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Matrix Isolation Applied to the ^{252}Cf Plasma-desorption Mass Spectrometry of Underivatized Oligosaccharides

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A number of different low molecular weight and volatile compounds have been tested and compared as matrices in ^{252}Cf plasma-desorption mass spectrometry (PDMS) measurements of oligosaccharides. Some heteroaromatic amines (2-aminothiazole and 3-aminopyridine), hydroxyanthraquinones (alizarin and quinalizarin) and phthaleins (fluorescein) proved to be especially suited to enhancing the quasimolecular ion intensity of oligosaccharides. Discrimination effects could be reduced by matrix addition and thus, quantitative results of PDMS could be improved. Oligosaccharides with broad molecular weight distribution (dextrin 10 and dextran T 1.5) have been successfully analyzed by PDMS. The mass spectra obtained compared quite well with ion chromatograms of the respective oligosaccharides.

^{252}Cf plasma-desorption mass spectrometry (PDMS)[1] is already a valuable tool for protein chemists.[2] A suitable procedure for preparing PDMS samples is the deposition of analytes onto a substrate surface. For example, adsorption to nitrocellulose has been proven to be very effective for analyzing proteins.[3] Recently, Wolf and Macfarlane[4] suggested that the basic concept of using a volatile matrix to influence the internal excitation and the ionization of an involatile analyte, as successfully applied in matrix-assisted laser desorption (MALD),[5] could be also of importance in ^{252}Cf PDMS. They showed that small volatile molecules can be used successfully as PDMS adsorption matrices, in the same way as the polymeric matrices which have been used in the past. When 9-anthroic acid was used as substrate for proteins (insulin), sharper peaks and a lower background were observed if compared to spectra obtained from a nitrocellulose substrate, and the molecular ion peak $[M+H]^+$ is somewhat higher. The better effect of this volatile compound is assigned to a reduction of internal excitations and of the initial kinetic energy of the molecular ion and to a support of ionization in the course of the desorption process. 2-Aminoanthracene was also used as matrix to study the influence of the functional group on desorption and ionization. The result was a drastic reduction of the molecular ion yield, whereas the background was comparable to that of 9-anthroic acid. The different effect of these two compounds was attributed to the fact that the carboxyl functional group plays an important role in the protonation of insulin. Wolf and Macfarlane focused their discussion on the results obtained with 9-anthroic acid, but other small molecules such as anthrarobin, purpurogallin, purpurin, alizarin, quinalizarin and anthraquinone-2-carboxylic acid gave good-quality protein spectra as well. For optimizing the assisting matrix effect, it is important to modify the functionality, solubility, hydrophilicity and volatility of the substrate or matrix by selecting molecules that have the desired characteristics.

An impressive improvement of quantitative PDMS measurements, achieved by developing a matrix procedure, has been reported by Jungclas *et al.*[6] They analyse as a matter of routine the cytostatic drug Etoposide from patient blood serum, and have expanded the concentration range recorded by PDMS after thin-layer chromatography (TLC) separation as far as four orders of magnitude. Together with an internal standard, they added in excess sucrose octaacetate and urotropin to the PDMS sample. The improvement was attributed to the fact that an isolation of analytes in the matrix material and a suppression of sodium attachment to the molecular ions is achieved by this sample preparation procedure.

Oligo- and polysaccharides represent a group of biologically important compounds, either as free carbohydrates or as constituents of glycoconjugates. Some of the more recently developed mass spectrometric desorption/ionization techniques are proving to be of considerable utility for the analysis of carbohydrates.[7] Aduru and Chait[8] reported on their ^{252}Cf PDMS investigation of underivatized and peracetylated maltooligosaccharides, ranging in length from four to seven glucose units. They investigated systematically the effects on the spectra (1) of peracetylation, (2) of sample preparation by electrospray deposition onto metallic substrates versus adsorption onto nitrocellulose films, and (3) of sodium addition to, or elimination from, the sample. They observed: (1) that peracetylation enhances the sensitivity for measuring oligosaccharides by a factor of 2–3 when compared to the underivatized compounds, and obtained mass spectra showing substantial informative fragmentation; (2) that underivatized maltooligosaccharides deposited on nitrocellulose

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yielded mass spectra showing only very weak $[M+Na]^+$ ion peaks, indicating poor adsorption to nitrocellulose. But deposition on metallized Mylar foil by electrospray produced mass spectra consisting of dominant $[M+Na]^+$ and of series of weak fragment-ion peaks; (3) that the extent and type of fragmentation of peracetylated oligosaccharides adsorbed on nitrocellulose could be controlled by the amount of sodium in the sample. The positive-ion mass spectrum is dominated by a peak corresponding to the sodium-cationized molecule when a large molar excess of sodium salt is added. But when the sample is completely depleted of sodium, the spectrum shows no quasimolecular ion and is instead totally dominated by fragment-ion species.

It would be of great importance if it were possible to measure by mass spectrometry the important compound class of polysaccharides such as starch, glycogen or cellulose. These polysaccharides exhibit a broad molecular-weight distribution in contrast to, for example, enzymes, which are well-defined molecules. There are only a few papers dealing with the determination of molecular-weight distribution of polymers,[9,10] especially of polysaccharides. Series of linear polymers like polystyrene and polyethyleneglycol, of average molecular weight $M_n < 10\,000$ Da, were studied mainly with secondary-ion mass spectrometry (SIMS)[9] and recently also with MALD-MS.[10] MALD-MS of lignines, a class of cross-linked and irregular biopolymers, yielded the moleculear-weight distribution directly.[11] Direct chemical ionization mass spectrometry (DCI-MS) investigations on short-chain polysaccharides showed that the largest masses accessible so far by this technique are approximately 7000 Da.[12] Hillenkamp, Karas and coworkers[13] reported quite recently on MALD-MS analyses of maltodextrins (α-1, 4-linked oligoglucans) and of dextrans (α-1, 6-linked oligoglucans). With both analytes, series of monosodium and monopotassium oligoglucan ions were observed with 2,5-dihydroxybenzoic acid as matrix. The masses of the maltodextrins ranged from 500 to 3500 Da, equivalent to a degree of polymerization (DP) of up to 22. With dextrans, masses up to 7000 Da were observed, equivalent to a DP of up to 43. It could be shown in one case that the signal pattern of the mass spectrum is comparable to that of a chromatogram using ion chromatography (Dionex) and pulsed amperometric detection.

We investigated the assisting effect of a variety of volatile compounds used as isolating matrices on the quasimolecular ion intensity of oligosaccharides. In a first series, experiments with a linear and a cyclic oligosaccharide (maltoheptaose and α-cyclodextrin, respectively) were performed. Secondly, equimolar mixtures of three oligosaccharides were analysed to prove discrimination effects. Finally, the preparation technique was applied to commercially available maltodextrins and dextrans, and the PD mass spectra were compared with ion chromatograms obtained with these oligosaccharide samples. Some first results have been given elsewhere.[14]

EXPERIMENTAL

Materials. The cyclodextrins were obtained from Professor P. Köll, Carl von Ossietzky-Universität, Oldenburg, Germany. Maltoheptaose was obtained from Boehringer Mannheim GmbH, Germany. Dextrin 10, dextran T 1.5, 3-aminopyridine, and 3-aminoquinoline were purchased from FLUKA, Buchs, Switzerland, alizarin and quinalizarin from Merck, Darmstadt, Germany, fluorescein from Serva Feinbiochemica, Heidelberg, Germany, and 2-aminothiazole from Jannsen Chimica, Brüggen, Germany. All compounds tested as matrices (Table 1) were commercially available and used without any further purification.

Sample preparation. The oligosaccharides were dissolved in a 1:2 mixture of water + methanol at a concentration of 1 mg/mL. The matrix was added to this solution at a sugar/matrix ratio of 1:2 (w/w) in the case of cyclodextrins and maltoheptaose, and 1:5 (w/w) in other cases. For sample preparation, 10 µL of solution was deposited onto an aluminized polyester foil (2.5 µm thick) by the use of a nebulizer. By this procedure, a thin, almost homogeneous layer is produced on the foil surface. The nebulizer consists of a glass capillary, a 10 µL microsyringe, and a tube for feeding nitrogen as carrier medium. Some samples have also been prepared by electrospray deposition and no significant change of the quasimolecular ion intensities has been observed. The amount of sodium on the sample has been monitored only by inspection of the spectra. The result was that the intensities of the Na^+ signal do not vary very much. The influence of sodium addition was tested when using amino compounds as matrix, and only a decrease of the molecular ion signal was observed. The preparation of maltoheptaose in 2-aminothiazole has proven to be highly reproducible with the corresponding spectrum showing approximately the same quasimolecular ion intensity (10-fold increase when compared to preparation without matrix addition). Consequently, each measurement using other matrices and analytes has been made using a maltoheptaose + 2-aminothiazole sample as a standard.

^{252}Cf PDMS. The PD mass spectra were obtained with a linear time-of-flight mass spectrometer[15] which was built in cooperation with Professor K. Wien, TH Darmstadt, Germany. The spectrometer is equipped with a ^{252}Cf source (effective fission fragments during the time of this study: about 250/s), 2 multichannel plate (MCP) detectors for the start and stop signals, respectively, a 0.8 m drift tube, and a time/digital converter CTN/M2 from IPN, Orsay, France. The data were collected and stored in an IBM PC/AT computer. Usually, the run time was 10 min, and the acceleration voltage +14 kV. Mass resolution is about 600. Therefore, only isotopically averaged molecular weights were determined for the oligosaccharides. The half-width of the molecular-ion peak observed in the maltoheptaose or in the α-cyclodextrin spectra was between 2 and 3 mass units independently of the sample preparation.

Ion chromatography. The dextrin 10 and dextran T 1.5 were separated by anion exchange chromatography in a basic medium using an AS-6 column (250 × 4 mm, Dionex Corp., Sunnyvale, CA, USA. The detection was achieved by pulsed amperometry. Initially, the eluent was 100 mmol NaOH. After 5 min, a linear

Table 1. List of compounds tested as matrices for PDMS measurements of oligosaccharides

2,5-Dihydroxybenzoic acid	−	2-Aminophenol	−
Sinapic acid	−	2-Aminopyridine	−
Syringic acid	−	3-Aminopyridine	+
Gallic acid	−	4-Aminopyridine	−
Nicotinic acid	−	3,4-Diaminopyridine	−
Ferulic acid	−	1-Naphthylamine	−
Caffeic acid	+	3-Aminoquinoline	−
β-Alanine	−	2-Aminothiazole	+ +
Tryptophane	−	2-Aminobenzothiazole	+
		2-Amino-4-methyl-thiazole	−
		2-Aminothiazoline	−
		Aminopyrazine	−
Phenol	−	3-Nitroaniline	−
Quercetin	−	1,4-Diaminoanthraquinone	+
Alizarin	+ +	1-Aminoanthraquinone	−
Quinalizarin	+ +	2-Aminoanthraquinone	−
Fluorescein	+ +	3-Aminopyrazole	−
1,4-Dihydroxyanthraquinone	+	Pyrazole	−
9-Fluorenone	+	Imidazole	−
		Benzidine	+
		Benzimidazole	+
		Anthrachinone	−
Dithiothreitol	+	Urea	−
		Acetamide	−
		Thioacetamide	−

− no or low enhancement; + approximately 5-fold enhancement; + + approximately 10-fold enhancement.

gradient from 10 mmol to 500 mmol sodium acetate was applied.

RESULTS AND DISCUSSION

The compounds chosen as matrices for this study were required to be soluble in water or water + methanol mixtures so that they could give homogeneous aqueous or aqueous-methanolic solutions and could be deposited together with the analyte onto the aluminized polyester foil by our spraying equipment. Secondly, the compounds were required to be volatile but also compatible with the high vacuum conditions in the spectrometer. Finally, the compounds were chosen for their ability to form hydrogen bonds to the sugar, so that analyte molecules could be isolated from each other. Several classes of compounds were investigated. A list of compounds together with a classification of suitability is given in Table 1. The classification of suitability was made by comparing the quasimolecular-ion intensity of the analyte (maltoheptaose or α-cyclodextrin) with that observed when the same amount of sugar was sprayed onto the aluminized foil without addition of matrix compounds. A maltoheptaose spectrum obtained without matrix addition is given in Fig. 1(*a*).

First, we tested aromatic acids such as 2.5-dihydroxybenzoic acid and nicotinic acid, which are typical MALD-MS matrices.[13] In PDMS, this class of compound produced no assisting matrix effect with oligosaccharides. The only exception was caffeic acid, but the enhancement of the quasimolecular-ion intensity produced by this compound was rather low.

Then, we tested compounds with an amino functional group, based on the premise that these compounds should be able to break hydrogen bonding between the sugar molecules and to form proton donor–acceptor complexes with them. Some very interesting matrix effects were observed when using heteroaromatic amines that dramatically enhanced molecular-ion yields. For example, 2-aminothiazole as an isolating matrix increased the quasimolecular-ion yield of maltoheptaose by a factor of 10 (Fig. 1(b)). The influence of the matrix is drastically affected by varying the molecular structure. For example, 2-aminothiazoline which is derived from 2-aminothiazole by hydrogenation of one double bond, or 2-amino-4-methylthiazole derived by introduction of one methyl group in position four of the heterocycle produces no molecular-ion intensity enhancement at all. On the other hand, 2-aminobenzothiazole does give an enhancement, but less than that given by 2-aminothiazole.

When we began our study, the first effective matrix we found was 3-aminopyridine, and several measurements were made using this matrix. A maltoheptaose spectrum obtained with 3-aminopyridine as matrix is given in Fig. 1(c). In later measurements, we found that 3-aminopyridine was less effective than 2-aminothiazole. Using 3-aminopyridine as the matrix and α-cyclodextrin as the analyte, we studied the influence of the analyte/matrix molar ratio on the analyte molecular-ion intensity. The amount of analyte sprayed onto the sample varied from 60 nmol to 0.06 nmol, whereas the amount of 3-aminopyridine was kept constant at 60 nmol. Thus, the analyte/matrix molar ratio decreased from 1:1 to 1:1000. But the quasimolecular-ion intensities observed in the spectra of the series were reduced only to 14% by this drastic analyte reduction (see Table 2).

The effect of varying the molecular structure of the matrix molecule proved to be quite informative. Using 2-aminopyridine, 4-aminopyridine or 3,4-diaminopyridine instead of 3-aminopyridine as

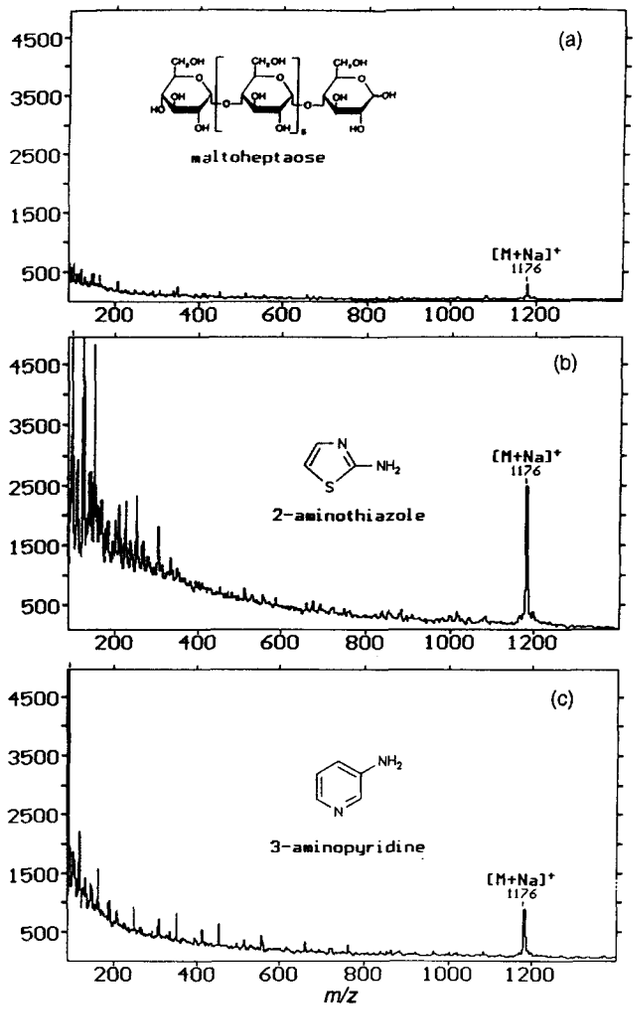

Figure 1. Positive-ion spectra of maltoheptaose: (a) preparation without matrix addition, total number of recorded quasimolecular ions: 339; (b) in 2-aminothiazole as isolation matrix, total number of recorded quasimolecular ions: 2982; (c) in 3-aminopyridine as isolation matrix, total number of recorded quasimolecular ions: 1210.

matrices, less intense quasimolecular-ion peaks of α-cyclodextrin were observed. The decreasing peak intensities could be correlated with increasing gas-phase basicities of the respective pyridine derivatives (Fig. 2). These results suggest that the basicity of the matrix compounds is an important feature of their suitability as matrix and must match that of the analyte. Probably, the matrix should have a somewhat higher basicity than the analyte to break the intermolecular hydrogen bonds of the sugar. But if the basicity of the matrix is too high, the analyte/matrix complex desorbed may not dissociate to give quasimolecular

Table 2. Peak height of quasimolecular ion correlated with the mole fraction of α-cyclodextrin in the sample[a]

Amount of α-cyclodextrin (nmol)	Mole fraction of α-cyclodextrin	Peak height intensity (%)
60	1:1	100
6	1:10	32
3	1:20	27
0.6	1:100	20
0.06	1:1000	14

[a] Amount of the 3-aminopyridine matrix was kept constant at 60 nmol.

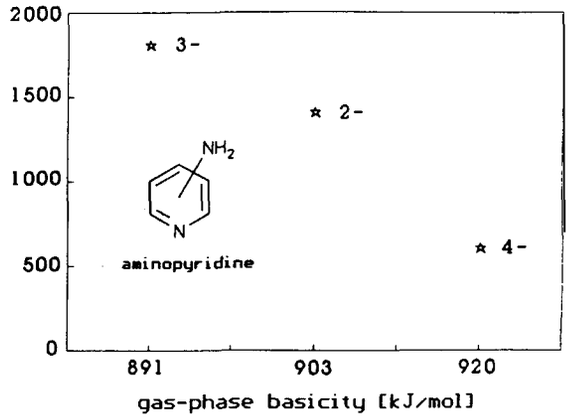

Figure 2. Peak height of the α-cyclodextrin quasimolecular ion correlated with the gas-phase basicity of different aminopyridine positional isomers.

ions. Our findings suggest that the most effective matrix should have a gas-phase basicity in the range between 800 kJ/mol the approximate gas-phase basicity of the sugar—and 890 kJ/mol—the value of 3-aminopyridine. When we expanded our study to include molecules that

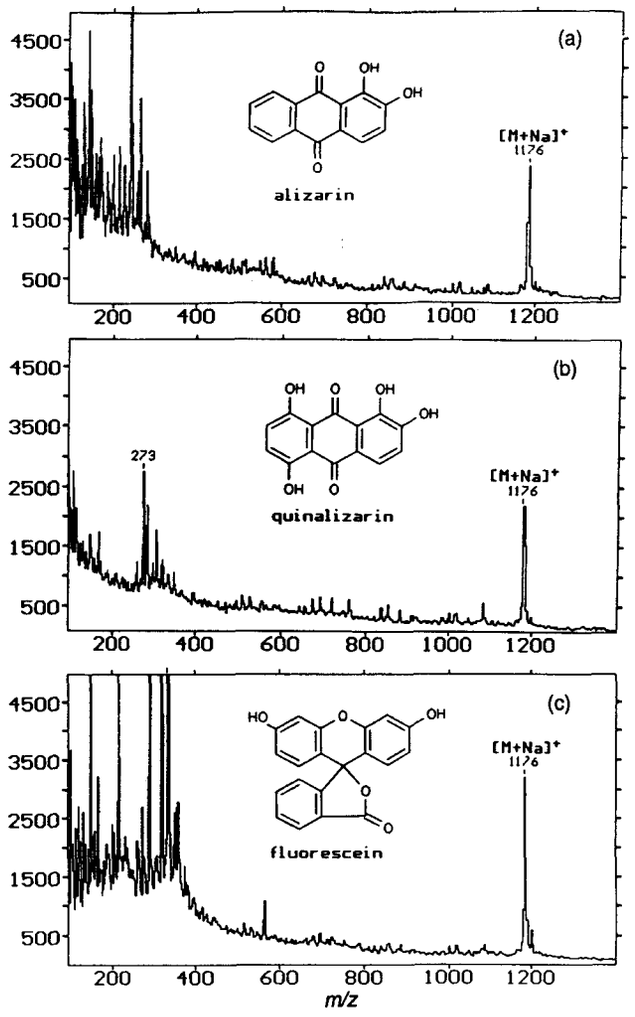

Figure 3. Positive-ion spectra of maltoheptaose: (a) in alizarin as isolation matrix, total number of recorded quasimolecular ions: 2821; (b) in quinalizarin as isolation matrix, total number of recorded quasimolecular ions: 3927; (c) in fluorescein as isolation matrix, total number of recorded quasimolecular ions: 4850.

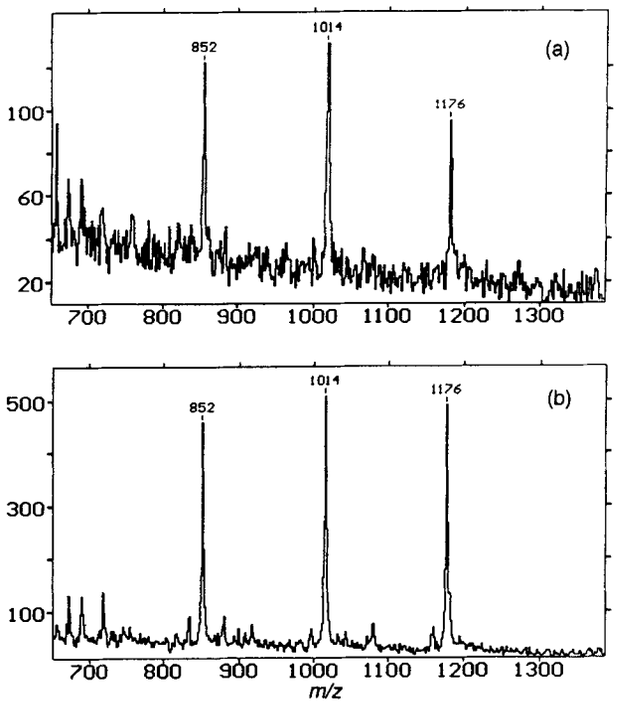

Figure 4. Quasimolecular-ion peaks obtained with an equimolar mixture of maltopentose (m/z 852), -hexose (m/z 1014) and -heptose (m/z 1176): (a) preparation without matrix; (b) in 3-aminopyridine as isolation matrix.

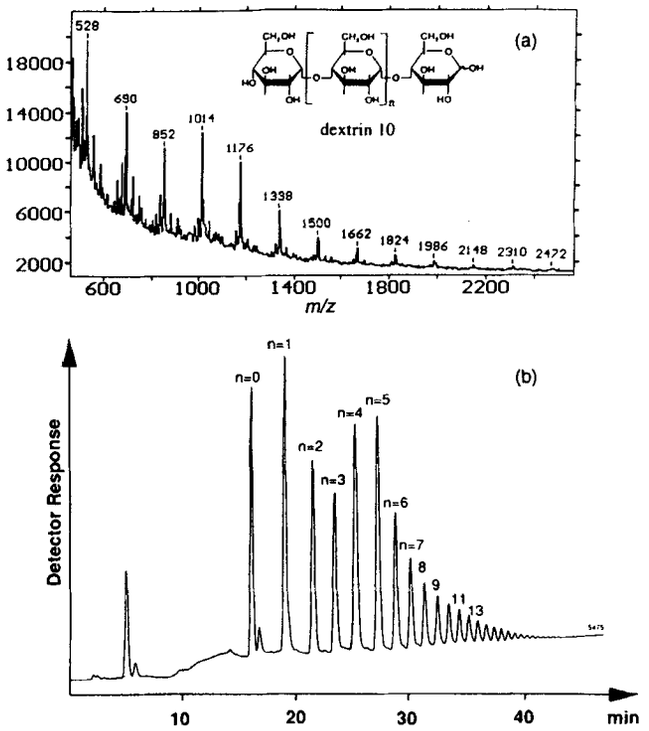

Figure 6. (a) PD mass spectrum (matrix: 2-aminothiazole) and (b) ion chromatogram of dextrin 10.

did not have basic functional groups, the importance of hydrogen bonding between individual analytes was strengthened.

Hydroxyanthraquinone derivatives have also been investigated. Alizarin (Fig. 3(a)) and quinalizarin (Fig. (b)) proved to be very effective matrices for oligosaccharides. These compounds applied as adsorption matrices have already been mentioned by Macfarlane[4] to give good-quality protein spectra with sharper peaks

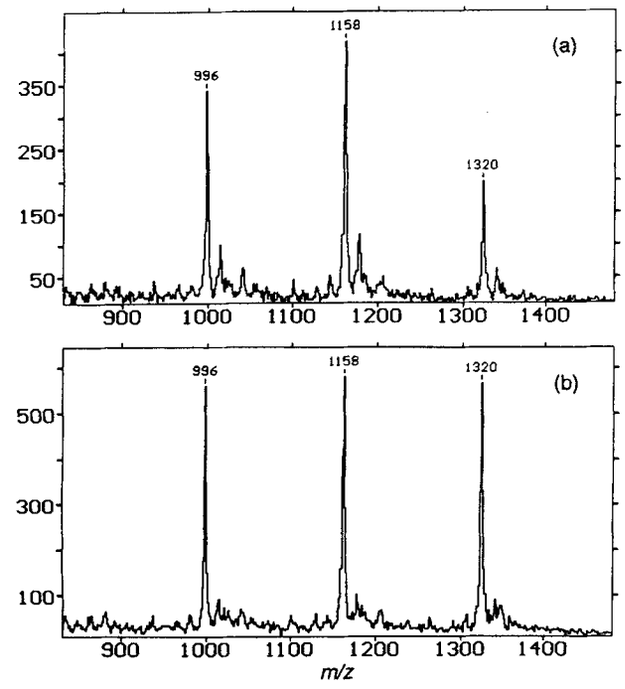

Figure 5. Quasimolecular-ion peaks obtained with an equimolar mixture of α-, β- and γ-cyclodextrins (m/z 996, 1158, 1230): (a) preparation without matrix; (b) in 3-aminopyridine as isolation matrix.

but with only minor peak enhancement. With oligosaccharides, we observed that the peak intensity is enhanced by one order of magnitude. Anthraquinone itself is not effective as matrix. Thus, it is apparent that hydroxyl groups on the matrix molecule are necessary for an enhancement effect, probably because they can

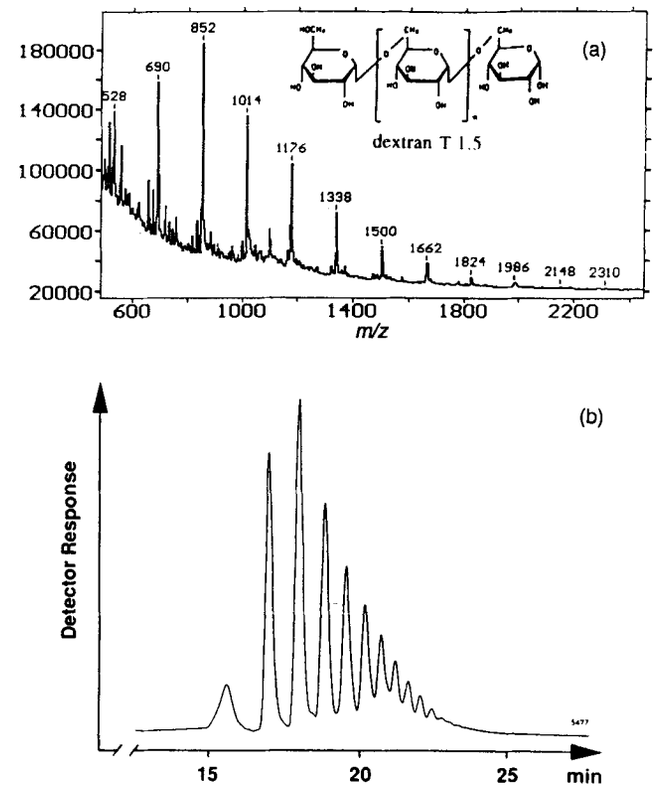

Figure 7. (a) PD mass spectrum (matrix: 3-aminopyridine) and (b) ion chromatogram of dextran T 1.5.

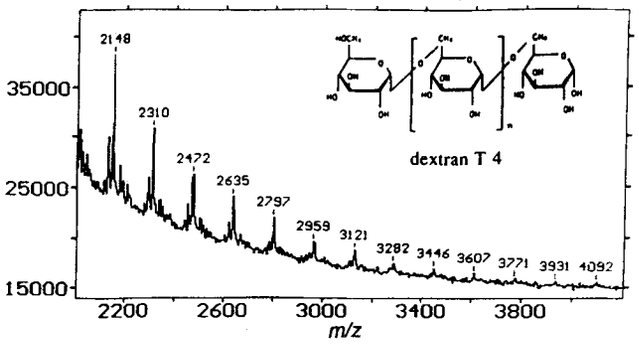

Figure 8. Positive-ion spectrum, from *m/z* 2000 to *m/z* 4200, of dextran T 4 in 3-aminopyridine.

form hydrogen bonds with the sugar molecules. This conjecture is supported by the remarkable result that we observed a very high enhancement of the quasimolecular-ion peak using fluorescein (Fig. 3(c)) as a matrix. In this case, the acidity of the matrix seems to be of importance, in contrast to the discussion of the gas-phase basicity of amino compounds reflecting the fact that hydrogen bonding involves proton-donating and -accepting functional groups. With dithiothreitol, a matrix introduced by Roepstorff,[16] we observed an assisting effect which is comparable to that of 3-aminopyridine, but much less than that of 2-aminothiazole or of alizarin.

The results presented show clearly that the addition of low molecular weight and volatile compounds influences considerably the molecular-ion intensity of oligosaccharides. The next objective was to study mixtures of oligosaccharides dispersed in a matrix to determine whether there is a discrimination against sugars having different molecular masses. We prepared two samples, with equimolar solutions of three linear and three cyclic α-glucans, respectively. The spectra obtained with these samples are given in Figs 4 and 5. Discrimination against highest molecular weight α-glucans, i.e. maltoheptaose and γ-cyclodextrin, respectively, is observed when the samples have been prepared without matrix addition. But with matrix addition, i.e. 2-aminothiazole and 3-aminopyridine, respectively, all three oligosaccharides are observed at equal intensity. These tests clearly show that quantitative results are also improved by matrix addition.

Finally, we applied the method of matrix assistance to the analysis of commercially available oligosaccharides having broad molecular mass distributions. Fig. 6(a) shows a spectrum of dextrin 10 with 2-aminothiazole as matrix. A series of sodium-attached oligoglucan ions is observed. The molecular weights of the compounds giving the quasimolecular ions range from 528 Da up to 2472 Da, equivalent to a DP (degree of polymerization) of 3 up to 15. The ion chromatogram of the same maltodextrin sample is depicted in Fig. 6(b). The signal pattern of the PD mass spectrum is closely connected to that of the chromatogram. The chromatogram shows oligomers with a DP > 15 at very low concentration, but these oligomers are not observed in the PD mass spectrum due to the background. Dextran T 1.5, an oligosaccharide sample of different structure but with a similar average molecular weight, gave a similar correspondence between mass spectrum and ion chromatogram. The results are given in Fig. 7. A spectrum of a dextran having an average molecular weight of 4000 Da (dextran T 4) shows oligomers up to DP = 25 (Fig. 8), but in this case high

CONCLUSION

The suitability of a variety of low molecular weight and volatile compounds as PDMS matrices for improving the analysis of oligosaccharides has been tested. An approximately 10-fold enhancement of the molecular weight oligosaccharides are obviously discriminated against. A MALD-MS analysis[12] of a similar dextran sample (dextran T 5) showed masses up to 7000 Da, equivalent to a DP up to 43.

quasimolecular-ion intensity has been observed by adding, in excess, heteroaromatic amines or hydroxyanthraquinones. Variations of the molecular structure of the matrix compounds lead to drastically different matrix effects. The suitability of a matrix for oligosaccharide analysis is discussed mainly with regard to an efficient isolation of the analytes, especially directed to reducing aggregation of oligosaccharides in the matrix by hydrogen bonding. But the same interactions are probably also important to the formation of quasimolecular ions. The basicity of the matrix compounds may play an important role, probably in the ionization step. Addition of aromatic acids, which are very successful MALD-MS matrices, do not generally enhance the quasimolecular-ion intensities of oligosaccharides.

By matrix addition, discrimination against oligomers is clearly reduced when mixtures of oligosaccharides are measured. Dextrin 10 and dextran T 1.5 have been successfully analyzed by PDMS. With oligosaccharide samples having a higher average molecular weight, we observed oligomers up to DP = 25, but the ion intensities do not correspond to the expected distribution.

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