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Characterization of modified cyclodextrins applied in capillary electrophoresis and high-performance liquid chromatography as chiral selectors by matrix-assisted laser desorption ionization curved field reflectron mass spectrometry

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Abstract

The use of ultraviolet (UV) matrix-assisted laser desorption ionization mass spectrometry combined with a curved field reflectron analyzer is demonstrated for the characterisation of samples of β-cyclodextrin (**1**) and five modified cyclodextrins (**2**–**6**) often applied in chiral separation techniques. Sodiated and sometimes potassium-adduct molecular ions are observed in the reflectron mode for all compounds studied with a mass accuracy between −0.02% and +0.09%. Characteristic molecular ion patterns for the heterogeneous samples **3**–**6** [methylated, (2-hydroxy)-propylated and carboxymethylated β-cyclodextrins] appear in the positive-ion spectra corresponding to the various degrees of substitution. Two different matrices, α-cyano-4-hydroxycinnamic acid with the thin-layer deposition technique and 2,5-dihydroxybenzoic acid with the volume preparation technique have been used. © 1997 Elsevier Science B.V.

Keywords: Matrix assisted laser desorption ionization mass spectrometry; Chiral selectors; Cyclodextrins

1. Introduction

Cyclic oligosaccharides such as the naturally occurring β-cyclodextrins are torus-shaped molecules consisting of seven 1,4-linked glucopyranose units. They have the ability to selectively incorporate guest analytes of appropriate size in its non-polar cavity. In aqueous solution hydrophobic interactions are the main contributions to the free energy of host–guest complexation. Cyclodextrins have been used in a wide variety of applications in pharmaceutical and chemical industries [1]. Commercially available cyclodextrins and their derivatives have been exploited for separation of enantiomers, diastereomers (geometrical) and positional isomers without specific derivatization. In particular, capillary electrophoresis (CE) has been established as a method for enantiomeric analysis in addition to enantioselective high-performance liquid chromatography (HPLC) and gas chromatography (GC), because of its various advantages (e.g., high separation efficiency, low sample amount needed). In CE and HPLC cyclodextrins and their modified forms are used as chiral selectors for enantiomeric separation of basic and acidic compounds. Non-ionic as well as charged cyclodextrins have been applied [2–5]. Many of the popular modified cyclodextrins are statistically O-substituted compounds and character-

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ized by the degree of substitution (DS) [6]. These chiral selectors are often mixtures of modified cyclodextrins with different DS [7]. As a consequence, different selectivities were observed when modified cyclodextrins from different suppliers and different batches were used [3,8]. The pattern of substitution and DS of the modified cyclodextrins show considerable influence on the enantiomeric selectivity in CE [9,10]. Therefore it is an analytical need for fast and reliable techniques to determine the degree of substitution and purity of modified cyclodextrins.

In the last two decades several new desorption/ionization techniques have been used to characterize cyclic oligosaccharides without prior derivatization as it was necessary for electron impact mass spectrometry (MS). Mass spectra of cyclodextrins and their derivatives have been reported using a variety of mass spectrometric methods, including field desorption (FD) [11], Californium-252 plasma desorption (PD) [12,13], fast atom bombardment (FAB) [14–17], secondary ion mass spectrometry (SIMS) [18], laser desorption ionization (LDI) [19–21] and ion spray (IS) [22,23]. Ultraviolet (UV) matrix-assisted laser desorption ionization (MALDI) MS, even though especially suited to the studies of high-molecular-mass and non-volatile polysaccharides [24], has not found wide use in studies of cyclic carbohydrates [10,25–28] despite only requiring a small amount of sample and no stringent sample preparation requirements. Further advantages of the MALDI technique are usually short times of data acquisition, detection of mainly single charged ions and good sensitivity.

This contribution demonstrates that UV-MALDI-MS in combination with a curved field reflectron can be applied to obtain the molecular mass of cyclic carbohydrate derivatives with high mass accuracy and good mass resolution. This allows to establish the exact DS as well as the heterogeneity with respect to the DS of substituted β-cyclodextrins frequently applied in CE or HPLC as chiral selectors.

2. Experimental

2.1. Chemicals

Trifluoroacetic acid (HPLC/spectrograde, TFA) was purchased from Pierce (Rockford, IL, USA). Acetonitrile (LiChrosolv) and acetone (analytical grade) were supplied by Merck (Darmstadt, Germany). Water (18 $M\Omega^{-1}$ cm^{-1}) was prepared with an Elgastat apparatus (Elga, Bucks., UK). α-Cyano-4-hydroxy-*trans*-cinnamic acid (97%, CHCA) and 2,5-dihydroxy benzoic acid (99%, DHB) were obtained from Aldrich (Steinheim, Germany). Both matrices were used without any further purification. The peptides angiotensin III, substance P and bovine insulin were all obtained from Sigma (St. Louis, MO, USA).

2.2. Samples

β-Cyclodextrin (**1**) was a gift from the Department of Chemistry of the Polish Academy of Sciences (Warsaw, Poland). Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin [21 methoxy groups per β-cyclodextrin ring] (**2**), heptakis(2,6-di-O-methyl)-β-cyclodextrin [between thirteen and fifteen methoxy groups per β-cyclodextrin ring] (**3**), (2-hydroxy)-propyl-β-cyclodextrin with a DS of approx. 4.3 [between four and five (2-hydroxy)-propyl groups per β-cyclodextrin ring] (**4**), (2-hydroxy)-propyl-β-cyclodextrin with a DS of approx. 6.3 (**5**) and carboxymethylated β-cyclodextrin with a DS of approx. 2.5 to 3 (**6**) were obtained from Cyclolab R&D Laboratory (Budapest, Hungary). Compound 3 was purchased additionally from Aldrich. The DS data provided by the company (Cyclolab R&D Laboratory) were based on nuclear magnetic resonance (NMR) spectroscopy.

2.3. MALDI–MS

Measurements were performed with a Kompact MALDI IV (Shimadzu Kratos Analytical, Manchester, UK) laser desorption time-of-flight (TOF) instrument in the reflectron mode equipped with a nitrogen laser (λ=337 nm, 3 ns pulse width) and a coaxial curved field reflector [29,30]. The ions were accelerated to a final potential of +24 kV or −24 kV. The detector signal was digitized at a sampling rate of 300 MHz. Reflectron mass spectra were generated by signals averaging from 40 up to 100 unselected laser shots. The lowest possible laser power was applied for inducing the desorption and ion formation.

During laser firing, the low-molecular-mass ions ($m/z \leq 200$) were deflected by an ion gate and did not enter the reflector for preserving the annular dual microchannel plate (MCP) detector. Conversion of flight time to mass/charge was achieved by external calibration using angiotensin III, substance P and bovine insulin.

2.4. Sample preparation

The sample preparation was done either with CHCA (20 mg/ml in acetone containing 1% water) according to the recently reported thin-layer procedure [31] or with DHB [32] [10 mg/ml or saturated solution in acetonitrile–water (30:70) with 0.1% TFA] according to the commonly applied volume procedure. Sample solutions were prepared with water except for the unmodified β-cyclodextrin (**1**) which was prepared with plain acetonitrile. Aliquots (0.5 μl) of these sample solutions were used for sample preparation.

3. Results and discussion

3.1. Unmodified β-cyclodextrin (1)

The positive-ion MALDI mass spectrum (matrix: DHB) of β-cyclodextrin (**1**) exhibits cationization of the intact molecule with mainly one sodium ion at m/z 1158.27 (calculated average m/z value: 1157.99) and to a very low degree with one potassium ion, both present in the sample or as impurities on the stainless steel target and matrix material. Doubly charged molecular ions could not be detected at all despite their description in ISMS [23]. No significant fragment ions above m/z 200 due to e.g., cleavage of glycosidic bonds could be found at the applied laser power. Fig. 1 shows the molecular ion region of the mass spectrum of **1** in the reflectron mode applying a curved field reflector. Mass resolution of approx. 2200 (full width at half maximum, FWHM) for β-cyclodextrin and modified compounds could be achieved by applying a laser power near the desorption/ionization threshold level and the thin-

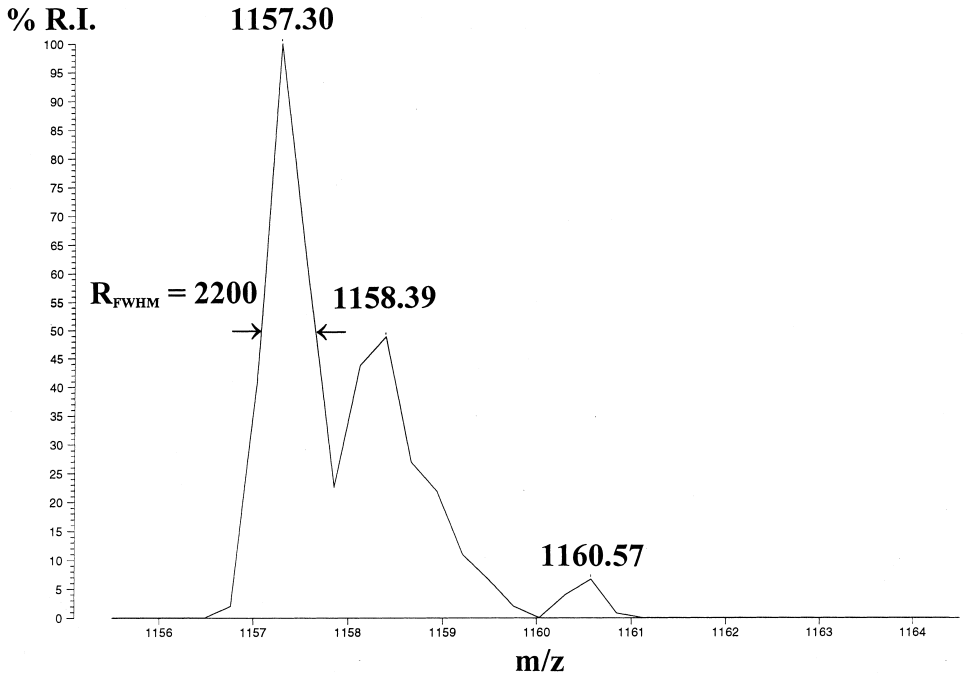

Fig. 1. Sodiated molecular ion $[M+Na]^+$ region of the curved field reflectron MALDI mass spectrum of compound **1** applying the matrix CHCA with the thin-layer technique (100 shots averaged). Calculated monoisotopic m/z value: 1157.36

layer sample preparation technique [31]. Both things in combination with a coaxial curved field reflector allowed to resolve the major stable isotope peaks with excellent mass accuracies (for **1**, ±0.01% external calibration). The reduction of the well-known energy spread of the formed molecular ions by the above mentioned steps generated this mass resolution and accuracy which is nowadays at the limit of a small-scale TOF instrument equipped with a 300 MHz digitizer without time-delayed extraction.

3.2. Modified β-cyclodextrins (2–6)

The observation of abundant molecular ions for 1 suggests that modification of the hydroxyl groups can be easily followed by MALDI-MS. To explore the potential of analysis of, for example an alkylated cyclodextrin, the positive-ion MALDI mass spectrum of compound **2**, a completely methylated (based on data of ^{1}H and ^{13}C NMR spectroscopy) cyclodextrin, was recorded in the reflector mode. A singly sodiated molecular ion was observed at *m/z* 1452.65 (calculated average *m/z* value: 1452.55) indicating the successful derivatization of all 21 hydroxy groups. Incompletely methylated derivatives were absent for this batch. Fragmentation was not detected at all especially when the laser power was optimized for maximum sodiated molecular ion yield.

Hitherto, the characterization of the purity of partially alkylated β-cyclodextrins has been difficult. Because MALDI was shown to generate mostly molecular ion signals, especially in matrices chosen for this characteristic, it seemed worthwhile to investigate its usefulness for determining the DS of β-cyclodextrins present in commercial products and to compare the results with other methods applied by the manufacturer. For this purpose, heptakis(2,6-di-O-methyl)-β-cyclodextrin with a DS of approx. 14 (**3**) from two companies, as well as two (2-hydroxy)-propylated β-cyclodextrins with a DS of 4.3 (**4**) and 6.3 (**5**) were used. The reflectron MALDI mass spectrum of **3** purchased from Cyclolab R&D Laboratory exhibited sodiated molecular ions starting with a DS of 13 (thirteen methoxy groups attached to β-cyclodextrin, *m/z* 1340.20) and increasing to a DS of 17 (seventeen methoxy groups attached to β-cyclodextrin, *m/z* 1397.70). Fragmentation of any kind was not detected. The relative intensity (R.I. %) of peaks can be expected to be a rough measure of the relative concentration of the various molecular species in the sample under the assumption of a similar mass spectrometric response. The most abundant peak in this mass spectrum was a sodiated molecular ion at *m/z* 1368.18 which corresponds to a modified β-cyclodextrin carrying fifteen methoxy groups. Additionally, compound **3** was obtained from Aldrich without any specified DS. In the mass spectrum of this material sodiated molecular ions corresponding to β-cyclodextrin with a DS of 14 (*m/z* 1354.77), 15 (*m/z* 1368.34) and 16 (*m/z* 1382.27) were detected. In this second commercial sample now the ions at *m/z* 1354.77 (DS 14) dominate the spectrum. Furthermore, it exhibits a lower degree of heterogeneity. This is an important point for the application of such chiral selectors in CE or HPLC obtained from different sources. The positive-ion MALDI mass spectrum of **4** revealed sodiated molecular ions starting with a DS of 3 [three (2-hydroxy)-propyl groups attached to β-cyclodextrins, *m/z* 1332.08] and increasing to a DS of 10 [ten (2-hydroxy)-propyl groups attached to β-cyclodextrins, *m/z* 1739.45]. No fragment ions were present, confirming that MALDI is a very soft desorption/ionization technique. The most intense peak in the mass spectrum was a sodiated molecular ion at *m/z* 1564.66 which corresponded to a modified β-cyclodextrin with a DS of 7. This observation is not in strict accordance with the mean DS (4.3±5%) specified by the manufacturer on the base of ^{1}H NMR spectroscopy. Table 1 exhibits all detected $[M+Na]^{+}$ ions of **4**. Fig. 2 shows the positive-ion MALDI mass spectrum of **5** exhibiting a similar broad distribution of sodiated molecular ions (*m/z* 1390.36 up to *m/z* 1738.22, Table 1) which parallels that observed for **4**. Again, the dominating sodiated molecular ion corresponded to a modified β-cyclodextrin with a DS of 7. This observation is now rather close to the DS (6.3±5%) specified by the company (based on ^{1}H NMR spectroscopy data).

In order to evaluate MALDI-MS for the analysis of differently modified β-cyclodextrins, a partially carboxymethylated β-cyclodextrin (**6**) with a DS between 2.5 and 3 (based on NMR data) was selected. The MALDI mass spectrum of **6** (Fig. 3) revealed a singly carboxymethylated product at *m/z* 1216.03 but also ion species with up to six additional

Table 1
Calculated (average) and measured m/z values for the sodiated molecular ions of compounds 1–6

Compound	Source and batch number	m/z		Δm[a]	Error (%)[b]	Type of ion	Degree of substitution (DS)
		Calculated	Measured				
1	Warsaw, PL	1157.99	1158.27	+0.28	+0.024	$[M+Na]^+$	0
2	Cyclolab, H CYL-389/2	1452.55	1452.65	+0.10	+0.007	$[M+Na]^+$	21
3	Cyclolab, H CYL-276/3	1340.34	1340.20	−0.14	−0.011	$[M+Na]^+$	13
		1354.36	1354.20	−0.16	−0.012	$[M+Na]^+$	14
		1368.39	1368.18	−0.21	−0.015	$[M+Na]^+$	15 (most abundant peak)
		1382.42	1382.70	+0.28	+0.020	$[M+Na]^+$	16
		1396.44	1397.70	+1.26	+0.090	$[M+Na]^+$	17
	Aldrich, D	1354.36	1354.77	+0.41	+0.030	$[M+Na]^+$	14 (most abundant peak)
		1368.39	1368.34	−0.05	−0.004	$[M+Na]^+$	15
		1382.42	1382.27	−0.15	−0.011	$[M+Na]^+$	16
4	Cyclolab, H CYL-242	1332.20	1332.08	−0.12	−0.008	$[M+Na]^+$	3
		1390.29	1390.72	+0.43	+0.031	$[M+Na]^+$	4
		1448.37	1448.78	+0.41	+0.028	$[M+Na]^+$	5
		1506.46	1506.16	−0.30	−0.020	$[M+Na]^+$	6
		1564.54	1564.66	+0.12	+0.008	$[M+Na]^+$	7 (most abundant peak)
		1622.63	1622.33	−0.30	−0.018	$[M+Na]^+$	8
		1680.71	1680.38	−0.33	−0.020	$[M+Na]^+$	9
		1738.80	1739.45	+0.65	+0.037	$[M+Na]^+$	10
5	Cyclolab, H CYL-226/2	1390.29	1390.36	+0.07	+0.005	$[M+Na]^+$	4
		1448.37	1448.23	−0.14	−0.010	$[M+Na]^+$	5
		1506.46	1506.66	+0.20	+0.013	$[M+Na]^+$	6
		1564.54	1564.66	+0.12	+0.008	$[M+Na]^+$	7 (most abundant peak)
		1622.63	1622.79	+0.16	+0.010	$[M+Na]^+$	8
		1680.71	1680.34	−0.37	−0.022	$[M+Na]^+$	9
		1738.80	1739.22	+0.42	+0.024	$[M+Na]^+$	10
6	Cyclolab, H CYL-570	1216.05	1216.03	−0.02	−0.002	$[M+Na]^+$	1
		1238.04	1237.62	−0.42	−0.034	$[M+2Na-H]^+$	1
		1274.09	1274.21	+0.12	+0.009	$[M+Na]^+$	2
		1296.08	1296.01	−0.07	−0.005	$[M+2Na-H]^+$	2
		1332.12	1332.58	+0.46	+0.035	$[M+Na]^+$	3
		1354.11	1354.29	+0.18	+0.013	$[M+2Na-H]^+$	3
		1390.16	1390.46	+0.30	+0.022	$[M+Na]^+$	4 (most abundant peak)
		1412.15	1412.33	+0.18	+0.013	$[M+2Na-H]^+$	4
		1448.20	1448.51	+0.31	+0.021	$[M+Na]^+$	5
		1470.19	1470.98	+0.79	+0.054	$[M+2Na-H]^+$	5
		1506.24	1506.81	+0.57	+0.038	$[M+Na]^+$	6

[a] Mass difference between measured and calculated $[M+Na]^+$ ion values.
[b] [(measured mass − calculated mass)/calculated mass] × 100.

carboxymethyl groups (m/z 1506.81) in the form of $[M+Na]^+$ and with lower abundance $[M+2Na-H]^+$ ions. Fragment ions were not detected at all. The most intense peak in this mass spectrum was a sodiated molecular ion at m/z 1390.46 which corresponded to a carboxymethylated β-cyclodextrin with a DS of 4 (Table 1). This value is not in agreement with the DS of 2.5 to 3 given by the manufacturer

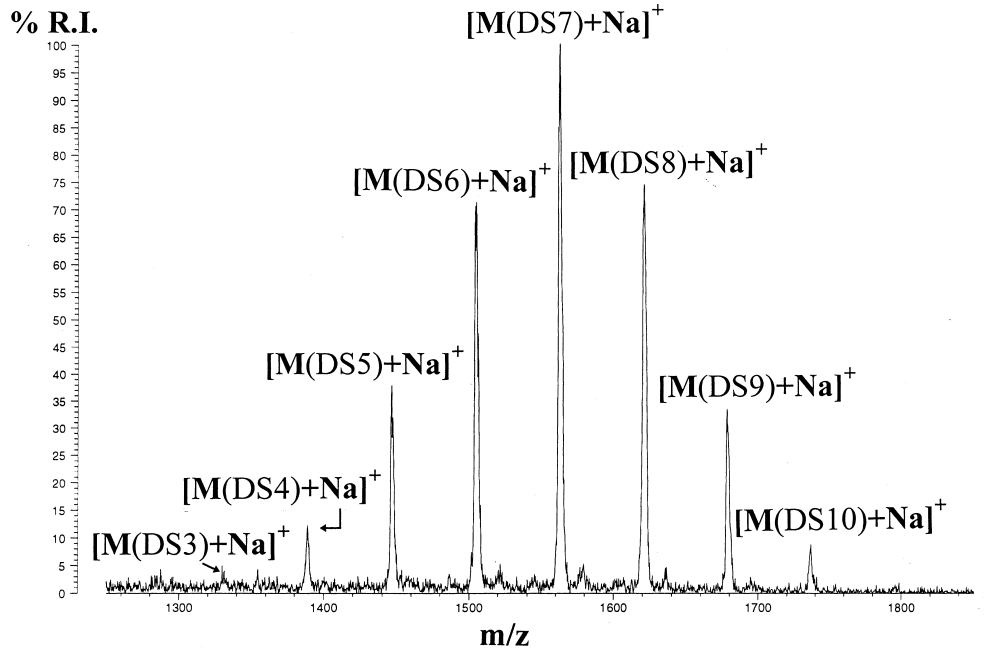

Fig. 2. Positive-ion curved field reflectron MALDI mass spectrum of compound **4** applying the matrix CHCA with the thin-layer technique (100 shots averaged).

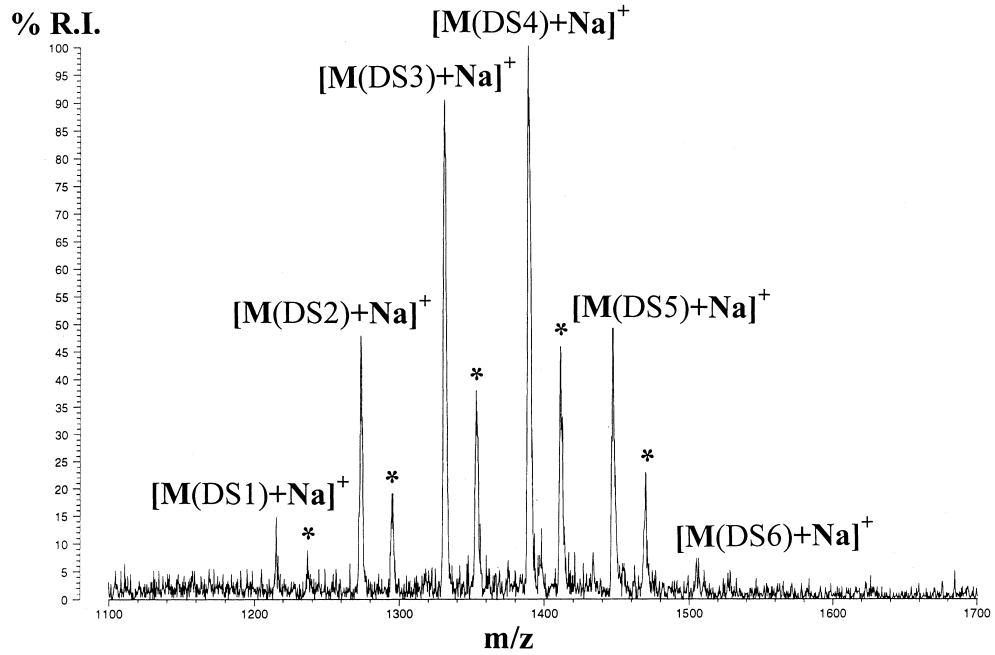

Fig. 3. Positive-ion curved field reflectron MALDI mass spectrum of compound **6** applying the matrix DHB with the volume technique (100 shots averaged). Peaks labelled with an asterisk correspond to $[M+2Na-H]^+$ ions.

(derived from NMR data). In the negative-ion MALDI spectrum of **6** (data not shown) abundant $[M-H]^-$ as well as $[M+Na-2H]^-$ ions were also observed.

Thus, MALDI-MS can be used to determine directly the composition of complex mixtures of incompletely modified β-cyclodextrins. The suppression of certain components in mixtures, as it is common in FAB- or PD-MS, has not been observed with the two applied MALDI matrices CHCA and DHB so far working at the lowest possible laser power. This is corroborated by the limited studies that have been published for carbohydrates [25,27,33] but the absence of discrimination effects is not completely proven. The obtainable mass accuracy was between −0.02% and +0.09% (Table 1) applying a curved field reflector.

4. Conclusions

Accumulated experience in CE and HPLC applying modified cyclodextrins [3,7,8] as chiral selectors heightened awareness of the influence of the cyclodextrin purity. This study has shown that MALDI combined with a curved field reflector TOF analyzer is a powerful technique for the study of modified cyclodextrins, generating well-resolved ($R_{FWHM} \approx$ 2000) sodiated molecular ions as dominating ion species at the sub-picomole level. Occurrence of fragmentation could be avoided by working at the threshold of the molecular ion formation. MALDI-MS offers detailed knowledge on the composition of mixtures and the degree of substitution (as demonstrated for compounds **3** to **6**) at least two orders of magnitude more sensitive than other mass spectrometric or spectroscopic methods. The high yields of sodiated molecular ions from permethylated, hydroxypropylated or carboxymethylated β-cyclodextrins suggest the feasibility of a highly sensitive approach to structural studies by employing post source decay fragment ion analysis [28–30,34] for locating modifications on the cyclodextrin ring system.

Any attempts to correlate enantioselectivity in CE or HPLC with the modification of the cyclodextrin selector have to be aware of the fact, that commercially available selector samples are far from uniform. Different degrees of substitution (DS) might have great impact on stereorecognition.

Acknowledgements

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