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Analysis of charged cyclomalto-oligosaccharides (cyclodextrin) derivatives by ion-spray, matrix-assisted laser-desorption/ionization time-of-flight and fast-atom bombardment mass spectrometry, and by capillary electrophoresis

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Abstract

The use of electrospray-ionization mass spectrometry (ESIMS), matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDITOFMS), and fast-atom bombardment mass spectrometry (FABMS) for the rapid determination of molecular weight, degree of substitution (ds), and purity is demonstrated for charged derivatives of cyclomalto-heptaose (βCD) and -octaose (γCD). The access to anionic sulfoalkyl ethers (alkyl: ethyl, *n*-propyl, and *n*-butyl) and to βCD-2-hydroxy-3-trimethylammoniumpropyl ether chlorides (HTAP-βCD) leads only to mixtures of products, the compositions of which can be determined directly from ESI and MALDITOF mass spectra. All charged derivatives consist of a mixture of unreacted and higher substituted compounds. The substitution patterns obtained by MS are in good agreement with the results of experiments on the separation of βCD-sulfoalkyl ethers by capillary electrophoresis (CE). © 1996 Elsevier Science Ltd.

Keywords: Cyclodextrin derivatives; ESIMS; MALDITOFMS; FABMS; Capillary electrophoresis (CE)

Abbreviations: IS, ion-spray (i.e., pneumatically assisted electrospray); ESIMS, electrospray-ionization mass spectrometry; MALDITOFMS, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; FABMS, fast-atom bombardment mass spectrometry.

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1. Introduction

The inclusion of pharmaceutical compounds in CDs can improve their solubility, stability, bioavailability, and pharmacokinetics, which makes these complexes potential candidates for novel drug carriers [1–3]. Unfortunately, βCD, which forms the most stable inclusion compounds with many drugs, possesses a low water solubility, preventing their complexes to be soluble at therapeutic concentrations. Therefore negatively charged βCD derivatives have been synthesized and used as clathrate compounds in pharmaceutical [4] or technical applications, such as sewage flocculation, paper finishing or as binder [5,6]. Sulfation is considered to empower βCD with unique biological activity similar and sometimes superior to that of heparin [7,8]. Less toxic and hemolytic effects of sulfated βCD in comparison with native βCD and sulfated dextran have been reported [9,10]. Recently, membrane-disrupting abilities of βCD-sulfobutyl ether (SBE-βCD) lower than those of the parent βCD and 3-hydroxy-*n*-propyl-βCD were described [10]. An excellent bioavailability of the vasodilator, antihistaminic and antiallergic agent, cinnarizine, complexed with SBE-βCD was also observed [11]. A qualitative assignment of the composition of the compounds is of current importance for the validation, optimization, and standardization of pharmaceutical formulations containing charged CDs. More recently, charged βCDs were found to be versatile chiral additives in capillary electrophoresis [12–14] due to the combined enantioselectivity and increased water solubility.

Lammers and co-workers [15] first systematically analyzed the sodium salts of α- and β-cyclodextrin carboxymethyl ethers and the corresponding sulfo-*n*-propyl ethers by comparing several different nonspectroscopic methods, such as hypoiodite oxidation, titration of chlorine, and carboxyl groups, as well as oxidation to sulfate and subsequent sulfur determination and UV absorption. They calculated the mean values of the molecular weights from the results so obtained. The methods employed do not give any insight into the real distribution of the derivatives; therefore, we explored two different methods, mass spectrometry and capillary electrophoresis, for the characterization of charged CD derivatives.

The comparison of three independent mass spectrometric methods for several charged derivatives of βCD and the parent compound made the reliable determination of molecular weight, purity, and degree of substitution possible.

Nonvolatility and high molecular weight render all cyclodextrins and their derivatives unsuitable for conventional mass spectrometry, i.e. EIMS and CIMS [17]. However, various mass spectrometric techniques can be applied to CDs and their derivatives, such as fast-atom bombardment [18–20], secondary-ion [21], laser-assisted field desorption [22], laser desorption [23], ^{252}Cf-plasma-desorption [24], matrix-assisted laser desorption [25–27], and electron ionization–flash desorption [28]. Some of the above techniques use home-built mass spectrometers and ion sources, which are not available for routine analysis. Some require a complicated and occasionally difficult procedure for the preparation of samples. Therefore routine ESI, MALDITOF, and FAB mass spectra of several charged cyclodextrin derivatives were recorded and compared in order to find similarities and fundamental differences between these three widespread methods.

The results obtained were compared with experiments on the separation of several

β-cyclodextrin sulfo-*n*-alkyl ethers by capillary electrophoresis, with direct and indirect UV detection [16]. The separation is based on the different electrophoretic mobilities of the negatively charged CDs caused by a difference of their overall charge as a result of substitution.

It should be noted that individual peaks of the electropherogram represent unresolved charged βCD regioisomers with the same number of substituents. It is obvious that these regioisomers give the same molecule ion peak, no matter what mass spectrometric methods are applied.

2. Experimental

Equipment.—The ESI mass spectra were recorded with a Scietex API III triple–quadrupole mass spectrometer, with a mass range of 2.4 kD, equipped with an IS ion source (Scietex, Toronto, ON, Canada). The mass spectrometer was operated under conditions of unit-mass resolution, and profile spectra were obtained by acquiring data points every 0.1 D. The IS voltage was 5 kV, and the orifice voltage was 80–180 V. The curtain gas flow was 0.7 L/min, and the nebulizer gas flow was 1.1 L/min. The interface heat was 58 °C. βCD was dissolved in 3:7 H_2O–MeOH and all other derivatives measured were dissolved in water. The final concentration of the substrates was approx. 100 μM. The solution was introduced into the IS source at a constant flow rate of 2–10 μL/min, using a medical infusion pump model 22 (Harvard Apparatus, South Natick, USA).

The MALDITOF mass spectra were recorded with a prototype of a Hewlett–Packard HP G2025A MALDITOF system [27] with a mass range of 340 kD, equipped with a N_2 laser (337 nm, 3 ns pulsewidth, 5.9 μJ) and an ion optic with ±28 kV. The mass spectrometer consisted of a 1.0 m flight tube and was operated in the positive-ion mode. The data acquisition rate was 200 MHz, and 50 spectra each were accumulated. The analytes were dissolved in water (1 μg/mL), and 5–10 μL of the solutions were mixed with aliquot amounts of 30 mM NaCl solution and the matrix [1:3 DHB–HIC, prepared by mixing 0.2 mol 2,5-dihydroxybenzoic acid and 0.6 mol 1-hydroxyisoquinoline in 1:1 H_2O–CH_3CN (1:1, v/v)].

The FAB mass spectra (carrier gas: Xe; ion source: temp 50 °C, 10 kV, 2 mA emission current) were recorded with a MAT 711A instrument modified by AMD Intectra, Beckeln, Germany. The data acquisition was performed with AMD Intectra software. The detection unit was operated in the negative-ion mode. Samples could only be prepared by dissolving the analytes in H_2O (3–5 μg/mL). A sample (1 μL) was mixed on the probe tip with a 25% soln of glycerol in H_2O and then transferred to the probe vacuum lock.

In CE for the sulfoethyl ethers of βCD (SEE-βCD) (**2**) and the sulfobutyl ethers of βCD (SBE-βCD) (**4a**, **4b**), a Grom capillary electrophoresis System 100 (Grom, Herrenberg, Germany), equipped with a Linear Instrument (Reno, NV, USA) UV-vis 200 detector and a HP 3396A integrator (Hewlett–Packard, Avondale, PA, USA) was used. The samples were injected by the hydrostatic (10 cm) method. The electric field was 400 V/cm, and the temperature was 21 ± 1 °C. The anode and cathode buffer had a

pH of 4.6 and molarity of 50 mM KH_2PO_4 (direct detection; 190 nm) or 30 mM TRIS–benzoic acid (indirect detection; 254 nm). Untreated fused-silica capillaries (Grom, Herrenberg, Germany), 50 cm total length, 41 cm effective length, 50 μm i.d., were used for the electrophoretic characterization of the additives.

In the case of the sulfopropyl ethers of βCD (SPE-βCD) (**3a**, **3b**) a "PRINCE" capillary electrophoresis system (Bischoff, Leonberg, Germany), equipped with a Linear UV-vis detector (Bischoff, Leonberg, Germany) and a chromatopac CR6 A integrator (Bischoff, Leonberg, Germany) was used. The samples were injected by the pressure-driven (40 mbar, 5 s) method. The electric field was 400 V/cm, and the temperature was 20 ± 1 °C. The anode and cathode buffer had a pH of 6.0 and molarity of 30 mM TRIS–benzoic acid. Untreated fused-silica capillaries (Ziemer, Mannheim, Germany), 75 cm total length, 60 cm effective length, 50 μm i.d., were used for all measurements.

Reagents and chiral additives.—The βCD (**1**) was obtained from Roth, Darmstadt, Germany. βCD-sulfoethyl ethers (SEE-βCD) (**2**) were a gift from the Consortium für Elektrochemische Industrie GmbH, Munich, Germany. Two specimens of βCD-sulfo-*n*-propyl ethers (SPE-βCD) (**3a**, **3b**) were synthesized by modifying a method described previously [5,16]. γCD-sulfo-*n*-propyl ether (SPE-γCD) (**6**) was synthesized according to ref. [4]. βCD-sulfo-*n*-butyl ethers (SBE-βCD) (**4a**) were synthesized according to ref. [5], and **4b** was obtained from Higuchi Biosciences Center, Lawrence, KS, USA. βCD-2-hydroxy-3-trimethylammoniumpropyl ether chloride (HTAP-βCD) (**5**) was synthesized according to ref. [29]. γCD was a gift from the Consortium für Elektrochemische Industrie GmbH, Munich, Germany. 2,5-Dihydroxybenzoic acid (DHB), 1-hydroxyisoquinoline (HIC), 1,3-propane sultone (98%), and glycidyltrimethylammonium chloride (90%) were obtained from Aldrich Chem. Co., Milwaukee, WI, USA. TRIS-(hydroxymethyl)-aminomethane was purchased from Sigma Chem. Co., St. Louis, MI, USA. Benzoic acid was purchased from Aldrich-Chemie, Steinheim, Germany, and KH_2PO_4 (analytical grade) was purchased from E. Merck, Darmstadt, Germany.

3. Results and discussion

The following CD derivatives were investigated: βCD (**1**, mol wt 1135.0), βCD-sulfoethyl ethers (**2**, mol wt 1265.0, 1395.0, and 1525.0), βCD-sulfo-*n*-propyl ethers (**3a** and **3b**, mol wt 1279.0, 1423.0, 1567.0, 1711.0, 1855.0, 1999.0, 2143.0, and 2287.0), γCD-sulfo-*n*-propyl ether (**6**, mol wt 1441.0), βCD-sulfo-*n*-butyl ethers (**4a** and **4b**, mol wt 1293.0, 1451.0 1609.0, 1767.0, and 1925.0), βCD-2-hydroxy-3-trimethylammoniumpropyl ether chlorides (**5**, mol wt 1286.5 and 1438), cf. Table 1. Compound **1** was used as reference for all measurements. All spectra were recorded once, and the conditions were optimized (cf. Experimental section) according to previous investigations. In the case of ESIMS, measurements from an autosampler run, without any optimization, were also included (cf. Fig. 2b).

Ion-spray (IS), i.e. pneumatically assisted electrospray, is a soft method of ionization for nonvolatile and thermolabile molecules. The method is based on the ion-evaporation process. When a solution of the sample is sprayed through a capillary that is maintained at a few kV relative to the charge at the entrance of the mass spectrometer, charged

Table 1
Designations, abbreviations, and sources of compounds analyzed

Compound no.	Compound	Source
1	βCD	Roth, Germany
2	SEE-βCD	Wacker, Germany
3a	SPE-βCD	synthesized [5,16]
3b	SPE-βCD	synthesized [5,16]
4a	SBE-βCD	synthesized [5]
4b	SBE-βCD	Higuchi Bioscience, USA
5	HTAP-βCD	synthesized [29]
6	SPE-γCD	synthesized [4]

droplets are produced, from which the preformed solute ions are emitted directly without further heating [2] into the gas phase. The occurrence of multiply charged quasi-molecular ions $[M + nH]^{n+}$ and $[M + nNa]^{n+}$ is characteristic for this method.

The intensity of the peaks observed in ISMS depends on the parameters of the IS interface. An increase in the voltage of the orifice enhances the intensity of single charged ions and lowers that of the doubly charged ions (cf. esp. Fig. 6). Therefore the mass spectra only allow a qualitative determination of the composition of a mixture [17]. A quantitative determination is possible if calibration curves (obtained with mixtures of known composition) are used. Due to the fact that the separation of the investigated compounds has not yet been achieved [4], the quantitative determination of the degree of substitution (ds) was not possible. In the negative mode the distributions were very similar.

In matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDITOFMS), a laser pulse is directed onto a solid matrix, consisting of, e.g., aromatic acids in which the analyte molecules are ideally separated, leading to an explosive disintegration and ejection of the top layers of the sample into the gas phase. Analyte molecules are charged $[M + H]^+$ and $[M + Na]^+$ on addition of NaCl. Fragmentation is generally not *observed*, because the MALDITOF operates in the linear mode.

The intensity of the peaks observed in the MALDI experiments can yield information on the molar mass distribution [30], but the unknown mechanism of the desorption/ionization step, the importance of the choice of matrix material, and the suppression of lower masses also render a quantitative determination of the mass distribution based on MALDI experiments ambiguous. We could not observe ions in the negative mode.

FAB mass spectra are produced by directing a beam of atoms (Ar, Xe) or ions (Cs^+) of high translational energy (5–10 keV) towards a probe tip, where in a desorption/ionization process the sample, which is usually dissolved in a matrix consisting of glycerol, is ionized. The atom beam produces quasi-molecular ions, $[M + H]^+$ and $[M + Na]^+$, as well as fragment ions of the samples and matrix. Even pyrolytic

[2] IS is a variant of ESI for low flow rates; it should also not be confused with thermospray, where a capillary is inserted into a heated prechamber where via evaporation a spray of droplets enters the ion optic.

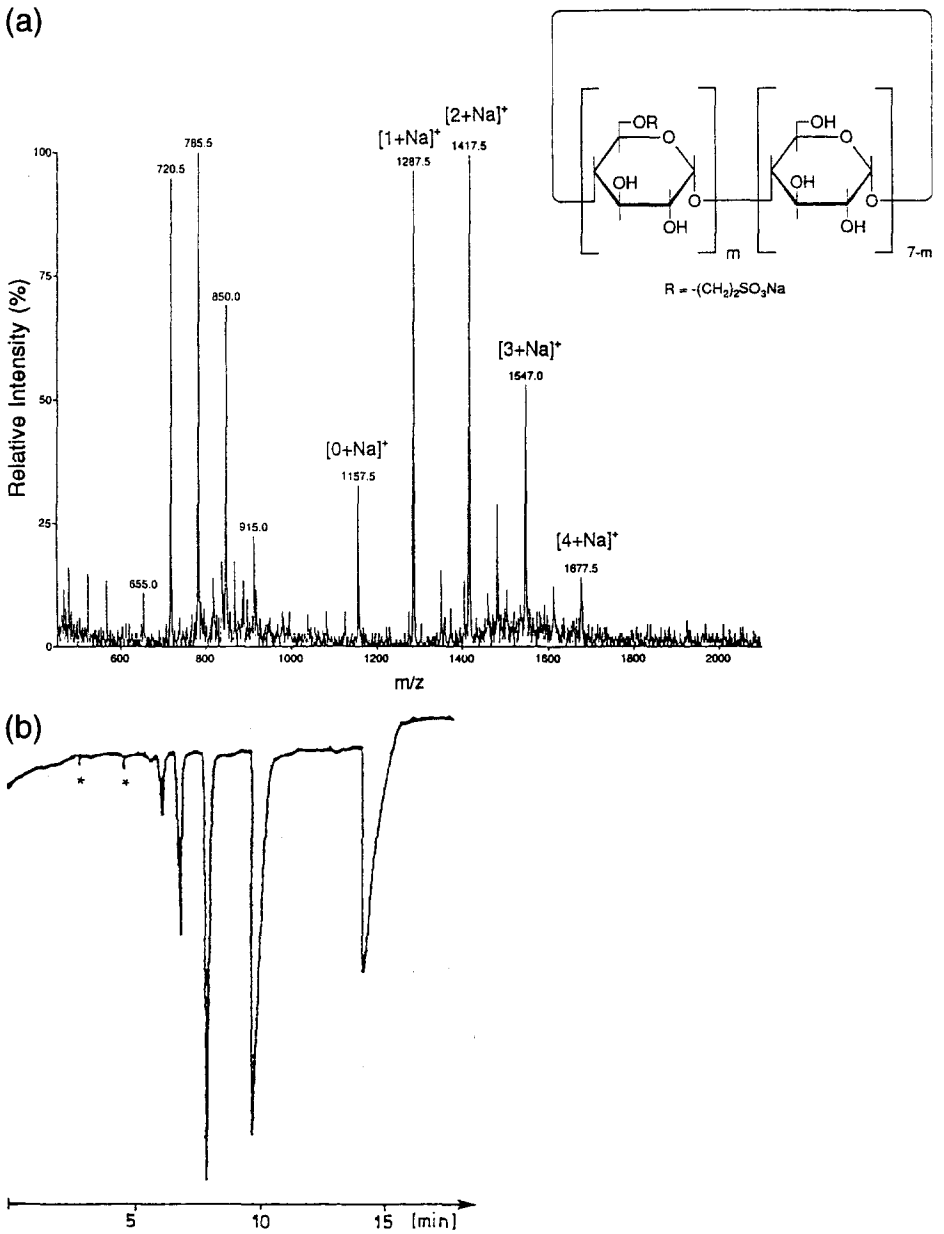


Fig. 1. Comparison of (*i*) the IS mass spectrum (a), containing the sodium adducts of the unsubstituted βCD and the one-to-fourfold substituted derivatives of **2**, and (*ii*) the CE electropherogram (b), five peaks, indirect detection at 254 nm, 30 mM benzoic acid buffer (pH 4.6) of **2**. Artefacts are marked with an asterisk.

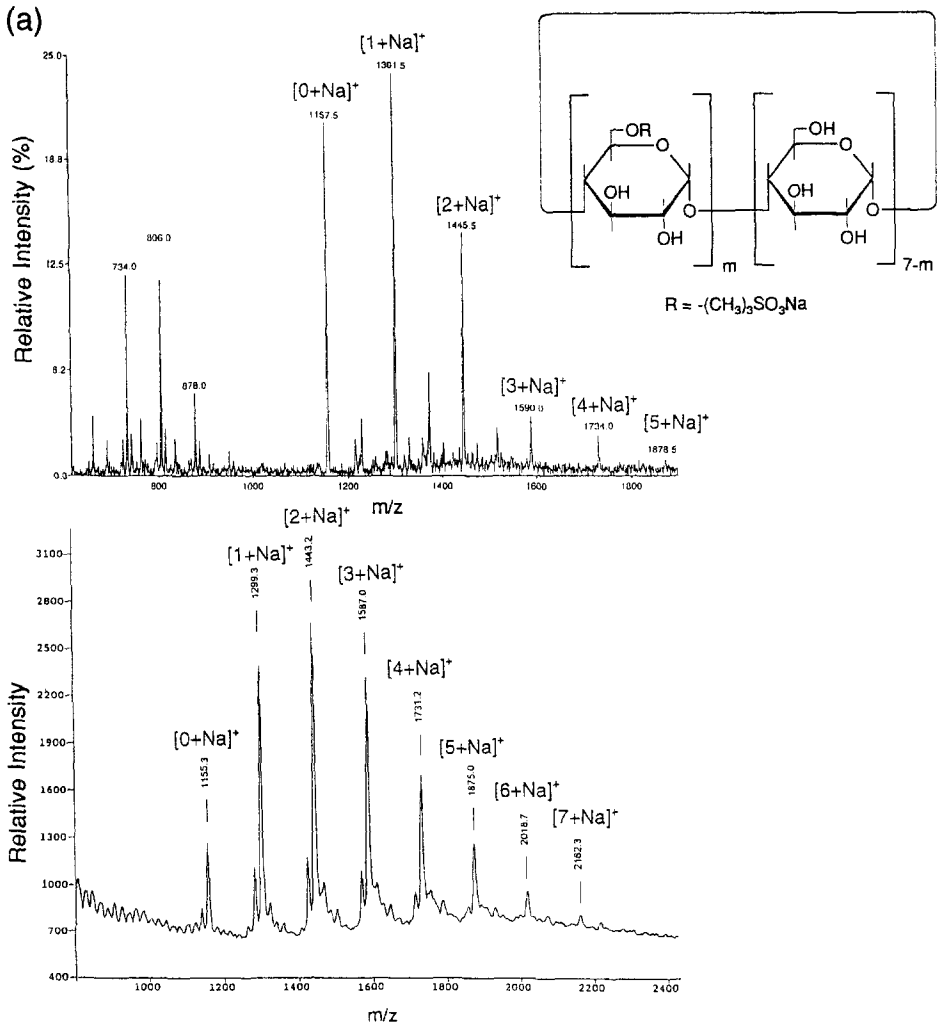


Fig. 2. Comparison of (*i*) the IS mass spectrum (a, top), containing the sodium adducts of the unsubstituted βCD and the one-to-fivefold substituted derivatives of **3a**, (*ii*) the MALDITOF-mass spectrum (a, bottom), containing the sodium adducts of the unsubstituted βCD and the one-to-sevenfold substituted derivatives of **3a**, and (*iii*) the CE electropherogram (b), indirect detection at 254 nm, 30 mM TRIS–benzoic acid buffer (pH 6.0) of **3a**, containing the underivatized βCD **1** (marked with O), and four substituted compounds. Artefacts are marked with an asterisk. Comparison of (*i*) the IS mass spectrum (c, top), containing the sodium adducts of the one-to-eightfold substituted derivatives of **3b** (autosampler run), (*ii*) the MALDITOF mass spectrum (c, bottom), containing the sodium adducts of the unsubstituted βCD and the one-to-eightfold substituted derivatives of **3b**, and (*ii*) the CE electropherogram (d), indirect detection at 254 nm, 30 mM TRIS–benzoic acid buffer (pH 6.0) of **3b**, containing the underivatized βCD **1** (marked with 0), and six substituted compounds. Artefacts are marked with an asterisk.

processes, which give rise to further ions and $[M + n\text{-glycerol}]^+$ peaks, are observed [31]. On addition of NaCl the $[M + Na]^+$ ions become predominant. In the negative-ion mode $[M + H]^-$ and in our case $[M - Na + H]^-$ peaks are observed. Mixed samples should give rise to mixed FAB spectra; however, competitive effects on the matrix surface may lead to preferential ionization of individual compounds irrespective of concentration. Indeed, complete ion suppression of some components in a mixture is often encountered. An additional disadvantage of FAB ionization is the relatively high background noise of generating ions at any mass in the spectrum in addition to intense cluster ions from the matrix. This leads to a much lower sensitivity of FAB ionization in comparison with IS or MALDI. Nevertheless, the FAB method is included for the comparison of the ds of one negatively charged cyclodextrin derivative, **4a**. Sample preparing and recording turned out to be lengthy, with poor results. Therefore, only one sample was analyzed for this study. The compound could not be detected in the positive mode.

In CE, the separation capillary is placed between two electrode reservoirs filled with the background electrolyte. Platinum electrodes serve to connect the high-voltage power supply (HVPS), which delivers approximately up to 100 μA and 30 kV. The sample is injected by pressure or electrophoretically into the capillary, and zones of separated substances are analyzed by a suitable detector (mainly UV, "oncolumn") at the opposite end of the capillary. Charged CDs are separated because of their different electrophoretic mobilities caused by their different charges. The detection of the derivatives is performed (*i*) by indirect UV detection in a benzoic acid buffer at 254 nm or (*ii*) by direct UV detection in a phosphate buffer at low wave length (190 nm). The indirect UV detection is based on the observed hypochromic shift due to inclusion of benzoic acid as the benzoate anion in the βCD cavity. The optical systems used for indirect detection are identical to those used for direct UV absorbance detection. According to ref. [4] it can be observed that the response factor (determined by relative

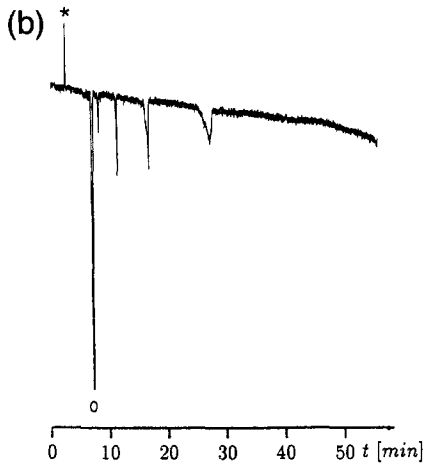
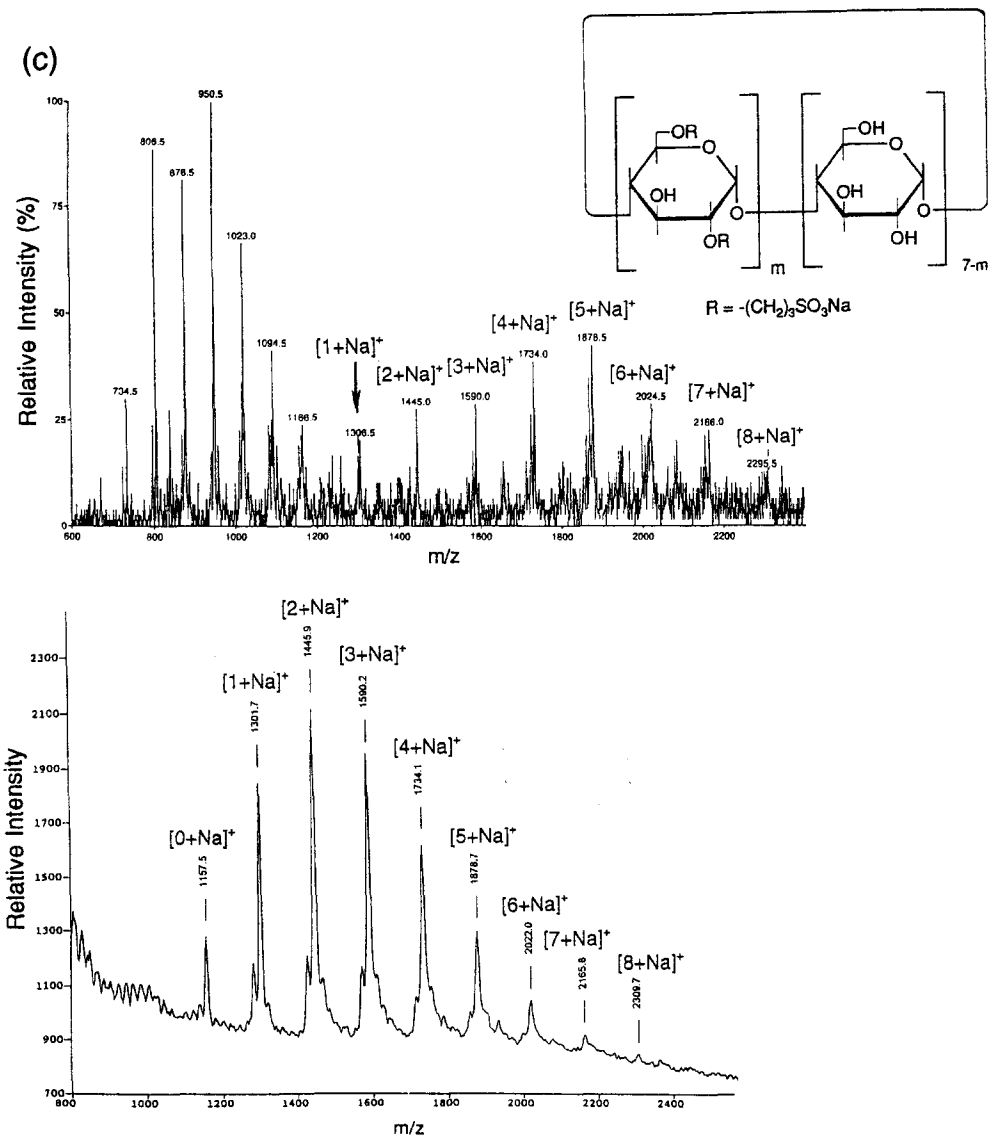


Fig. 2 (continued).

peak areas) increases with the degree of substitution. This indicates that the detection response is dependent on the degree of substitution, and unfortunately prevents the quantitative determination. In CE the electropherograms for the separation of SEE- (**2**), SPE- (**3a** and **3b**) and SBE-βCD (**4a** and **4b**) clearly show that the compounds are a mixture of derivatives with many degrees of substitution (ds) present. The peak assignments were based upon the assumption that the parent compound **1** is eluted first,



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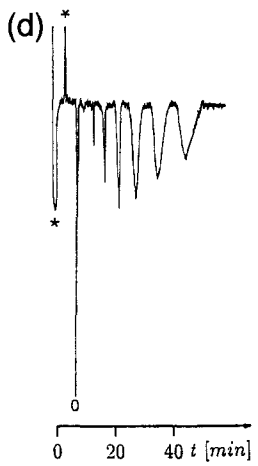

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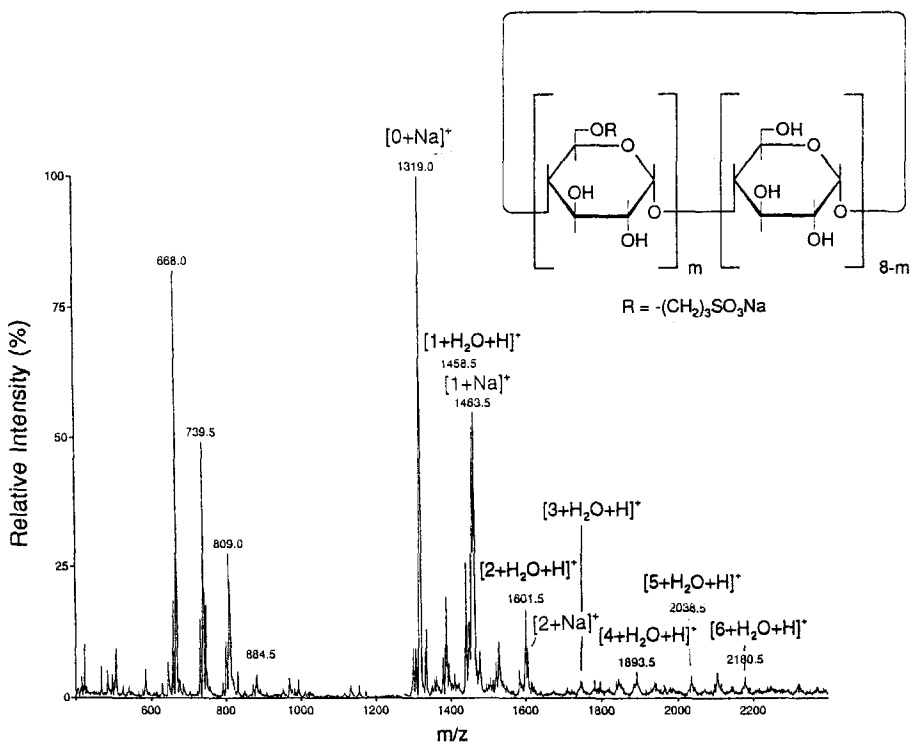

Fig. 3. IS mass spectrum of **6**, containing the water and the sodium adducts of the unsubstituted and the one- to sixfold substituted γCD.

which was verified for compounds **3a** and **3b** by admixing βCD to the sample. The later eluted peaks represent fractions of higher ds.

The following results were obtained: all figures show the molecular formula and contain peak assignments for the *charged* ions of the unsubstituted ($m = 0$; $[M]^+$) and manifold substituted ($m = 1-8$; $[M']^+$) compounds for MS experiments. Doubly charged ions (ESIMS) are not labelled. In CE the first-eluted peak was identified by admixing **1**.

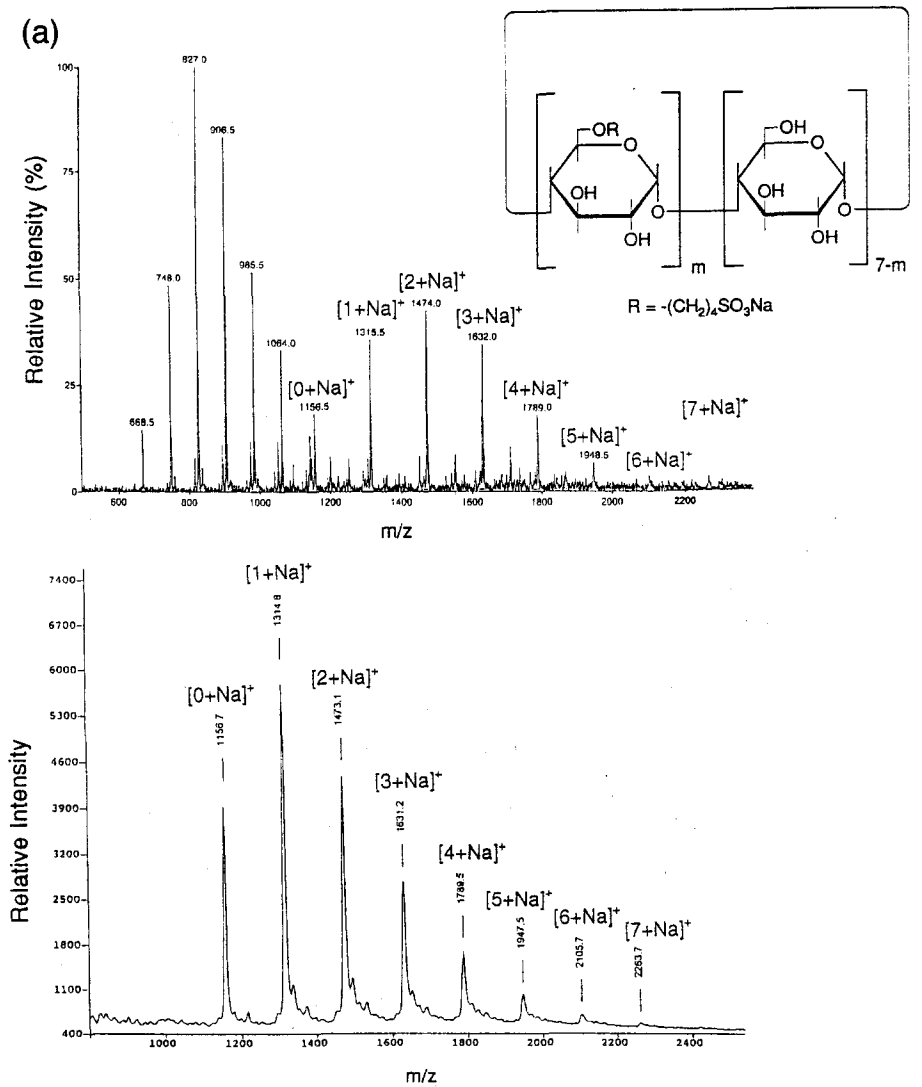

Fig. 4. Comparison of (*i*) the IS mass spectrum (a, top), containing the sodium adducts of the one-to-sevenfold substituted derivatives of **4a**, (*ii*) the MALDITOF mass spectrum (a, bottom), containing the sodium adducts of the unsubstituted βCD and the one-to-sevenfold substituted derivatives of **4a**, and (*iii*) the FAB mass spectrum (b), containing the one-to-fourfold substituted derivatives (loss of one or more sodium cations) **4a**.

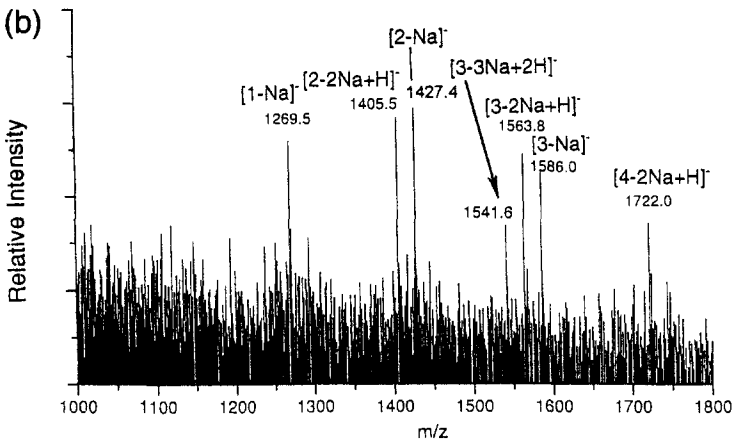

Fig. 4 (continued).

βCD (**1**). The IS mass spectrum of βCD (**1**) contained the ions $[M + H]^+$ and $[M + Na]^+$. The MALDITOF mass spectrum contained only the $[M + Na]^+$ ion.

βCD-sulfoethyl ethers (**2**). The IS mass spectrum of the βCD-sulfoethyl ethers (**2**) contained ions $[M + Na]^+$ and $[M' + Na]^+$ that are assigned to the parent compound **1** and one- to fourfold substituted derivatives. The one- to fivefold substituted βCD-sulfoethyl ethers could also be detected as doubly charged ions (cf. Fig. 1a). The electropherogram of the indirect detection in CE for **2** showed five peaks (artefacts are marked with an asterisk), which represent the unsubstituted and the one- to fourfold substituted βCD-sulfoethyl ether (cf. Fig. 1b).

*βCD-sulfo-*n*-propyl ethers* (**3a** *and* **3b**). The IS mass spectra of the βCD-sulfo-*n*-propyl ethers (**3a** and **3b**) contained ions $[M + Na]^+$ and $[M' + Na]^+$ which are assigned to the parent compound **1** and one- to eightfold substituted derivatives. Compound **3a** was found to contain mostly the one- to fourfold substituted sulfo-*n*-propyl ethers (cf. Fig. 2a, top), whereas the sample of **3b** contained mostly ions that are assigned to two- to eightfold substitution (cf. Fig. 2c, top). The orifice voltage was slightly lower for the ESI spectrum of **3b** (autosampler run); therefore, more doubly charged ions were formed, and the signal/noise ratio for the onefold charged ions became poorer. All ions could also be detected as doubly charged ions. The MALDITOF mass spectra contained the $[M + Na]^+$ and the $[M' + Na]^+$ ions of the one- to sixfold **3a** (cf. Fig. 2a, bottom), or in the case of **3b** (cf. Fig. 2c, bottom), eightfold substituted derivatives. Only negligible amounts of higher charged ions could be detected. The electropherograms of the indirect detection showed in the case of **3a**, five (cf. Fig. 2b), in the case of **3b**, eight peaks (artefacts are marked with an asterisk) (cf. Fig. 2d), which were in analogy to Fig. 1b assigned to the unsubstituted and one- to fourfold, and one- to sevenfold, substituted βCD-sulfo-*n*-propyl ethers, respectively.

*γCD-sulfo-*n*-propyl ether* (**6**). The IS mass spectrum of γCD-sulfo-*n*-propyl ether (**6**) contained ions which are assigned to the parent compound γCD $[M + Na]^+$ and the mono-substituted derivative, i.e. plus 145 D $[M' + H_2O + H]^+$, $[M' + Na]^+$ in equal

amounts (cf. Fig. 3). Small and negligible quantities of higher substituted derivatives and water adducts, i.e. plus *m* times 145 D $[M' + H_2O + H]^+$, could also be detected.

*βCD-sulfo-*n*-butyl ethers* (**4a** *and* **4b**). The IS mass spectra of the βCD-sulfo-*n*-butyl ethers (**4a** and **4b**) contained ions which are assigned to the parent compound **1** $[M + Na]^+$ and one- to sevenfold substituted derivatives $[M' + Na]^+$ (cf. Fig. 4a and

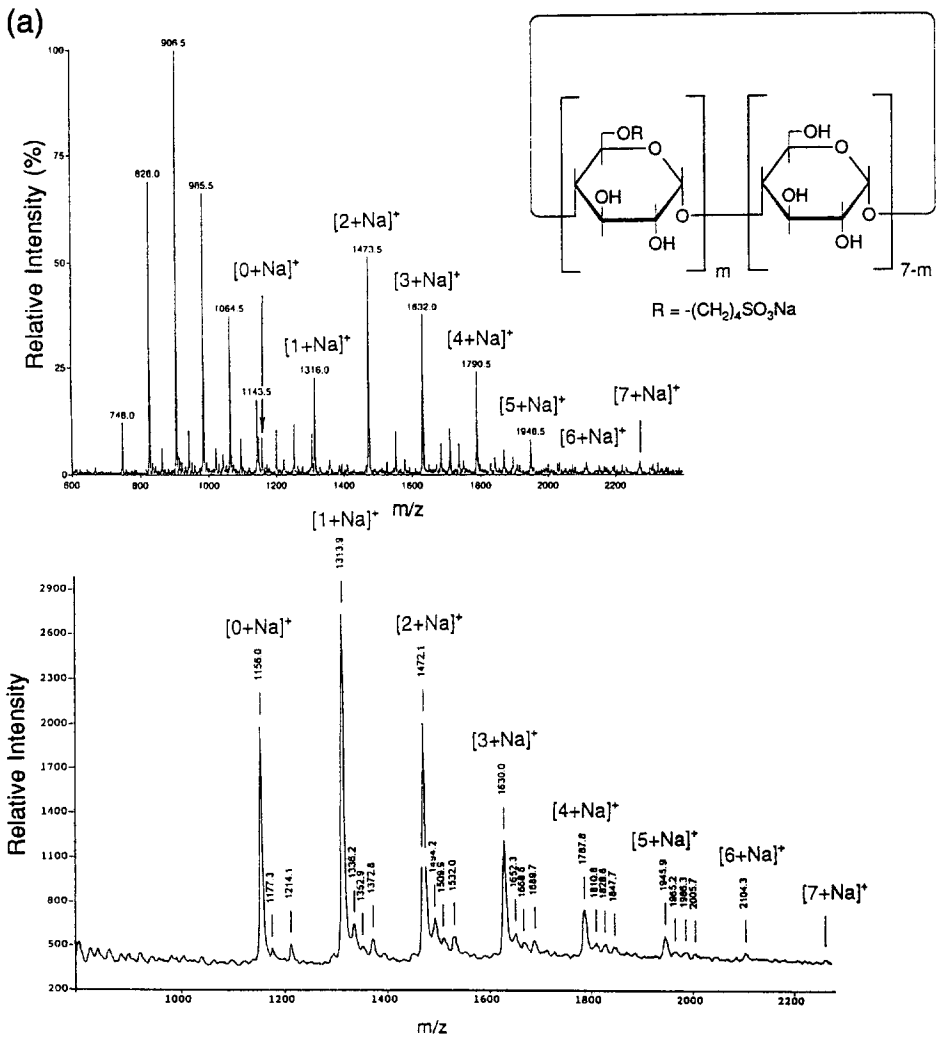

Fig. 5. Comparison of (*i*) the IS mass spectrum (a, top), containing the sodium adducts of the unsubstituted βCD and the one-to-sevenfold substituted derivatives of **4b**, (*ii*) the MALDITOF mass spectrum (a, bottom), containing the sodium adducts of the unsubstituted βCD and the one-to-sevenfold substituted derivatives of **4b**, and (*iii*) the CE electropherograms, direct [b (i)] and indirect [b (ii)] UV detection. KH_2PO_4 buffer (50 mM), pH 4.6, 190 nm (direct detection); 30 mM benzoic acid buffer, pH 4.6, 254 nm (indirect detection) of **4b**, seven peaks each.

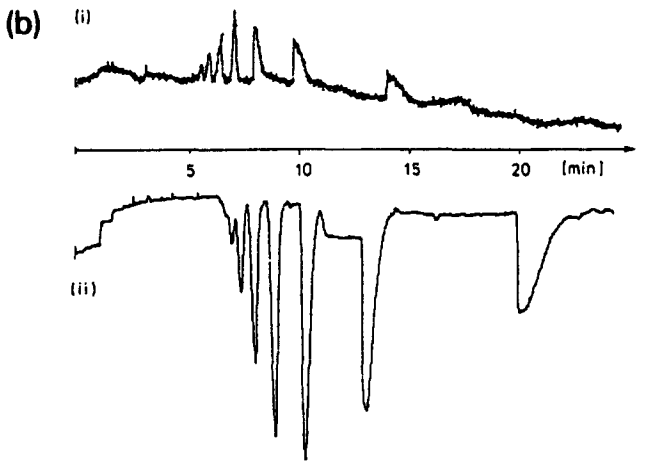

Fig. 5 (continued).

Fig. 5a, top). The one- to sevenfold substituted βCD-sulfo-*n*-butyl ethers could also be detected as doubly charged ions. The MALDITOF mass spectrum contained the $[M + Na]^+$ ion of the unsubstituted **1** and the $[M' + Na]^+$ ions of the one- to sevenfold compound (**4a**) (cf. Fig. 4a and Fig. 5a, bottom). Small amounts of higher adducts $[M' + 2Na]^+$, $[M' + 2H_2O]^+$ could also be detected. The FAB mass spectrum showed the $[M' - Na + H]^-$, $[M' - 2Na + H]^-$ and $[M' - 3Na + 2H]^-$ ions for the one- to fourfold substituted derivatives of **4a** (cf. Fig. 4b). The parent compound, βCD, could not be detected. The electropherograms for the direct [cf. Fig. 5b (*i*)] and indirect [cf. Fig. 5b (*ii*)] detection of **4b** also showed the peaks corresponding to one- to sixfold substitution and small amounts of the native βCD (**1**).

βCD-2-hydroxy-3-trimethylammoniumpropyl ether chlorides (**5**). Here the importance of the orifice voltage and the impossibility of quantification of the ds in ESIMS can be demonstrated very easily: Fig. 6, top, shows an ISMS of **5** with an orifice voltage of 180 V (increase of single charged ions); Fig. 6, bottom, shows one with an orifice voltage of 140 V (increase of doubly charged ions). The IS mass spectrum of βCD-2-hydroxy-3-trimethylammoniumpropyl ether chlorides (**5**) contained ions which are assigned to the parent compound **1** βCD $[M + Na]^+$ with very low intensity, and with high intensities to the one- and twofold substituted derivatives. The IS mass spectra for these cationic compounds become complicated, not only due to the fact that one- and twofold substituted derivatives can no longer be detected within the same m/z range, but also because part of the chloride anions are split off as the compounds leave the capillary of the interface. Thus the IS mass spectra show with high intensities peaks that are assigned to the *cations* of the one- and twofold derivatives, i.e., plus m times 116 D $[M']^+$. Nevertheless, the NaCl present allows the formation of NaCl adducts $[M' + n\text{NaCl}]^+$. We also observed the elimination of H_2O out of the doubly charged compound $[M' - H_2O + H]^+$, a phenomenon not encountered with the anionic species we examined. In CE the compounds could not be detected; several capillaries used lost

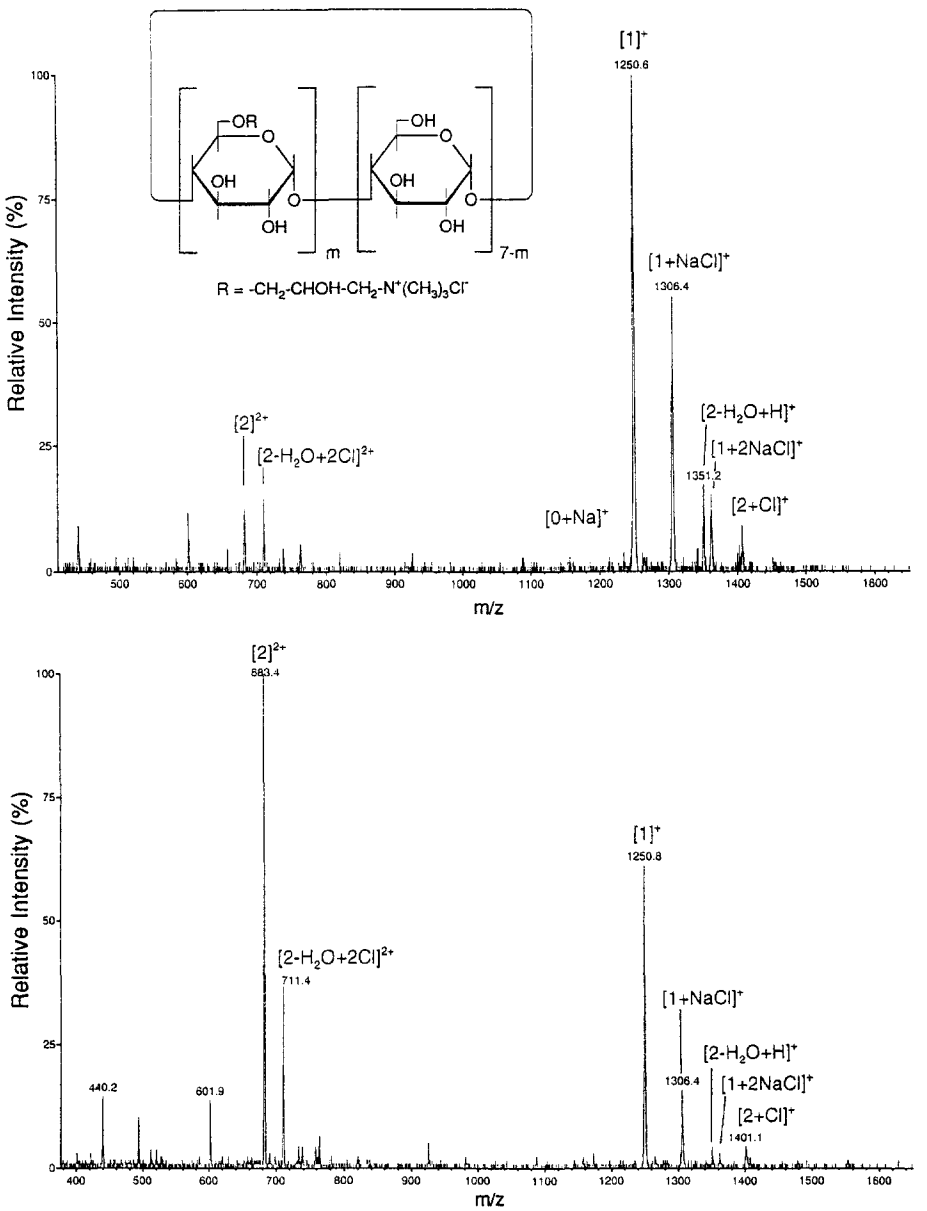

Fig. 6. Comparison of IS mass spectra of **5** (top: orifice voltage 180 V, bottom: orifice voltage 140 V), containing NaCl and water adducts of the one-and-twofold substituted derivative.

their performance very fast when compound **5** was injected. It seems that the negatively charged surface of the fused-silica capillary interacts strongly with the cationic compounds, a fact often encountered in the case of proteins used as chiral additives in CE.

4. Summary

In summary, ISMS and MALDITOFMS are suitable methods for the rapid analysis of the degree of substitution (ds) and purity, i.e., determination of the presence of starting material in charged cyclodextrin derivatives. The FAB method may be suitable for pure molecules. In our case the results, especially for the higher substituted species, were very poor and could lead to the wrong conclusion that the sample is substituted to a lesser extent than determined by other methods.

The ds determined by ISMS and MALDITOFMS was verified by the capillary electrophoretic separation of the individual charged cyclodextrin derivatives. Almost all species detected in the MS experiments could also be found in CE; however, only a CE–MS coupling will allow the final proof for the exact correlation of the results. The nearly bell-shaped distribution of the observed peak intensity (slightly positively skewed), centered around the largest peak, occurred both in ISMS and MALDITOFMS, as well as in CE. This "skewing" is expected, for no species with a lower mass (or a higher electrophoretic mobility) than the starting material can be formed during the synthesis. Also, the apparent differences between peak intensity in ESI and MALDITOF experiments are not unexpected: the totally different methods for forming ions should lead to a dissimilar distribution of the preferentially ionized species. The central peaks matched the average ds expected from the molar ratios of the reactants; however, it accounted for no more than 25% of the total material detected. Surprisingly, masses for the compounds **3b** in the ISMS and MALDIMS could be detected, which are equivalent to an eightfold substituted βCD. This degree of substitution can only be explained by the reaction of a *secondary* hydroxyl group in excess of all primary hydroxy groups, which are assumed to react first.

5. Conclusions

The negatively charged CD derivatives investigated are mixtures of the unreacted parent compound and derivatives with various degrees of substitution, depending on the concentration of the starting materials. While manufacturers only give an average degree of substitution based on the data of the elemental analysis, a more detailed characterization of these derivatives is possible by refined routine methods of mass spectrometry, even for complex mixtures. The results of the mass spectrometric analysis are confirmed by the separation of the derivatives by CE.

Given that differently substituted cyclodextrins show different solubilities and electrophoretic mobilities, and will (at least) be metabolized at different rates, the present investigation implies the need for thorough standardization (batch-to-batch, source-to-source) of charged cyclodextrins used for pharmaceutical applications and as chiral additives for enantiomer separation in CE.

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References

[1] K. Uekama and M. Otagiri, *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 3 (1987) 1–40.
[2] J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akadémiai Kiadò, Budapest, (1982) 95–115.
[3] B.W. Müller, U. Brauns, and T. Backensfeld, *Proc. 4th Int. Symp. Cyclodextrins*, (1988) 369–382.
[4] R.J. Tait, D.J. Skanchy, D.P. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella, and J.F. Stobaugh, *J. Pharm. Biomed. Anal.*, 10 (9) (1992) 615–622.
[5] S.M. Parmerter, E.E. Allen, and G.A. Hull, U.S. Pat. 3,426,011; *Chem. Abstr.*, 71 (1969) 13331z.
[6] S.M. Parmerter, E.E. Allen, and G.A. Hull, U.S. Pat. 3,453,257; *Chem. Abstr.*, 71 (1969) 91821y.
[7] J. Folkman, P.B. Weisz, M.M. Joullie, W.W. Li, and W.R. Ewing, *Science*, 243 (1989) 1490–1493.
[8] K. Fukunaga, S. Hijikata, K. Ishimura, R. Sonoda, T. Irie, and K. Uekama, *J. Pharm. Pharmacol.*, 46 (1994) 168–171.
[9] E.J. Macarak, K. Kumor, and P.B. Weisz, *Biochem. Pharmacol.*, 42 (1991) 1502–1503.
[10] K. Shiotani, K. Uehata, T. Irie, K. Uekama, D.O. Thompson, and V.J. Stella, *Pharm. Res.*, 12 (1995) 77–83.
[11] T. Järvinen, K. Järvinen, N. Schwarting, and V.J. Stella, *J. Pharm. Sci.*, 84 (1995) 295–299.
[12] S. Mayer, M. Schleimer, and V. Schurig, *J. Microcol. Sep.*, 6 (1994) 43–48.
[13] S. Mayer, *Thesis*, Universität Tübingen (1993).
[14] B. Chankvetadze, G. Endresz, and G. Blaschke, *Electrophoresis*, 15 (1994) 804–807.
[15] J.N.J.J. Lammers, J.L. Koole, and J. Hurkmans, *Starch / Stärke*, 23 (1971) 167–171.
[16] H. Grosenick, S. Mayer, M. Juza, H. Jakubetz, and V. Schurig, *Seventh Int. Symp. High Performance Capillary Electrophoresis*, Würzburg, Germany, 1995, Abstr. 128.
[17] J.W. Metzger, M. Jung, D. Schmalzing, E. Bayer, and V. Schurig, *Carbohydr. Res.* 222 (1991) 23–35.
[18] A. Dell, J.E. Oates, H.R. Morris, and H. Egge, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 415–418.
[19] S.K. Sethi, D.L. Smith, and J.A. McCloskey, *Biochem. Biophys. Res. Commun.*, 112 (1983) 126–131.
[20] S. Verma, S.C. Pomerantz, S.K. Sethi, and J.A. McCloskey, *Anal. Chem.*, 58 (1986) 2898–2902.
[21] K. Harada, M. Suzuki, and H. Kambara, *Org. Mass. Spectrom.*, 17 (1982) 386–391.
[22] H.-R. Schulten, T. Komori, K. Fujita, A. Shinoda, T. Imoto, and T. Kawasaki, *Carbohydr. Res.*, 107 (1982) 177–186.
[23] B. Lindner and U. Seydel, *Anal. Chem.*, 57 (1985) 895–899.
[24] D.W. Armstrong, W. Li., C.-D. Chang, and J. Pitha, *Anal. Chem.*, 62 (1990) 914–923.
[25] M. Karas, U. Bahr, and F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes*, 92 (1989) 231–234.
[26] M. Karas, U. Bahr, A. Ingendoh, and F. Hillenkamp, *Angew. Chem.*, 101 (1989) 805–806.
[27] K.O. Boernsen, M. Schär, and H.M. Widmer, *Chimia*, 44 (1990) 417–427.
[28] D.G. Daves, Jr., W.R. Anderson, Jr., T.D. Lee, H.G. Boettger, and C.E. Giffin, in W. Voelter and D.G. Daves (Eds.), *Biologically Active Principles in Natural Products*, Georg Thieme Verlag, Stuttgart, Germany, 1984, pp 187–194.
[29] A. Deratani, G. Lelièvre, T. Maraldo, and B. Sébille, *Carbohydr. Res.*, 192 (1989) 215–222.
[30] R.-P. Krüger, *GIT Fachz. Lab.*, 39 (1995) 189–194.
[31] M. Hesse, H. Meier, and B. Zeeh, *Spektroskopische Methoden in der Organischen Chemie*, 3th ed., Georg Thieme Verlag, Stuttgart, 1987, pp 231–232.