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Applications of matrix-assisted techniques in plasma desorption mass spectrometry[1]

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Abstract

A report on our research activities regarding applications of matrix-assisted techniques in PDMS is presented. Analyte–analyte interactions between adsorbates on nitrocellulose are studied. The suitability of 3-aminopyridine as matrix for carbohydrates is shown. The detection of negative C_{60} ions from a femtomole-sample is made possible by matrix assistance.

Key words: Plasma desorption mass spectrometry; Matrix-assisted techniques; Analyte–analyte interactions.

Introduction

A report on research activities in Oldenburg is presented regarding applications of matrix-assisted techniques in PDMS. The isolation of analyte molecules in a matrix or on a substrate makes PDMS spectra more reproducible and often enhances the detection sensitivity, especially if the formation of stable analyte ions is supported by process-induced interactions between analyte and matrix or substrate. These interactions have a strong influence on the ion yield, especially for larger molecular ions [1–4].

The PDMS yield is also strongly dependent on analyte–analyte interactions if the applied analyte concentration is high. Also, high local concentrations produced, for example, by self-aggregation or by cluster formation have a considerable effect on the PDMS yield [5]. Precise information about the amount and the arrangement of molecules on the sample are required for studying these analyte–analyte interactions. We apply optical spectroscopy methods for characterizing our samples. The absorption of light from the near IR to UV via diffuse reflectance and the sample fluorescence can be determined. Much information about sample composition and structure is obtained by evaluating the optical spectra.

The determination of analyte concentrations on the sample is one of the very useful advantages of this application. For example, a coverage in the range from 6×10^{12} to 6×10^{15} adsorbates per cm^2 can be determined precisely enough by the diffuse reflectance method. Results with a varied coverage of dyes on nitrocellulose are presented.

The mass spectrometry of underivatized carbohydrates has been an intensive object of research in the last few years [6–13]. An extension of the accessible mass range is one important aspect of applying matrix-assisted techniques. PDMS results on a maltooligosaccharide and on dextrans are pre-

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sented showing clearly the supporting effect of a heteroaromatic amino compound as matrix.

Many research activities on the carbon cluster compounds fullerenes have been started since production of macroscopic amounts was made possible in 1990 [14]. Mass spectrometry is one important method for characterizing this new class of compounds. We present PDMS spectra of fullerenes which we obtained by preparing a C_{60}/C_{70} mixture in a matrix.

Experimental

The PDMS spectra of the samples were recorded by our spectrometer OLDA 1. This linear time-of-flight instrument has a 10 μCi ^{252}Cf source, an 80 cm flight tube, two double stage multichannel plates for start or stop signals, and a time/digital converter CTM/M2 from the IPN in Orsay. The data were collected in an IBM PC/AT computer by a direct memory incremental card. Mass resolution is about 700.

Dyes were dissolved in 2-propanol at varied concentrations. The dye samples were prepared by applying 5 μl solution to a 1 cm^2 nitrocellulose film which was made by electrospraying 40 μl of a 2 mg ml^{-1} acetone solution on an aluminized polyester foil. The amount of dyes adsorbed on the substrate were determined by diffuse reflectance and varied in the range from 6×10^{12} to approximately 6×10^{15} cm^{-2}. The sample fluorescence has been measured by a fluorimeter, excitation wavelength was 460 nm with rhodamine 6G. For recording the mass spectra, the spectrometer was operated with +10 kV, the run time was 30 min.

α-Cyclodextrin was dissolved in a 1 : 2 mixture of water and methanol at a concentration of 0.1 mg ml^{-1}. The matrix was added to this solution at a ratio (w/w) of 1 : 2. Dextran T 1.5 and T4 were dissolved in a 1 : 2 water/methanol mixture at a concentration of 1 mg ml^{-1}. In this case the matrix was added at a ratio of 1 : 10 (w/w). For sample preparation, 10 μl of solution was sprayed onto an aluminized polyester foil by an air-brush-like apparatus. This apparatus consists of a glass capillary, a 10 μl microsyringe and a pipe for feeding N_2 as carrier medium. By this procedure, a very thin, almost homogeneous layer was produced on the foil. The mass spectra of α-cyclodextrin were collected within 15 min (operating voltage, +10 kV). The dextran spectra were recorded in a 12 h run (operating voltage, +16 kV).

The fullerene samples were prepared by spraying 15 μl of benzene solution onto an aluminized polyester foil using the air-brush equipment. The amount of β-carotene was approximately 28 nmol on all samples. The amount of fullerenes was varied in a range of less than 5 nmol. For comparison, a sample of 5 nmol fullerenes without β-carotene was also prepared. Acceleration voltage was ±14 kV.

Results and Discussion

Analyte–analyte interactions between adsorbates on nitrocellulose

PDMS spectra of nitrocellulose samples with varied amounts of adsorbed rhodamine 6G molecules were recorded. The intensity of the molecular ion signal centered at m/z 443 was determined by integrating over all peaks observed from m/z 440 to 446. The molecular ion intensity divided by the number of start events, i.e. the relative ion

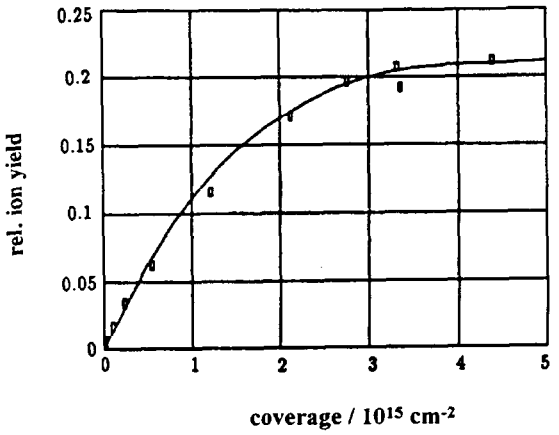

Fig. 1. Relative yield of rhodamine 6G ions as a function of coverage.

yield, is depicted in Fig. 1 as a function of the rhodamine 6G coverage. As shown in this figure, the relative molecular ion yield increases linearly with the amount of adsorbed rhodamine 6G molecules at coverages of less than $8 \times 10^{14}\,cm^{-2}$ and becomes saturated at larger values.

A linear increase of the relative molecular ion yield at coverages of less than $8 \times 10^{14}\,cm^{-2}$ and a saturation at larger values was also obtained with the dyes malachite green and chlorophyll-a (figures are not presented). The chlorophyll-a spectrum is dominated by a lot of characteristic fragment peaks and the relative fragment ion yield turns out to show a very similar dependence on the chlorophyll-a coverage. The relative molecular ion yields of rhodamine 6G and malachite green are nearly the same, but with chlorophyll-a a much lower value was obtained for the molecular ion. These results reflect the fact that rhodamine 6G and malachite green are cations of an organic salt and that the fragmentation rate is rather high with chlorophyll-a.

However, the results indicate that the observed saturation is not due to single ion counting effects or to other setup parameters. It is concluded that the similar dependence of the relative ion yield on the amount of adsorbed molecules is more correlated with the limited surface area offered by the nitrocellulose. The microscopic surface area of the nitrocellulose is much larger than the macroscopic $1\,cm^2$ sample area due to the roughness of nitrocellulose. But it is obviously impossible to adsorb on the nitrocellulose more than 10^{15} analytes per cm^2 without clearly increasing interactions between the adsorbed species.

No indications for increasing aggregation or cluster formation of analytes were found in the absorption spectra evaluated from the diffuse reflectance of the samples. Position and shape of the analyte absorption are the same and only the intensity varies in the range of coverage investigated. But the sample fluorescence shows a clear dependence on the coverage. In Fig. 2, the spectral fluorescence integral of rhodamine 6G samples is presented as a function of the coverage. The fluorescence increases linearly with the amount of rhodamine 6G on nitrocellulose at coverages of less than $5 \times 10^{14}\,cm^{-2}$. The fluorescence shows a distinct maximum at $1 \times 10^{15}\,cm^{-2}$ and then decreases with larger values.

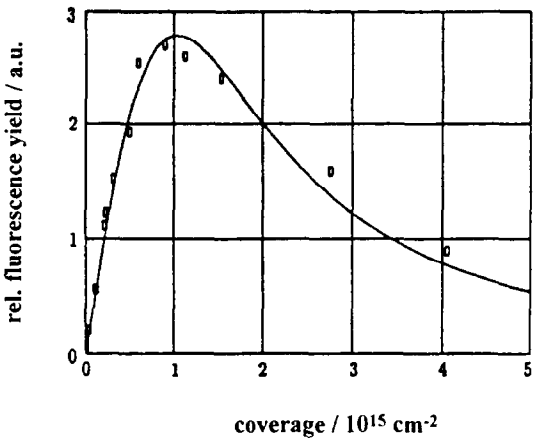

Fig. 2. Rhodamine 6G fluorescence as a function of coverage.

The linearly increasing rhodamine 6G fluorescence at low coverage demonstrates clearly the existence of isolated rhodamine 6G molecules on the nitrocellulose. The fluorescence saturates and decreases at larger coverages owing to a growing probability of energy transfer processes between neighbouring dye molecules at smaller distances. These processes are produced by dipole–dipole interaction and finally give rise to a decreasing fluorescence yield of the samples. In consideration of the fluorescence data, the mean distance between adjacent rhodamine 6G molecules is estimated to be about 6 nm at $8 \times 10^{14}\,cm^{-2}$, i.e. it slowly approaches the molecular size [15].

Thus, the linear dependence of the relative ion yields on the amount of analytes, observed at low coverage, is attributed to the linearly increasing probability that isolated adsorbates are "hit" by a passing fission fragment. The nonlinear behaviour observed at larger coverages is obviously due to increasing interactions between the analytes, probably via excited electronic states. The resulting effect is a competition between adjacent analytes leading to a considerable modification of the relative ion yields.

Very similar results have been obtained in a study of ion adsorption on polypropylene and Mylar surfaces [16]. The ion intensities deduced from PDMS spectra in this study were found to be a function of solution concentration reaching a maximum value at full monolayer coverage. From our results obtained with nitrocellulose it is not necessarily concluded that a full monolayer is reached within the applied range of coverages.

3-Aminopyridine as matrix for carbohydrates

The development of suitable matrices for carbohydrates is one of our objects of research which we are following up together with J.O. Metzger and his collaborators in Oldenburg. Heteroaromatic amino compounds have been proven to give better results than, for example, nitrocellulose or sinapinic acid [11]. PDMS spectra of α-cyclodextrin are depicted in Fig. 3 demonstrating the matrix effect of 3-aminopyridine. The upper spectrum has been taken without matrix, the lower one was obtained with α-cyclodextrin embedded in 3-aminopyridine. The peak at m/z 996 represents the quasimolecular ion $[M + Na]^+$ of α-cyclodextrin. A small amount of β-cyclodextrin is also present on the sample and leads to the peak at m/z 1158. Fragments of the cyclodextrins are observed at m/z 127, 145, 163, and 326. In comparison, many more fragments than quasimolecular ions are obtained in the spectrum taken without matrix, whereas in the spectrum obtained with 3-aminopyridine as matrix the quasimolecular ion yield is much larger.

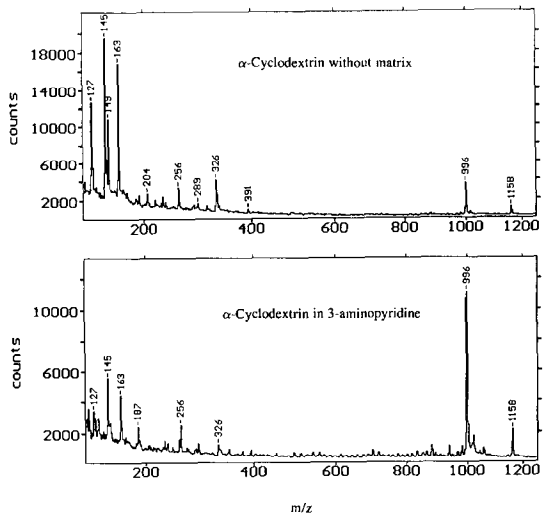

Fig. 3. α-Cyclodextrin spectra with and without matrix.

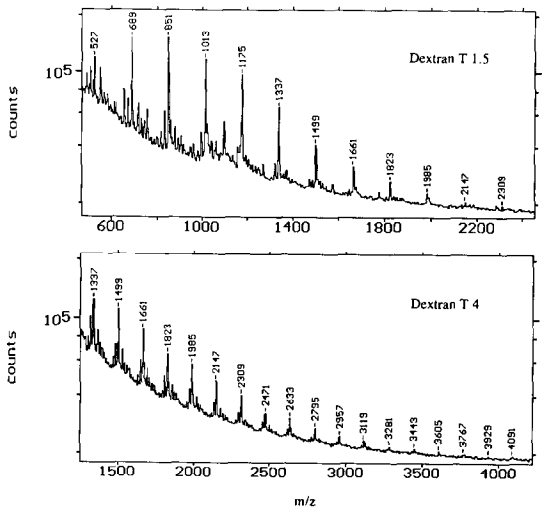

Fig. 4. Spectra of dextrans in 3-aminopyridine.

Figure 4 shows PDMS spectra of dextran T 1.5 and T 4 in 3-aminopyridine. These dextrans are branched 1,6-D-glucose polymers with an average molecular weight of 1500 and 4000 Da, respectively. Thus, dextrans are suitable compounds for testing the mass range made accessible by matrix assistance. The spectra of Fig. 4 show the quasimolecular ion series of oligomers with mass increments of 162 Da up to 2309 and 4091 Da for dextran T 1.5 and dextran T 4, respectively. The intensity pattern observed for the oligomer ions might be correlated with the abundances of oligomers in the case of dextran T 1.5, but certainly not for dextran T 4.

Thus, the suitability of 3-aminopyridine as matrix for carbohydrates has been proven by the results presented, but the reasons for the limited detection of carbohydrates in the range of higher masses are still not clear. An elucidation of the mechanism, by which the formation of quasi-

molecular ions is supported when applying matrix assisted techniques, is obviously necessary to solve the problem.

Detection of C_{60} from a femtomole-sample

Figure 5 shows the molecular ion region of a positive and negative ion spectrum which were taken from a sample prepared by spraying a pure fullerene solution on an aluminized polyester foil. The amount of fullerenes in the sample was 5 nmol, the run time in both modes was 60 min. The positive molecular ions of C_{60} are observed as $[M]^+$ and the negative as $[M]^-$, but in the negative ion spectrum $[M + H]^-$ is also slightly present. Comparing both spectra in Fig. 5, the most striking feature is that the relative ion yield for positive C_{60} ions is much larger than for the negative molecular ions.

In contrast, if the fullerenes are embedded in β-carotene as matrix, many more negative than positive molecular ions are observed. Figure 6

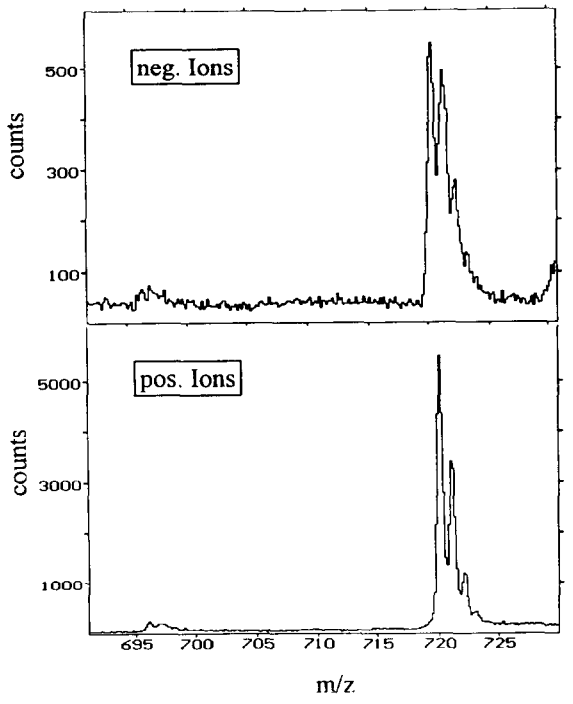

Fig. 5. Positive and negative C_{60} ion peak obtained without matrix.

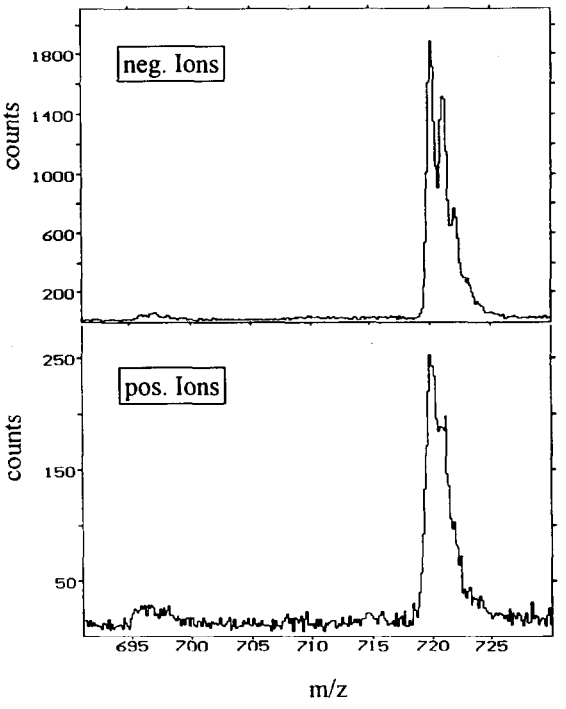

Fig. 6. Positive and negative C_{60} ion peak obtained with β-carotene as matrix.

shows spectra obtained from a sample prepared by spraying a fullerene/β-carotene solution. Five mmol fullerenes were present in the sample, as above, and 28 nmol β-carotene. Run time was, in this case, 15 min. The relative yield for negative C_{60} ions is dramatically enhanced by the addition of β-carotene, whereas the relative yield for the positive C_{60} ions is reduced, but not on the same scale. The count rate for the sum of positive and negative C_{60} ions is clearly increased if compared with the spectra of Fig. 5. The β-carotene ions are observed predominantly as $[M + H]^+$ and $[M + H]^-$, $[M]^+$ and $[M]^-$ are only slightly present in the spectra (not shown in the figure). Regarding the molecular ions of β-carotene, a much higher relative yield for the positive ions is obtained than for the negative ions. A growing amount of fullerenes results in a decrease of negative β-carotene ions.

Thus, the dramatic enhancement of negative C_{60} ions in favor of both the positive C_{60} and the negative β-carotene ions, raises the sensitivity for observing negative molecular ions of fullerenes.

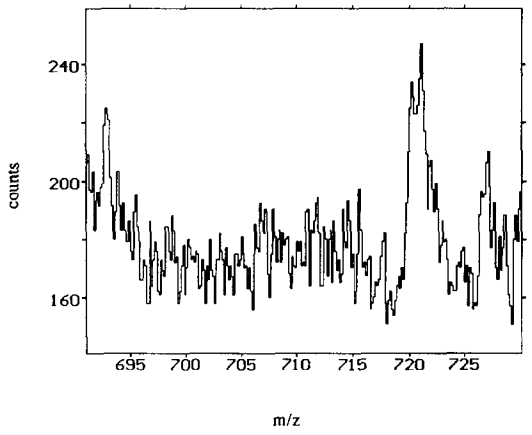

Fig. 7. Negative C_{60} ion peak obtained from a 250 fmol sample.

This is well proven by a spectrum which was taken from a sample prepared by spraying only 250 fmol fullerenes. This small amount of fullerenes was embedded in 28 nmol β-carotene. The result of a 150 min run is shown in Fig. 7. A distinct molecular ion signal at m/z 720 is obtained, indicating clearly the presence of C_{60} on the sample and thus showing that detection of fullerenes in the femtomole-range is made possible by applying matrix-assisted techniques. The mechanism of the formation of fullerene ions cannot be described precisely. We consider the high electron affinity of fullerenes to be the main reason for the dramatic enhancement of negative ion formation. The β-carotene molecules are assumed to serve as electron donors.

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