate was separated and discarded. Because less polar compounds were enriched in this phase and the highly polar cationic monomers left in the trifluoroacetic acid phase, a purification step was performed with a kind of reversed-cloud-point extraction [26].

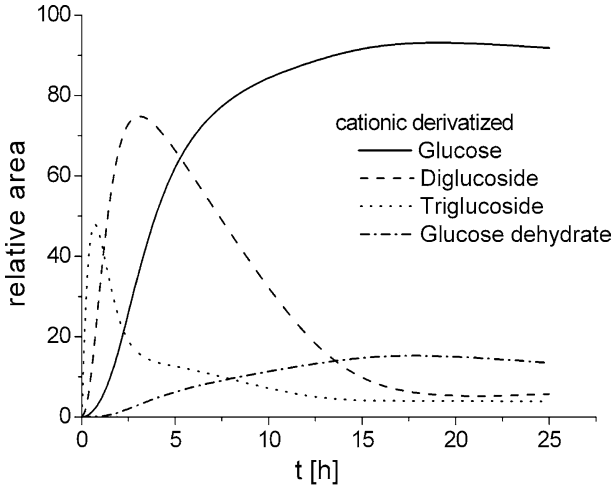



**Fig. 2** Dependence of signal areas from the products on hydrolysis time

After separating and discarding the surfactant, the hydrolysate was dried under a nitrogen stream to remove the trifluoroacetic acid which otherwise interferes with mass spectrometric detection. The dry residue was dissolved in methanol followed by chromatographic separation.

Because of the cationic charge it is not possible to retard the monomers under reversed-phase conditions. With the apolar mobile phases used in normal phase mode, no or little ionization occurs during electrospray. Accordingly, normal phase separation was performed on a commercial diol stationary phase using a mobile-phase gradient prepared from mixtures of *tert*-butyl methyl ether, methanol, and water. To reduce the interactions between the cationic group and free silanol of the stationary phase, formic acid was added. The composition of the mobile phase is shown in Table 1.

**Table 1** Mobile phase composition used for HPLC analysis on GromSil Diol as the stationary phase

| Time (min) | Methanol[a] (%) | Water[a] (%) | *tert*-Butyl methyl ether (%) | Flow rate (mL $min^{-1}$) |
|---|---|---|---|---|
| 0.0 | 60 | 40 | 0 | 0.3 |
| 1.5 | 100 | 0 | 0 | 0.3 |
| 4.0 | 50 | 0 | 50 | 0.3 |
| 7.0 | 50 | 0 | 50 | 0.3 |
| 9.0 | 100 | 0 | 0 | 0.4 |
| 10.0 | 60 | 40 | 0 | 0.5 |
| 14.0 | 60 | 40 | 0 | 0.5 |
| 14.1 | 60 | 40 | 0 | 0.3 |
| 14.5 | Stop time | | | |

[a]Containing 0.5% formic acid

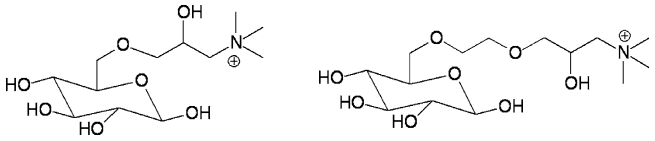


**Fig. 3** Simplified generic structure of the non-ethoxylated (*left*) and monoethoxylated (*right*) cationic monomers

## Mass spectrometric quantitation

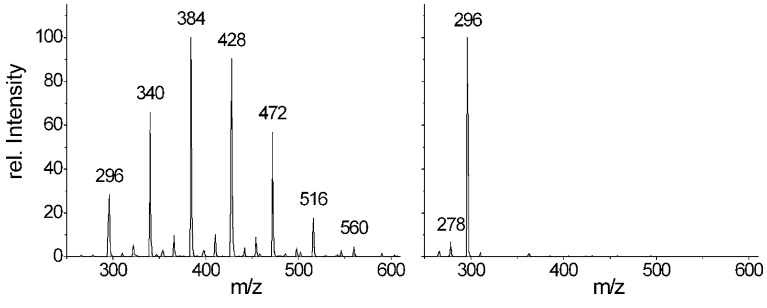
The mass spectrometric signals from non-ethoxylated and monoethoxylated cationic monomers (*m*/*z* 296 and 340, Fig. 3) were detected because the mass spectrometric sensitivity is much higher than with the underivatized monomers. This is shown by a 100-fold higher response factor. Also, the cationic monomers are characteristic for cationic polysaccharides, resulting in a high selectivity. Uncharged polysaccharides, which are widely used as thickeners, do not interfere with this analysis. The mono-ethoxylated glycoside is specific for Polyquaternium-10 because Guar hydroxypropyltrimonium chloride contains no ethylene glycol groups. In contrast, the signal of the non-ethoxylated species can come from both polymers (Fig. 4). Therefore, quantitation of the Polyquaternium-10 was based on the signal at *m*/*z* 340. The Guar hydroxypropyltrimonium chloride content was obtained from the signal at *m*/*z* 296 after subtraction of the amount resulting from Polyquaternium-10. It is supposed that this method of quantitation has a larger statistical error than common external calibration methods because signal variations of two measurements affect the results. Therefore the error originating from the inhomogeneity of hair material should be negligible, because both values are measured on the same hair sample. The statistical error in the quantitation of Guar hydroxypropyltrimonium chloride from one strand (relative standard deviation 8%, $n = 5$) is, in fact, comparable with that for Polyquaternium-10. If the five samples originate from different strands the relative standard deviation for quantitation of Polyquaternium-10 increases to 11% ($n = 5$) whereas that for Guar hydroxypropyltrimonium chloride does not change.

**Fig. 4** Mass spectra of Polyquaternium-10 (*left*) and Guar hydroxypropyltrimonium chloride (*right*) hydrolysate dilutions. Only signals from cationized monomers are visible. The Polyquaternium-10 hydrolysate contains several monomers differing in the degree of ethoxylation (mass difference 44 Da). In the Guar HPTMA hydrolysate spectrum only the non–ethoxylated monomer (*m*/*z* 296) is present

## Results and discussion

During electrospray ionization, fragmentation of *N*-(2,3-epoxypropyl)-*N*,*N*,*N*-trimethylammonium chloride-derivatized glycosides was observed, resulting in a mass loss of 60 Da. To investigate the fragmentation mechanism oligosaccharides with a chain length of seven monomer units and an average degree of substitution of 0.5 were used. Unlike in the polymer hydrolysate, multiply charged molecules are present. If fragmentation at the cationic group occurs, the residues of these multiply charged species would likewise be charged. Therefore their signals would appear in the mass spectrum, whereas uncharged residues are suppressed by the cationic oligomers. The mass spectrum of a methanolic solution (Fig. 5) shows signals of the monocationized oligosaccharide (*m*/*z* 1,250), the dicationized doubly charged oligosaccharide (*m*/*z* 683), the three times derivatized oligomer in a triply charged state (*m*/*z* 484), the four times etherified derivative in a fourfold charged state (*m*/*z* 399) and the five times derivatized species carrying five charges (*m*/*z* 343). All of these signals have satellite peaks with mass differences of several times 56 Da appearing at intervals of 56 Da divided by the charge state of the species. Accordingly the singly charged monocationized oligosaccharide has an additional signal pattern with differences of *m*/*z* 56, whereas for the doubly charged twofold etherified species a pattern with differences of *m*/*z* 28 exists.

Chromatographic investigations indicated that the signals at *m*/*z* 683 and 1,306 (1250 + 56 Da) in Fig. 5 originate from the same species. This was supported by the occurrence of the mass peak at *m*/*z* 1,306 in the fragment spectrum of the signal at *m*/*z* 683 (Fig. 6, performed in the MS–MS mode on a Quattro Micro triple quadrupole mass spectrometer). The peaks at *m*/*z* 494, 711 (683 + 56/2 Da) and 1362 (1,250 + 2×56 Da) in Fig. 5 also seemed to have the same source. On he basis of these results, a fragmentation model was proposed in which the additional peaks are fragments formed during the ESI process. It was assumed that in solution multiply cationized oligomers occur both as totally free multiply charged ions and as partial salts



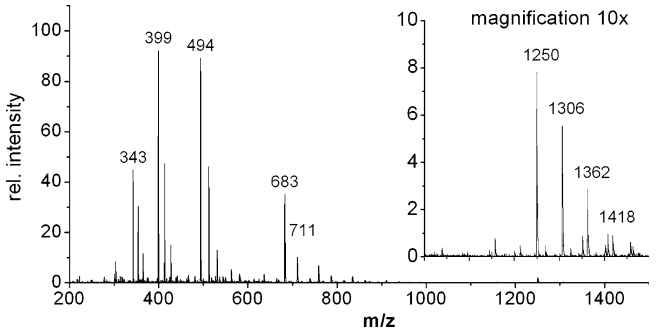



**Fig. 5** Mass spectrum of cationized oligosaccharide (degree of oligomerization 7)

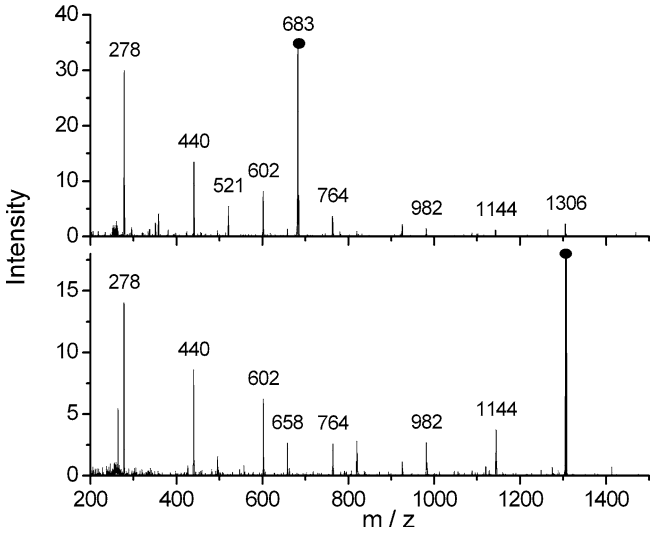



**Fig. 6** Fragment spectra of the signals at *m/z* 683 (*top*) and *m/z* 1306 (*bottom*) from the positive-ion-mode ESI mass spectrum of the cationic oligosaccharide recorded with a triple-quadrupole mass spectrometer

with available anions, usually hydroxide ions. During the electrospray process the cationic group, which is paired with the hydroxide ion, splits off trimethylamine and a proton, which forms water with the anion. Consequently, a mass loss of 60 Da and a charge reduction occurs. The interpretations of all signals observed in Fig. 5 are shown in Table 2. The influence of the hydroxide anion can be clarified by replacing it with other anions, for example formate or toluenesulfonate. Displacement with the formate anion results in reduction of fragment signals; displacement with the toluenesulfonate anion results in suppression of the signals (Fig. 7). The reduction efficacy is supposed to be dependent on the ability to form ion pairs with the quaternary ammonium group of the analyte [27, 28]. Unfortunately, ion pairing results in weaker signal intensities and hence poor sensitivity [29].

Because the anionic modifier may promote fragmentation, it is important for quantitation to have almost the same matrix in the calibration standard and in the sample. Hence the same sample preparation procedure has to be used for the standard dilutions and for the extraction dilutions.

Collision-induced fragmentation (CID) of the cationic monoglycosides was performed using a triple quadrupole mass spectrometer and an ion-trap mass spectrometer (Fig. 8). The fragment spectra differ significantly. Whereas dissociation inside the ion trap is characterized by successive degradation of the glycosidic carbon backbone, fragmentation in a triple quadrupole mass spectrometer gives the trimethylammonium ion (*m/z* 60) as the major fragment. Additional fragments appear in the low-mass area with mass differences of 14, 16, and 18 Da, suggesting further fragmentation of the glycoside after separation of the cationic amino group.

These fragmentation results can be ascribed, on the one hand, to the lower sensitivity of the ion trap at lower masses and, on the other hand, to the significantly greater fragmentation occurring inside the triple-quadrupole mass spectrometer. The latter was confirmed by higher intensities of high-mass fragments in the ion-trap mass spectrum than in the triple-quadrupole mass spectrum, although more complete decomposition of the

**Table 2** Mass spectrometric signals obtained from oligosaccharides substituted with hydroxypropyl trimethylammonium chloride (HPTMA)

| Compound | Mass (Da) | Loss of | *m/z* $M^{5+}$ | *m/z* $M^{4+}$ | *m/z* $M^{3+}$ | *m/z* $M^{2+}$ | *m/z* $M^{+}$ |
|---|---|---|---|---|---|---|---|
| $(Glucose)_7$-HPTMA | 1,250 | – | | | | | 1,250 |
| $(Glucose)_7$-HPTMA$_2$ | 1,366 | – | | | | 683 | |
| | | $N(CH_3)_3 + H^+$ (60 Da) | | | | | 1,306 |
| $(Glucose)_7$-HPTMA$_3$ | 1,482 | – | | | 494.3 | | |
| | | $N(CH_3)_3 + H^+$ (60 Da) | | | | 711 | |
| | | $2N(CH_3)_3 + 2H^+$ (120 Da) | | | | | 1,362 |
| $(Glucose)_7$-HPTMA$_4$ | 1,598 | – | | 399.5 | | | |
| | | $N(CH_3)_3 + H^+$ (60 Da) | | | 512.7 | | |
| | | $2N(CH_3)_3 + 2H^+$ (120 Da) | | | | 739 | |
| | | $3N(CH_3)_3 + 3H^+$ (180 Da) | | | | | 1,418 |
| $(Glucose)_7$-HPTMA$_5$ | 1,714 | – | 342.8 | | | | |
| | | $N(CH_3)_3 + H^+$ (60 Da) | | 413.5 | | | |
| | | $2N(CH_3)_3 + 2H^+$ (120 Da) | | | 531.3 | | |
| | | $3N(CH_3)_3 + 3H^+$ (180 Da) | | | | 767 | |
| | | $4N(CH_3)_3 + 4H^+$ (180 Da) | | | | | 1,474 |

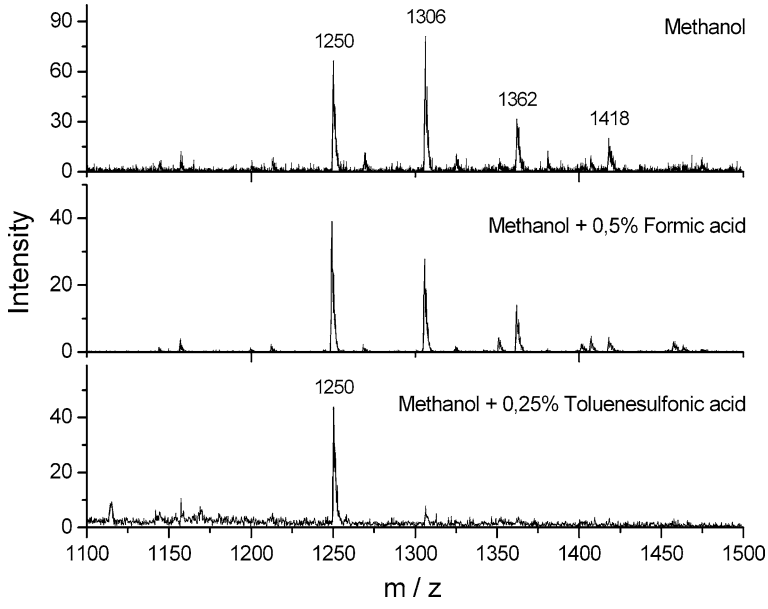



**Fig. 7** Fragment pattern of cationized oligosaccharides formed during electrospray ionization with different modifiers. Use of toluenesulfonic acid suppresses fragmentation (*bottom*)

precursor ion is indicated by the lower intensity of $m/z$ 296.

On the basis of these fragmentation experiments a tandem MS detection method was developed for the triple-quadrupole mass spectrometer using transition of the cationic monomer to the trimethylammonium ion. The limit of quantitation was 5.4 µg Polyquaternium-10 ($S/N$ ratio 10:1). For an initial hair weight of 0.5 g this would give a value of 10.8 µg $g^{-1}$ hair for the limit of quantitation. Because cationic monomers only were considered, this value depends on the degree of substitution of the polymer. Assuming an average degree of substitution of 0.1 and a solvent volume of 10 mL the determinable monomer concentration would be about 54 µg $L^{-1}$. In reality this value is even lower, because of the difference of the monomer mass of the Polyquaternium-10 with regard to the degree of ethoxylation.

This method was used to analyze undamaged and bleached hair tresses treated according to a standardized washing procedure. The undamaged hair contained 150 µg Polyquaternium-10 and 180 µg Guar hydroxypropyltrimonium chloride $g^{-1}$ hair. The bleached hair contents were determined to be 300 µg and 180 µg $g^{-1}$ hair, respectively. These values are significantly lower than those found in the literature. Jachowicz reported contents of up to 3 mg $g^{-1}$ hair [30] and Blanco and coworkers described values of up to 2.5 mg $g^{-1}$ hair [31] after immersing the hair in a pure polymer solution for a period of 30 min. However, conditions were optimized for high polymer content. In reality, hair is treated with a surfactant-rich shampoo-dilution for a maximum of 1 min, and consequently the achievable polymer content is lower. As far as we are aware this is the first time real-life investigations have been described.

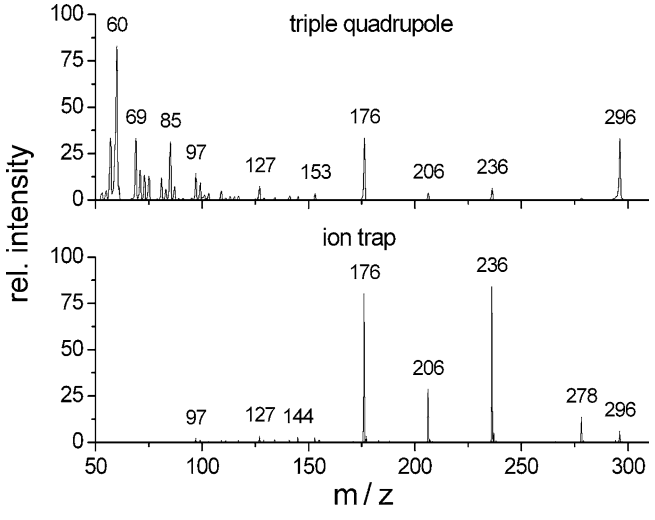



**Fig. 8** Comparison of the MS–MS spectra of glucose-hydroxypropyltrimethylammonium chloride recorded with triple-quadrupole (*top*) and ion-trap mass spectrometers

## Conclusion

This work presents a sensitive method for the quantitation of cationic polysaccharides on hair treated with a shampoo and rinsed with water without prior derivatization. Because of the small quantity of sample used even hair from volunteers can be analyzed, making it possible to obtain important information about the behavior of cationic polymers during real-life treatment procedures. Furthermore, simultaneous quantitation of different polymers makes it possible to obtain new information on synergetic effects. The information obtained on the underlying mechanisms of action enables faster and more systematic product development and optimization.

## References


1. Trüeb RM (1998) Hautarzt 49:895–901
2. Wu W, Alkema J, Shay GD (2001) J Cosmet Sci 52:51–65
3. Wilke O, Mischnick P (1995) Carbohydr Res 275:309–318
4. Wilke O, Mischnick P (1997) Starch/Stärke 49:453–458
5. DeMartino RN, Conciatori AB (1976) USA patent 4,031,307
6. Chudzikowski RJ (1971) J. Soc Cosmet Chem 22:43–60
7. Schueller R, Romanowski P (2000) Cosmet Toiletries 115:67–73
8. Kon R, Nakamura A, Takeushi K (1998) Int J Cosmet Sci 20:369–380
9. Romanowski P, Schueller R (1998) Cosmet Toiletries 113:51–56
10. Jachowicz J (1998) Cosmet Toiletries 113:45–59
11. Hössel P, Dienig R, Nörenberg R, Pfau A, Sander R (1998) Int J Cosmet Sci 22:1–10
12. Leidreiter HI, Jenni K, Jorbandt C (1994) SOFW 120:852–860
13. Goddard ED (1987) Cosmet Toiletries 102:71–80
14. Hutter JM, Clarke MT, Just EK, Lichtin JL, Sakr A (1991) J Soc Cosmet Chem 42:87–96
15. Goddard ED, Harris WC (1987) J Soc Cosmet Chem 38:233–246
16. Dalton JS, Allen GC, Heard PJ, Hallam KR, Elton NJ, Walker MJ, Matz G (2000) J Cosmet Sci 51:275–287
17. Gruber JV, Winnik FM, Lapierre A, Khaloo ND, Josh N, Lawrence A, Konish PN (1999) J Cosmet Sci
18. Goddard ED, Winnik, FM (1999) Cosmet Toiletries 114:63–81
19. Busch P (1989) Arztl Kosm 19:270–315
20. Covaci A, Schepens P (2001) Chromatographia 53:S366–S371
21. Regismond STA, Heng Y-M, Goddard ED, Winnik FM (1999) Langmuir 15:3007–3010
22. Arisz PW, Kauw HJJ, Boon JJ (1995) Carbohydr Res 271:1–14
23. Fengel D, Wegener G, Heizmann A, Przyklenk M (1977) Holzforschung 31:65–71
24. Novellino L, Napolitano A, Prota G (2000) Biochim Biophys Acta 1475:295–306
25. Materna K, Cote G, Szymanowski J (2004) J Colloid Interface Sci 269:466–471
26. Li L, Liu F, Kong X, Su S, Li KA (2002) Anal Chim A 452:321–328
27. Takacs-Novak K, Szasz G (1999) Pharm Res 16:1633–1638
28. Eckard PR (1998) Ph.D. thesis, Faculty of the Virginia Polytechnic Institute of the State University Blacksburg
29. Gustavsson SA, Samskog J, Markides KE, Langström B (2001) J Chromatogr A 937:41–47
30. Jachowicz J (1987) J Soc Cosmet Chem 38:263–286
31. Blanco B, Durost BA, Myers RR (1997) J Soc Cosmet Chem 48:127–131