RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 10, 1927–1933 (1996)

# Studies on the Selection of New Matrices for Ultraviolet Matrix-assisted Laser Desorption/ Ionization Time-of-flight Mass Spectrometry

**Joern Krause, Markus Stoeckli and Urs Peter Schlunegger***

Institute of Organic Chemistry, University of Berne, Freiestrasse 3, CH-3012 Berne, Switzerland

**A new group of compounds has been successfully tested as matrices for ultraviolet matrix-assisted laser desorption/ionization mass spectrometry (UV-MALDI MS). Several new matrices for UV-MALDI MS have been found by choosing, as potential matrices, compounds that perform an intramolecular proton transfer along an intramolecular H-bond under UV irradiation. Compounds of this type are, for example, salicylamide, salicylanilide, several *ortho*-hydroxyacetophenones and *ortho*-hydroxybenzophenones. The matrix activity of these compounds is compared to the corresponding *meta*- and *para*-isomers and to the matrix activity of such well known matrices as 2,5-dihydroxybenzoic acid and 2,4,6-trihydroxyacetophenone. It was found that *meta*- and *para*-substituted hydroxycarbonyl compounds show either a significantly lower or no matrix activity compared with the *ortho* isomers.**

When matrix-assisted laser desorption/ionization (MALDI) mass spectrometry was introduced in 1988,[1,2] the intention of further research was, at first, to improve resolution, and of course to widen the mass range in order to be able to analyse high-mass molecules. Moreover, MALDI was applied to the analysis of several different classes of chemical substances.[3,4] The search for new matrix compounds was, and still is, one important field of investigation. One reason for this was the result that, for different classes of analytes, very different matrix compounds have been proved to work best. Several reports about quite different classes of substances have been published so far (Ref. 3 and relevant citations therein; Refs. 4–15). There seems to be no limitation to the kind of substance that can be used as a matrix. Most of the matrices, which have been published until now, are solids, but there are also liquid compounds which work successfully as a matrix, such as nitrobenzyl-alcohol and others.[2,3]

Only few basic properties for a potential matrix compound have been identified so far. First, the matrix should have a strong absorbance at the wavelength with which the sample is irradiated. Secondly, other important properties investigated so far are the solubility and the vacuum stability of the potential matrix. Finally, it may be useful to look for a substance that can be sublimated at not too high a temperature.[16]

A subject attracting great interest was, and still is, the fundamental question of how a matrix works, or how the whole desorption and ionization process occurs. Attempts to model this process have also been made[17–21] but the process is not completely understood. That means, of course, that there are very few fixed rules for the selection of new matrices. Regarding the chemical structure of the matrices that have been identified so far, one can roughly distinguish between two different groups of substances. The first group is embodied by derivatives of cinnamic acid such as sinapic acid or α-cyano-4-hydroxycinnamic acid.[3,4,11] Derivatives of aromatic carbonyl compounds, such as 2,5-dihydroxybenzoic acid (DHB), salicylic acid or anthranilic acid, can be regarded as examples of the second group (Fig. 1).[3,4] The work presented in this paper deals with this second group of compounds.

Compounds such as salicylic acid (Fig. 1) which contain a phenolic hydroxy group *ortho* to a carbonyl function often show a special behaviour under UV irradiation. Thus, UV irradiation induces an intramolecular proton shift along the intramolecular H-bond in these molecules, yielding a metastable excited state.[22–32] The scheme in Fig. 2 describes the observed transition. Similar transitions are observed for many compounds that contain the structural element described.[22–32] The excitation wavelength of those transitions is, in general, located in the ultraviolet region. This means that compounds performing an intramolecular proton transfer under UV irradiation are also strong absorbers at the laser wavelength used, which is one major requirement for a matrix. This feature is combined with the observation that the acidity of phenol is enhanced significantly under UV irradiation whereas the acidity of the carboxylic group in hydroxybenzoic acids is reduced in the excited state.[27,33–36] Additionally, the excited state that results from an intramolecular proton transfer is a metastable state (lifetime 3 ns) which could perhaps enhance the probability

| | W | X | Y | Z |
|---|---|---|---|---|
| Benzoic acid | H | H | H | H |
| Salicylic acid | OH | H | H | H |
| 2,5-Dihydroxybenzoic acid (DHB) | OH | H | H | OH |
| Anthranilic acid | $NH_2$ | H | H | H |
| p-Aminobenzoic acid | H | H | $NH_2$ | H |

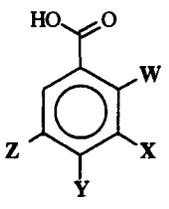



**Figure 1.** Hydroxycarbonyl compounds used as MALDI matrices.

* Author for correspondence

CCC 0951–4198/96/151927–07


*Received 20 August 1996*
*Accepted (revised) 25 October 1996*

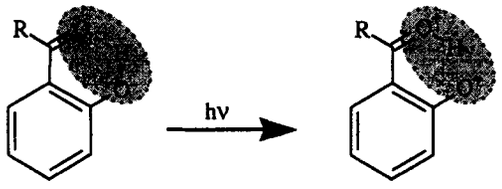



**Figure 2.** Intramolecular proton transfer under UV irradiation.

of a proton transfer from the matrix to analyte molecules.[33] Compared to the *ortho*-hydroxycarbonyl compounds, the lifetime for the excited state is shorter for the corresponding *meta*- or *para*-compounds.[33] Both the increased acidity of phenolic H-atoms as well as the special state of the phenolic H-atom in the type of molecule described may have an important influence on the propensity for a proton transfer from the matrix to the analyte molecules. In this respect, it is sensible to test compounds showing the described transition as new potential matrices. Several compounds, showing the described transition under UV irradiation have, in fact, been successfully used as new MALDI matrices.

## EXPERIMENTAL

### Mass spectrometry

The mass spectrometric measurements were carried out on a home-built linear MALDI time-of-flight instrument. A more detailed description of this MALDI-TOFMS instrument has been published earlier.[37] Desorption/ionization was achieved by a pulsed nitrogen laser (337 nm, pulse energy>250 μJ, pulse duration 3 ns FWHM). The irradiation intensity was regulated using an adjustable iris, placed between the laser and focusing optics. Data acquisition was carried out using a personal computer, running the LDI 1700 Data Acquisition Software (Biomolecular Separations Inc., Reno NV, USA) under Windows 3.1.

### UV spectrometry

For the determination of the UV absorption of any potential matrix compound, UV–Vis spectra of all tested compounds were measured in ethanolic solution ($10^{-4}$ mol/L) on a Perkin-Elmer (Foster City, CA, USA) UV–Vis spectrophotometer (Model 554). The absorption of tested matrix compounds at 337 nm wavelength is given in Tables 1 and 2.

### MALDI sample preparation

All tested analytes were dissolved in water to a final concentration of $10^{-4}$ mol/L in deionized water except the peptides and proteins which were dissolved in a 0.1% solution of trifluoroacetic acid in water. Nucleotide samples were desalted using Millipore VSWP 01300 membranes (Bedford, MA, USA).

The analyte molecules tested were all commercially available. Thus, $d(pA)_3$, $d(pA)_9$, and poly(ethyleneglycol) dimethyl esters (1000 and 4000) were from Fluka AG, Buchs, Switzerland; bovine insulin was from Sigma AG, Buchs, Switzerland; the oligopeptides $Glycine_3$, $Glycine_4$ and $Glycine_6$ were from Bachem AG, Bubendorf, Switzerland. The samples were used without any further treatment or purification.

All matrix compounds tested were purchased from Fluka AG, Buchs, Switzerland except for the following: **2** (Table 1), from Sigma AG, Buchs, Switzerland; **6**, **10**, **19**, **21** and **26** (Tables 1 and 2) from Aldrich AG, Steinheim, Germany, and **8**, **20** and **22** which were synthesized in-house. Each matrix was dissolved in the solvent that proved to work best in our experiments (see Tables 1 and 2). For each sample preparation, 10 μL of matrix solution and 5 μL of the diluted analyte were mixed. By this procedure, a solution with a matrix/analyte ratio of 2000:1 was obtained. A 1.5 μL droplet of this solution was deposited on a brass probe tip and finally dried in vacuum (fast evaporation). All samples were prepared and analysed at least three times. The measurements on our MALDI-TOF instrument were carried out using the following set of acquisition parameters:

Ion-repeller voltage: 28 kV positive-ion mode, 20 kV negative-ion mode. Extractor voltage: 11 kV positive-ion mode, 9 kV negative-ion mode. Accelerator voltage: 0 kV (ground) positive and negative ion modes).

## RESULTS AND DISCUSSION

Our results show that compounds which perform a proton transfer under UV irradiation (Fig. 2) are actually usable as matrices for UV-MALDI MS. This was found for the hydroxyacetophenone derivatives tested (Table 1) as for the hydroxybenzoic acid derivatives (Table 2). The values for resolution and sensitivity, given in Tables 1 and 2, were measured using bovine insulin ($M$=5733.5 Da, positive-ion mode) or the oligonucleotide $d(pA)_3$ ($M$=959.6 Da, negative-ion mode) as a sample. Measurements with other different analytes have also been carried out and yielded similar results.

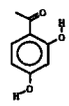
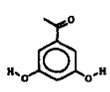
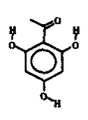
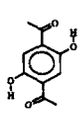
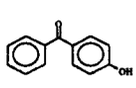
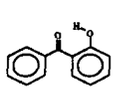
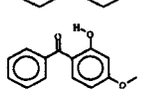
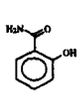
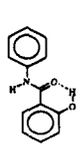
The matrix activity of 2-hydroxyacetophenone is only observable with a cooled probe tip which was designed especially for the investigation of liquid compounds as MALDI matrices. Unfortunately, the cooling of the probe tip was not efficient enough to prevent warming and evaporation of the sample. For this reason, it was not possible to obtain constant results for the resolution and sensitivity which can be achieved using 2-hydroxyacetophenone (**2**; Table 1) or 2-hydroxybenzophenone (**10**; Table 1). The *meta*- and *para*-compounds show a significantly lower matrix activity in our experiments.

Regarding the matrix activity of different dihydroxyacetophenones, it was found that 2,4-, 2,5- and 2,6-dihydroxyacetophenone (**3**, **4**, **5**; Table 1) are very good matrices for MALDI experiments in both the positive- and negative-ion modes (Figs 3 and 4). 2,5-Dihydroxyacetophenone, for example, allows a resolution of more than 1000 in the spectrum of bovine insulin. In our experiments, we did not find any matrix that showed a better resolution in this mass range. Also, in the negative-ion mode, a very good resolution and sensitivity was achieved using this new matrix. Comparable results have also been obtained using 2,6-dihydroxyacetophenone as a matrix. In contrast, 3,5-dihydroxyacetophenone (**6**; Table 1) shows no matrix activity although its absorbance at the wavelength used is very strong. 3,5-Dihydroxyacetophenone is the only isomer that contains no *ortho*-hydroxy group and therefore should offer no possibility for an intramolecular proton transfer under UV irradiation. 2,5-Dihydroxyacetophenone is a very powerful matrix for the analysis of peptides, proteins (Fig. 5) and oligonucleotides (Fig. 4). In our experiments, 2,5-dihydroxyacetophenone proved to be a better matrix for the analysis of oligonucleotides, such as $d(pA)_9$, than 3,4,6-trihydroxyacetophenone.

Another interesting group of compounds are the hydroxy-

benzophenones. The *ortho*-hydroxybenzophenones (**10** and **11**; Table 1) again show the structural element which should allow an intramolecular proton transfer under UV irradiation.[22–32] Additionally, the absorbance at 337 nm is very high, especially for **11** (Table 1). Unfortunately, *ortho*-hydroxybenzophenone is a very volatile liquid which could be used in our MALDI-TOF instrument only with a cooled probe tip (as described above). The compound shows matrix

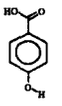
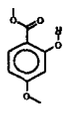
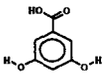
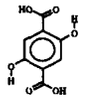
**Table 1. Hydroxycarbonyl compounds**

| | Structure | Chemical name | Solvent | $\varepsilon_{337}$ | Polarity | Intensity[a] $[M+H]^+/[M-H]^-$ | Resol.[b] $[m/\Delta m]$ |
|---|---|---|---|---|---|---|---|
| **1** |  | 4-Hydroxy acetophenone | $EtOH/H_2O$ 1:1 | 200 | positive | + | <20 |
| **2** |  | 2/Hydroxy acetophenone | liquid | 2100 | positive | – | – |
| **3** |  | 2,4-Dihydroxy acetophenone | $CH_3CN$ | 2900 | positive | ++ | 410 |
| **4** |  | 2,5-Dihydroxy acetophenone | EtOH | 1100 | positive<br>negative | ++<br>+++ | 1030<br>320 |
| **5** |  | 2,6-Dihydroxy acetophenone | EtOH | 2000 | positive<br>negative | ++<br>++ | 640<br>290 |
| **6** |  | 3,5-Dihydroxy acetophenone | EtOH | 1700 | positive | – | – |
| **7** |  | 2,4,6-Trihydroxy acetophenone | EtOH | 2500 | positive<br>negative | +<br>++ | 341<br>270 |
| **8** |  | 2,5-Dihydroxy 1,4-diacetylbenzene | EtOH | 1650 | positive | ++ | 324 |
| **9** |  | 4-Hydroxy benzophenone | $EtOH/H_2O$ 1:1 | 900 | positive | + | 55 |
| **10** |  | 2-Hydroxy benzophenone | liquid | 2850 | positive | – | – |
| **11** |  | 2-Hydroxy-4-methoxy-benzophenone | EtOH | 8000 | positive | – | – |
| **12** |  | Salicylamide | $EtOH/H_2O$ 1:1 | 100 | positive | ++ | 380 |
| **13** |  | Salicylanilide | EtOH | 1100 | positive | ++ | 600 |
| **14** |  | Tropolone | $EtOH/H_2O$ 1:1 | 6100 | positive | – | – |

[a] Key for the measured signal intensities
+++, very strong $[M+H]^+$ or $[M-H]^-$ signals; ++, intense $[M+H]^+$ or $[M-H]^-$ signals; +, good $[M+H]^+$ or $[M-H]^-$ signals; +–, weak intensity of $[M+H]^+$ or $[M-H]^-$ signals; –, very weak or no $[M+H]^+$ or $[M-H]^-$ signals.
[b] Resolution averaged over 10 laser shots

activity but the results were not quantitatively reproducible. We therefore looked for a derivative of 2-hydroxybenzophenone, that shows the same structural element but is less volatile under vacuum conditions. Such a compound was found in 2-hydroxy-4-methoxybenzophenone (**11**; Table 1). This compound absorbs strongly at 337 nm. Based on our idea that compounds which can perform an intramolecular proton transfer under UV irradiation are usable as MALDI matrices, this compound should show good matrix activity. In fact, it does not. This compound is the only *ortho*-hydroxycarbonyl compound that we tested so far that does not work as a matrix. The question why cannot be answered easily. Perhaps the very low melting point of the compound (66 °C[38]) is responsible, so that the compound melts instead of being desorbed. In contrast to this, the *para* isomer, 4-hydroxybenzophenone, is a solid com-

**Table 2. Hydroxybenzoic acids**

| | Structure | Chemical name | Solvent | $\varepsilon_{337}$ | Intensity[a] $[M+H]^+$ | Resol.[b] $[m/\Delta m]$ |
|---|---|---|---|---|---|---|
| **15** |  | 2-Hydroxybenzoic acid | EtOH/$H_2O$ 1:1 | 100 | + | 285 |
| **16** |  | 3-Hydroxybenzoic acid | EtOH/$H_2O$ 1:1 | 0 | − | − |
| **17** |  | 4-Hydroxybenzoic acid | EtOH/$H_2O$ 1:1 | 50 | − | − |
| **18** |  | 2-Hydroxy-5-chlorobenzoic acid | EtOH/$H_2O$ 1:1 | 750 | ++ | 260 |
| **19** |  | 2-Hydroxy-5-methoxybenzoic acid | EtOH/$H_2O$ 1:1 | 5000 | ++ | 290 |
| **20** |  | Methyl 5-methoxysalicylate | liquid | — | − | − |
| **21** |  | Methyl 2-hydroxy-4-methoxybenzoate | EtOH/$H_2O$ 1:1 | 200 | − | − |
| **22** |  | Methyl 5-chlorosalicylate | EtOH/$H_2O$ 1:1 | 1150 | +− | <200 |
| **23** |  | 2,5-Dihydroxybenzoic acid | EtOH/$H_2O$ 1:1 | 4250 | ++ | 240 |
| **24** |  | 2,6-Dihydroxybenzoic acid | EtOH | 700 | ++ | 478 |
| **25** |  | 3,5-Dihyroxybenzoic acid | EtOH | 100 | − | − |
| **26** |  | 2,5-Dihydroxy-terephthalic acid | EtOH/$H_2O$ 1:1 | 1250 | + | 91 |

[a] Key for the measured signal intensities
+++, very strong $[M+H]^+$ or $[M-H]^-$ signals; ++, intense $[M+H]^+$ or $[M-H]^-$ signals; +, good $[M+H]^+$ or $[M-H]^-$ signals; +−, weak intensity of $[M+H]^+$ or $[M-H]^-$ signals; −, very weak or no $[M+H]^+$ or $[M-H]^-$ signals.
[b] Resolution averaged over 10 laser shots

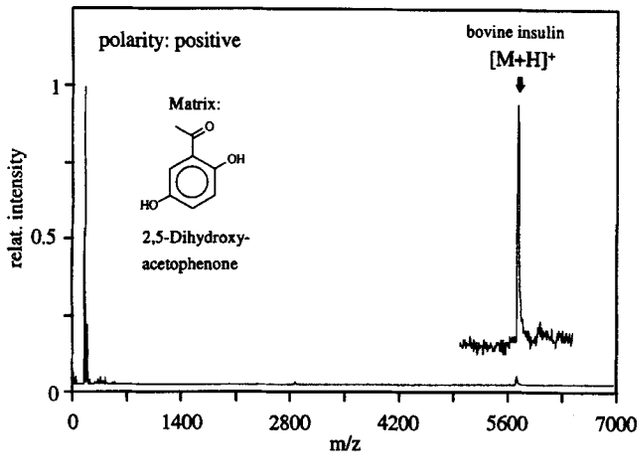



**Figure 3.** MALDI spectrum of oligonucleotide $d(pA)_9$, averaged over 10 laser shots.

pound which can be used under vacuum conditions and shows matrix activity with a low sensitivity and poor resolution in the positive-ion mode.

Another very useful matrix in the positive-ion mode is salicylamide (**12**; Table 1). Salicylamide also shows proton transfer under UV irradiation.[24] Its absorbance is comparatively weak, but its performance as a MALDI matrix is nevertheless very good. The low intensity of matrix (fragment) ions allows measurements with this matrix in a mass range from just above $m/z$ 150 up to $m/z$ 40 000 (Figs 4, 6 and 7). Figure 4 shows MALDI spectra of glycine oligomers (3, 4 and 6 glycine units). The detection of each molecule was easily possible whilst almost no fragmentation of the matrix can be seen. As well as the $[M+H]^+$ ions from the glycine oligomers, there are signals due to the protonated monomer and dimer of the matrix. Another example of the application of as a MALDI matrix is given in Fig. 6 which shows that the analysis of synthetic polymers such as poly(ethyleneglycol) dimethyl ether 1000 is easily possible. Again there are no matrix (fragment) ions in the region $m/z>500$. These results demonstrate that salicylamide offers the possibility of analysing small molecules, with a molecular weight of less than 200 Da, in the presence of large molecules of up to 30 000 Da molecular weight. Above 30 000 Da, the sensitivity decreases so that salicylamide is not usable as a matrix for very large molecules. This is perhaps due to the comparatively weak absorption of salicylamide compared to the absorption of matrices such as 2,5-dihydroxybenzoic acid. For the analysis of bovine insulin salicylamide provides a

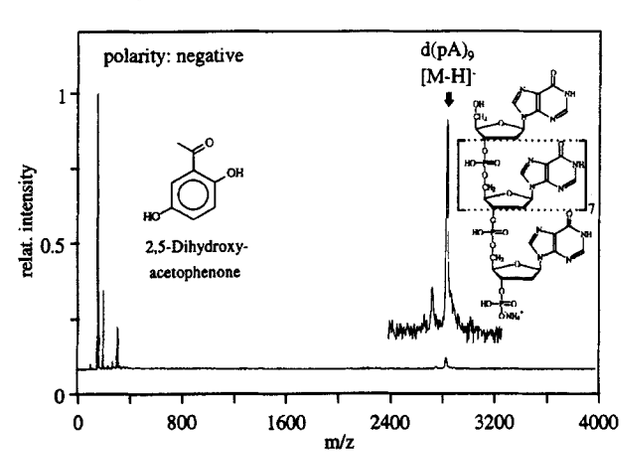



**Figure 4.** MALDI spectra of oligoglycines, averaged over 10 laser shots.

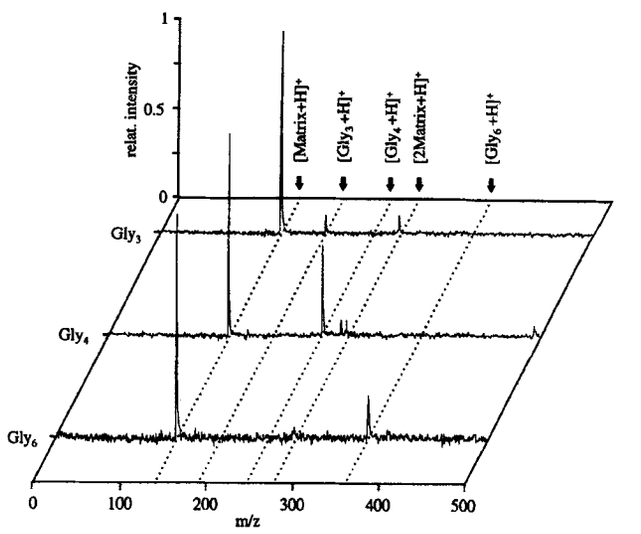



**Figure 5.** MALDI spectrum of bovine insulin, averaged over 10 laser shots.

resolution of 380 (FWHM, Table 1).

Another useful new matrix for experiments in the positive-ion mode is salicylanilide (**13**; Table 1). Resolutions up to 600 were obtained in the spectra of bovine insulin. Salicylanilide also showed very good matrix activity for the analysis of synthetic polymers as is demonstrated by the spectrum of poly(ethyleneglycol)dimethyl-ether 4000 (Fig. 7).

Results similar to those obtained with the hydroxyacetophenones were also obtained for hydroxybenzoic acid derivatives (Table 2). Again, the *ortho*-hydroxybenzoic acid derivatives (**15**, **18**, **19** and **22**; Table 2) showed high matrix activity whereas the *meta*- and *para*-isomers showed no matrix activity. The same result was found for the dihydroxybenzoic acids. The isomers containing an *ortho*-hydroxy group (**24**, **26**) are powerful matrices for the analysis of peptides and proteins in the positive-ion mode while the 3,5-isomer (**25**; Table 2) was again inactive as a MALDI matrix. The same result was observed for 3,5-dihydroxyacetophenone. Similar results have been observed with different hydroxybenzoic acids (Table 2).

In principle, the carboxylic acid derivatives should be more useful as matrices in the positive-ion mode and, in fact, none of the hydroxybenzoic acids tested proved to be a suitable matrix in the negative-ion mode. This is probably due to the comparatively stronger acidity of these compounds. The representatives of the hydroxybenzoic acids

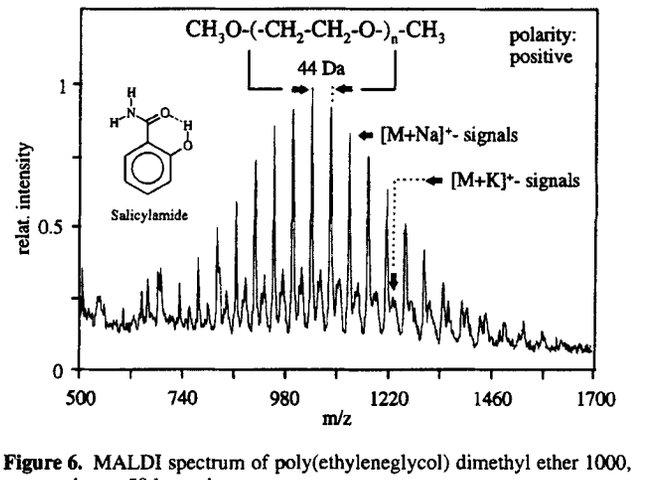



**Figure 6.** MALDI spectrum of poly(ethyleneglycol) dimethyl ether 1000, averaged over 50 laser shots.

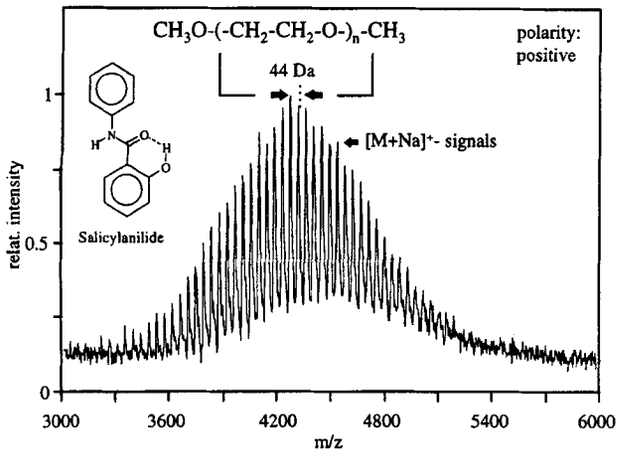



**Figure 7.** MALDI spectrum of poly(ethyleneglycol) dimethyl ether 4000, averaged over 40 laser shots.

are compounds **15**, **16** and **17**, i.e. salicylic acid and the *meta*- and *para*-isomers of salicylic acid. Salicylic acid is known to be a suitable matrix[27] but its UV absorption is very weak. Again, only the *ortho*-hydroxybenzoic acid shows matrix activity. This has to be related to the very weak absorption of the *meta*- and *para*-isomers and the fact that, in this case, salicylic acid is the only one of the three compounds for which an intramolecular proton shift has been reported.[27] Compounds **18–22** are derivatives of salicylic acid which have been chosen as potential matrices because they show an intramolecular proton transfer[22–32] and they absorb 337 nm radiation more strongly than salicylic acid itself. By introducing a chlorine atom or a methoxy group into the salicylic acid molecule the absorption is increased remarkably (**18**, **19**, **20** and **21**; Table 2). This leads to a better sensitivity, for example, in the spectrum of bovine insulin (Table 2). On the other hand, the introduction of a chlorine atom into the matrix molecule leads to a lower resolution and to a poorer peak shape which mainly appears as split peak tops. This could be due to the abstraction of chlorine atoms from the matrix molecules and adduct formation betweeen chlorine atoms and the analyte molecules. Better results are obtained by the use of a methoxy group to improve the absorption of a potential matrix as shown, for example, by 2-hydroxy-5-methoxybenzoic acid[26] (**19**; Table 2), already introduced as a matrix by Karas *et al.*[39] Karas *et al.* used mixtures of 2,5-dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic as a new, very-high sensitivity matrix especially for the analysis of high mass protein samples ('super DHB').[39] 2-Hydroxy-5-methoxybenzoic acid is itself a good MALDI matrix which shows a proton shift under UV irradiation.[26] Methyl 2-hydroxy-4-methoxybenzoate is another compound which shows a proton shift under UV irradiation. Like 2-hydroxy-4-methoxybenzophenone (**11**; Table 1), however, it shows no matrix activity, perhaps again due to its very low melting point (46 °C[40]).

Another very interesting group of substances are the dihydroxybenzoic acids. The most prominent compound of this type is 2,5-dihydroxybenzoic acid (**23**; Table 2) (DHB) which is one of the most commonly used MALDI matrices. This compound contains a structural element that is similar to those discussed above (Fig. 2). The matrix activity of the compound is very well known.[3,4] 2,6-Dihydroxybenzoic acid (**24**; Table 2) also shows very good matrix activity whereas 3,5-dihydroxybenzoic acid, the only one of these three compounds that has no possibility for an intramolecular proton shift, shows no matrix activity. The lower UV-absorption of 3,5-dihydroxybenzoic acid cannot be identified as the only reason for this behaviour since the same result was also found for the dihydroxyacetophenones and hydroxybenzoic acids (see above).

$$\text{matrix} + h\nu \longrightarrow \text{matrix}^*$$
$$\text{matrix}^* + \text{analyte} \longrightarrow [\text{matrix-H}]^- + [\text{analyte+H}]^+$$
$$\text{matrix}^* + \text{matrix} \longrightarrow [\text{matrix-H}]^- + [\text{matrix+H}]^+$$

**Figure 8.** Hypothetical mechanism for the formation of protonated analyte molecules in MALDI.

One important theme related to the use of *ortho*-hydroxycarbonyl compounds as matrices is the question of how the proton transfer described affects the ionization mechanism. Although it may not be possible to answer the question in detail at the present time, some relevant details can be mentioned. First, it should be said that the reported work on proton transfer[22–32] was done studying the properties of compounds like salicylic acid or salicylamide in the gas phase or in solution. Since we intend to desorb the compounds from the solid state into the gas phase, one has to keep in mind that it may not be possible to transfer the results from the gas phase to the solid state. At the moment, we cannot predict whether the absorption of the matrix will lead to the described transition in the solid state. Nevertheless, assuming that proton transfer will take place in the solid or in the dense gas phase immediately after desorption, the long lifetime of the excited state (about 3 ns) could increase the propensity for a proton transfer from the matrix to the analyte molecules and lead to an ionization mechanism as reported by Lia and Allison[41] (Fig. 8). Additionally, the bond between the matrix molecule and the proton is strongly influenced by the intramolecular proton shift[33–36] which should result in a higher propensity for a proton abstraction from the matrix.

Another important question is why analyte molecules, which tend to appear as cationized species in the MALDI spectrum (e.g. polyethylene glycols, Figs 6, 7) can be analysed using the matrices described. One possible explanation, from our point of view, is that the basic ionization step is the same in both cases, i.e. a proton transfer from the matrix to the analyte molecule. This would support an ionization mechanism for the formation of sodium- and potassium-adducts of glycols or carbohydrates in MALDI MS based on the protonation of sodium or potassium salts of analyte molecules. Lia and Allison[41] described a similar mechanism for the formation of cationized species in MALDI as one possible ionization pathway (Fig. 9) On this basis, it can be understood why the same matrices which prove to be useful for the analysis of peptides and proteins are also applicable to the analysis of glycols and carbohydrates.

Even if one assumes such a mechanism, however, it does not explain the formation of negative ions using matrices such as 2,5-dihydroxyacetophenone and other matrices of this type. Obviously, there has to be another mechanism which allows the deprotonation of the analyte molecules. This deprotonation seems to be related to the acidity of the

$$\text{matrix} + h\nu \longrightarrow \text{matrix}^*$$
$$\text{matrix}^* + [\text{analyte-H+Na}] \longrightarrow [\text{matrix-H}]^- + [\text{analyte+Na}]^+$$
$$\text{matrix}^* + \text{matrix} \longrightarrow [\text{matrix-H}]^- + [\text{matrix+H}]^+$$

**Figure 9.** Hypothetical mechanism for the formation of cationized species from analyte molecules in MALDI.[41]

matrix used. We observed that compounds containing a carboxylic acid group, such as 2,5-dihydroxybenzoic acid, show a worse performance in the negative-ion mode compared with compounds such as 2,5-dihydroxyacetophenone, although 2,5-dihydroxyacetophenone does not contain special basic groups. Our guess is that the matrix anion may be the proton accepting agent. Thus, as a first step, proton exchange between two matrix molecules can be considered. Such a reaction is very likely since we observed protonated matrix species in the positive-ion mode and deprotonated matrix species in the negative-ion mode. In a second step, a matrix anion could abstract a proton from an analyte molecule. This is, of course, just a hypothesis so far, but we intend to investigate this theme in more detail.

## CONCLUSIONS

Our results show that new matrices can actually be selected by choosing compounds which perform an intramolecular proton transfer under UV irradiation. Salicylamide, salicylanilide, 2,5-dihydroxyacetophenone and 2,6-dihydroxyacetophenone are particularly useful new matrices. Salicylamide is of special interest in the analysis of low mass analytes whereas 2,5-dihydroxyacetophenone is a very powerful matrix in the negative-ion mode. For both the hydroxyacetophenones and hydroxybenzoic acids we found a high matrix activity with the *ortho*-hydroxy isomers whereas the *meta*- and *para*-isomers showed no, or a significantly lower, matrix activity. As to whether the proton transfer described and the resulting metastable excited state are involved in the ionization process or whether it just offers an absorption band at the wavelength used is not absolutely clear so far. Further work, especially on the crystal structure specifications of the different matrices,[42] is going on.

### Acknowledgement

The authors thank the Swiss National Science Foundation for financial support. Grant No. 2-37'622.93/I.

## REFERENCES

1. M. Karas and F. Hillenkamp, *Anal. Chem.* **60**, 2299 (1988).
2. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida and T. Yoshida, *Rapid Comun. Mass Spectrom.* **2**, 151 (1988).
3. M. Karas, U. Bahr and U. Gießmann, *Mass Spectrom. Rev.* **10**, 335 (1991).
4. R. Caprioli and A. H. B. Wu, *Anal. Chem.* **65**, 471R (1993).
5. P. Juhasz, C. E. Costello and K. Biemann, *J. Am. Soc. Mass Spectrom.* **4**, 399 (1993).
6. T. Huth-Fehre, J. N. Gosine, K. J. Wu and C. H. Becker, *Rapid Commun. Mass Spectrom.* **6**, 209 (1992).
7. R. C. Beavis and B. T. Chait, *Rapid Commun. Mass Spectrom.* **13**, 436 (1989).
8. G. J. Currie and J. R. Yates III., *J. Am. Soc. Mass Spectrom.* **4**, 955 (1993).
9. K. Strupat, M. Karas and F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes* **111**, 89 (1991).
10. K. H. Wu, A. Steding and C. H. Becker, *Rapid Commun. Mass Spectrom.* **7**, 142 (1993).
11. R. C. Beavis, T. Chaudhary and B. T. Chait, *Org. Mass Spectrom.* **27**, 156 (1992).
12. N. I. Taranenko, K. Tang, S. L. Allman, L. Y. Chang and C. H. Chen, *Rapid Commun. Mass Spectrom.* **8**, 1001 (1994).
13. G. Montaudo, M. Montaudo, C. Puglisi and F. Samperi, *Rapid Commun. Mass Spectrom.* **8**, 1011 (1994).
14. U. Pieles, W. Zürcher, M. Schär and H. E. Moser, *Nucleic Acids Res.* **14**, 3191 (1993).
15. M. C. Fitzgerald, G. R. Parr and L. M. Smith, *Anal. Chem.* **65**, 3204 (1993).
16. A. W. Colburn, *Org. Mass Spectrom.* **27**, 53 (1992).
17. G. J. Currie and J. R. Yates III., *J. Am. Soc. Mass Spectrom.* **4**, 955 (1993).
18. A. Vertes and R. Gijbels, *Rapid Commun. in Mass Spectrom.* **4**, 228 (1990).
19. A. Vertes, *Methods and Mechanisms for Producing Ions from Large Molecules*, Plenum Press, New York, pp. 275–286 (1991).
20. J. Sunner, M. G. Ikonomou and P. Kebarle, *Int. J. Mass Spectrom. Ion Processes.* **82**, 221 (1988).
21. R. N. Zare and R. D. Levine, *Chem. Phys. Lett.* **136**, 593 (1987).
22. J. Catalan, F. Toribio and A. U. Acuna, *J. Phys. Chem.* **86**, 303 (1982).
23. P. J. Thistlethwaite and G. J. Woolfe, *Chem. Phys. Lett.* **63**, 401 (1979).
24. A. U. Acuna, A. Costela and J. M. Munoz, *Phys. Chem.* **90**, 2807 (1986).
25. F. Toribio, J. Catalan, F. Amat and A. U. Acuna, *J. Phys. Chem.* **87**, 817 (1983).
26. A. U. Acuna and F. Toribio, *J. Phytochem.* **30**, 339 (1985).
27. M. P. Chiarelli, A. G. Sharkey and D. M. Hercules, *Anal. Chem.* **65**, 307 (1993).
28. T. Nishiya, S. Yamauchi, N. Hirota, Y. Fujiwara and M. Itoh, *J. Am. Chem. Soc.* **108**, 3880 (1986).
29. A. Douhal, F. Amat-Guerri, M. P. Lillo and A. U. Acuna, *J. Photochem. Photobiol. A: Chem.* **78**, 127 (1994).
30. W. Frey and T. Elsaesser, *Chem. Phys. Lett.* **189**, 565 (1992).
31. P. Chou, M. L. Martinez and S. L. Studer, *J. Phys. Chem.* **95**, 10306 (1991).
32. M. Kasha, *Proceedings of the Society of Photo-optical Instrument Engineers.* **2**, 1637 (1992).
33. A. Weller, *Electrochemistry* **60**, 1144 (1956).
34. P. J. Kovi, C. L. Miller and S. G. Schulman, *Anal. Chim. Acta* **61**, 7 (1972).
35. W. G. Paul and S. G. Schulman, *Anal. Chim. Acta* **69**, 195 (1974).
36. D. D. Perrin and D. Boyd, $pK_a$ *Prediction for Organic Acids and Bases*, Chapman and Hall, New York (1981).
37. S. Schuerch, M. Schaer, K. O. Boernsen and U. P. Schlunegger, *Biological Mass Spectrom.* **23**, 695 (1994).
38. *Handbook of Chemistry and Physics*, 76th Edn, CRC Press, Boca Raton (1995).
39. M. Karas, H. Ehring, E. Nordhoff, B. Stahl, K. Strupat, F. Hillenkamp, M. Grehl and B. Krebs, *Org. Mass Spectrom.* **28**, 1476 (1993).
40. Lyman *et al.*, *J. Org. Chem.* **23**, 756 (1958).
41. P. C. Liao and J. Allison, *J. Mass Spectrom.* **30**, 408 (1995).
42. B. W. Liebich and E. Parthé, *Acta Cryst.* **30**, 2522 (1974).