Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 268 (2007) 131–138
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

A one-way hydrophobic surface foil as sample support for MALDI and *off-line* CZE/MALDI mass spectrometry: An alternative for low and high molecular mass compounds

Justyna Rechthaler[a], Andreas Rizzi[b], Günter Allmaier[a,*]

[a] *Institute of Chemical Technologies and Analysis, Vienna University of Technology, Getreidemarkt 9/164, A-1060 Vienna, Austria*
[b] *Department of Analytical and Food Chemistry, University of Vienna, Waehringer Str. 38, A-1090 Vienna, Austria*

Received 20 March 2007; received in revised form 7 July 2007; accepted 11 July 2007
Available online 25 July 2007

Abstract

A one-way multilayered flexible hydrophobic foil, fixed onto a standard microtiter-format MALDI target, has been utilized as a sample support for UV matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometric experiments as well as for the automated *off-line* coupling of capillary zone electrophoresis (CZE) with MALDI. Various matrices as well as preparation techniques on this polar solution-repellent foil were evaluated with regard to matrix suppression effects, sensitivity and crystallization of deposited samples as well as drop migration on the surface. The results were compared with those yielded on the conventional stainless steel targets. It turned out that this foil is a sample support of choice due to significant improvements in chemical noise reduction, spot area reduction, sensitivity and no drop migration dealing with low molecular mass compounds such as tryptic peptide mixtures, small organic synthetic biomolecules (200–700 Da) as well as intact proteins. Furthermore the foil was a useful MALDI sample surface for the deposition of CZE peptide fractions in proteomics applications.

Keywords: Peptides; Carbohydrates; MALDI; CZE/MALDI; Proteomics

1. Introduction

The matrix-assisted laser desorption/ionization (MALDI) technique was developed by Karas, Hillenkamp [1,2] and Tanaka et al. [3] in the 1980s, and allowed to overcome the limitations of laser desorption ionization introducing high molecular mass species (>5000 Da) into the gas phase as intact molecule ions [4]. The benefit of the intact molecule desorption/ionization is evident because a low degree of fragmentation considerably simplifies the mass spectrometric characterizations. However, the application of the MALDI technique to low molecular mass species (<1000 Da) leads to significant problems owing to interferences between matrix and analyte. The matrix ions, including clusters and fragment ions are similar to analyte molecular masses, and yield often-stronger signals than analyte ions due to the large molar excess. Under certain conditions, e.g., high analyte concentration and low laser fluence, the matrix background disappears, and MALDI mass spectra show exclusively analyte signals, despite the large excess of matrix [5]. Chan et al. observed suppression effects in the low *m*/*z* range using nicotinic acid as matrix [6], whereas Knochenmuss et al. [7,8] reported on full suppression of all matrix ions in MALDI mass spectra of analyte ions (1000–20,000 Da) at appropriate matrix/analyte mixing ratios. However, in general matrix suppression effects cannot be easily avoided when dealing with low concentrated, precious low molecular weight (<1000 Da) samples and conventional stainless steel targets, especially in case of the α-cyano-4-hydroxy cinnamic acid (CHCA) matrix, which is a so-called "hot" matrix leading to plenty of fragments and clusters [9]. In order to obtain a successful mass spectrometric performance dealing with less concentrated analytes in the low *m*/*z* range, not only the matrix suppression effects are crucial, but also the spot homogeneity. When sample/matrix droplets are deposited onto the conventional stainless steel surface, they tend to spread in an uncontrollable way on the metal due to strong interaction between the metal and solvent system used. As a consequence, the sensitivity of the MALDI MS analysis decreases and the location of the "sweet spots" becomes a time-

* Corresponding author. Tel.: +43 1 58801 15160; fax: +43 1 58801 15199.
E-mail address: guenter.allmaier@tuwien.ac.at (G. Allmaier).

1387-3806/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2007.07.010

consuming procedure, whereas by reducing of the spot area, the analyte becomes more concentrated, homogenous and then easier to locate. Therefore, over the past decade, various sample deposition devices have been introduced in order to create small (picoliter to nanoliter) analyte droplets for subsequent MALDI MS analysis [10–13]. Another possibility to minimize the sample spot area and improve the sensitivity is the application of the hydrophobic sample supports. During the last years, some polymeric membranes fixed onto the standard targets [14–19] and polymer-coated stainless steel targets [20–24] have been introduced as an alternative support for MALDI sample deposition.

In our previous work [25] we introduced a new sample support for MALDI mass spectrometry, a flexible hydrophobic foil, known and sold under the trade name DropStop™, which is used for the drop-free pouring out of drinkable liquids (e.g., orange juice or liquors), and is widely available. The main benefit described was the matrix suppression effect dealing with a standard peptide (250 fmol substance P) and CHCA as MALDI matrix [26] applying thin layer sample preparation technique [27] on the hydrophobic foil.

In the current study we report on further tests and benefits of this one-way hydrophobic foil (fixed on a conventional microtiter-format target) applying various MALDI matrices as well as samples. By switching from the stainless steel surface to the foil as a target surface we could achieve significantly better performance for the molecular mass determination of low molecular mass compounds such as tryptic peptides, a synthetic quinone derivate, a modified monosaccharide and disaccharide as well as intact high molecular proteins. The sensitivity and signal-to-noise ratio could be significantly improved due to the reduction of the distribution area of the sample droplet on the foil and of the chemical noise. These improvements could be additionally supported by using the novel Nanozyme Pipetter™ in order to create more reproducible spots from nanoliter sample volumes. Moreover, the advantages using the DropStop™ foil for the sample/matrix deposition supported considerably our development of an *off-line* CZE/Probot™/MALDI MS coupling for the analysis of tryptic digests.

2. Experimental

2.1. Materials and methods

The proteins bovine serum albumin (BSA) and cytochrome *C* (from bovine) as well as the MALDI matrices 2,5-dihydroxy benzoic acid (DHB) and CHCA were purchased from Sigma (St. Louis, MO, USA). Sinapic acid (SA) and 2,4,6-trihydroxyacetophenone (THAP) were from Fluka (Buchs, Switzerland). The ProteoMass™ peptide MALDI-MS calibration kit consisting of bradykinin fragment 1–7, angiotensin II, $P_{14}R$, ACTH fragment 18–39 (Sigma, St. Louis, MO, USA) was utilized for spot area studies on the two different types of surfaces applying a hand-held prototype pipetting device (Nanozyme Pipetter™, Eppendorf Instrumente, Hamburg, Germany), which allows reproducible handling of nanoliter volumes down to 20 nl. All used organic solvents were of analytical quality and the specific conductivity of the utilized ultra-high

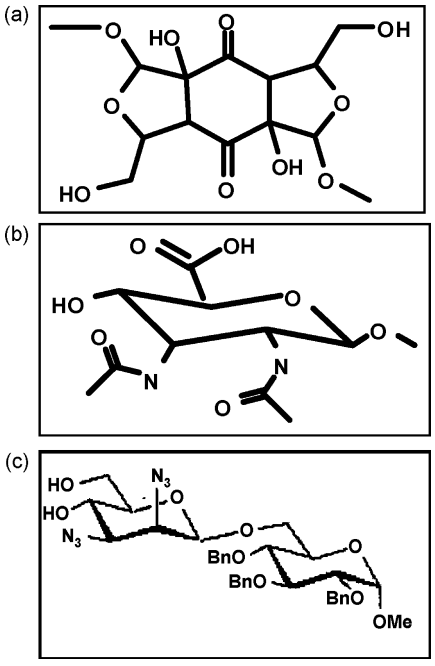

Fig. 1. Chemical structure of the investigated difuroquinone derivative: 3a,7a-dihydroxy-1,5-bis-hydroxymethyl-3,7-dimethoxy-octahydro-benzo [1,2*c*;4,5-*c*′] difuran-4,8-dione; $C_{14}H_{20}O_{10}$, MW: 348.1 Da (a), diaminoacetyl mannuronic acid; $C_{11}H_{18}O_7N_2$, MW: 290.1 Da (b), and D III Zemplen: Methyl-(2,3-diazido-2,3-dideoxy-*b*-D-mannosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-a-D-glucopyranoside. $C_{34}H_{40}O_9N_6$, MW: 676.3 Da (c).

quality (UHQ) water was 0.05 μS cm^{-1} (at 20 °C) generated by an EASYpure LF Compact system from Barnstead/Thermoline (Dubuque, IO, USA). The standard peptides were dissolved in ACN/0.1% aqueous TFA (1:9 v/v) at a concentration of 10^{-6} M. The CHCA as well as DHB matrices (10 mg/ml) were dissolved each in ACN/0.1% aqueous TFA (1:9 v/v). Droplets of peptide/matrix (1:2) were spotted in the range of 50–500 nl by means of the above-mentioned hand-held pipette and dried at room temperature (RT).

The compounds, difuroquinone derivative (DF), see Fig. 1(a), diaminoacetyl mannuronic acid (DM), see Fig. 1(b) and "DIII Zemplen" (Fig. 1(c)) were synthesized at Institute of Chemistry (DF, University of Natural Resources and Applied Life Sciences, Vienna, Austria) and Institute for Organic Chemistry (DM and DIII Zemplen, University of Vienna, Austria) [28].

The multilayered (hard lacquer, metal layer, PET, metal layer, hard lacquer) one-way hydrophobic foil, named DropStop™, was obtained from Schur (Vejle, DK) or local wine stores. The stainless steel sample targets were obtained from Shimadzu Biotech-Kratos Analytical (Manchester, UK).

2.1.1. Tryptic digestion of the proteins

Prior to tryptic digestion protein solutions of BSA and cytochrome *C* were purified using the ZipTip™ C_4 for BSA and C_{18} tips for cytochrome *C* (Millipore, Bedford, MA, USA). After denaturation, reduction of disulfide bonds and lyophilization, protein samples as well as trypsin (sequencing grade, TPCK modified, Roche, Mannheim, Germany) were dissolved in 50 mM NH_4HCO_3 (Merck, Darmstadt, Germany). The pro-

teins were digested by adding 8×10^{-6} M trypsin solution (protein/trypsin, 10:1 v/v). After 18 h incubation at 37 °C and subsequent lyophilization, the tryptic digests were dissolved in ACN/0.1% aqueous TFA (1:9 v/v) and an aliquot (e.g., 2×0.25 μl) was used for MS analysis.

2.2. MALDI RTOF mass spectrometry

All positive-ion MALDI reflectron MS experiments concerning low (below 2500 Da) molecular weight compounds were performed on the curved field reflectron instrument (AXIMA CFR, Shimadzu Biotech-Kratos Analytical, Manchester, UK), equipped with a N_2 laser ($\lambda = 337.1$ nm, 4 ns pulse width) from Spectra Physics (Mountain View, CA, USA), applying all the time compound-optimized delayed extraction (delayed time: 120–390 ns). The acceleration voltage was set to 24 kV. In the few cases of intact protein analysis the same mass spectrometer was applied, but in the linear mode with an acceleration voltage of 20 kV. The DropStop™ foil was cut to the appropriate size and mounted with a double-sided tape (Scotch™, ATG 700; 3 M, St. Paul, MN, USA) on a microtiter-format, stainless steel MALDI target (instrument calibration has to be adjusted) prior to sample deposition.

2.2.1. Sample preparation of low-mass compounds

For the MALDI-TOF MS analyses saturated solutions of DF (in methanol), DM (in water) and DIII Zemplen (in methanol) were prepared and used either with the thin layer technique applying CHCA (7 mg/ml in acetone) or dried droplet technique using DHB (20 mg/ml in methanol).

2.2.2. Thin layer sample preparation of tryptic digested BSA

The CHCA matrix was dissolved in acetone (7 mg/ml). Usually a 0.5 μl droplet of the matrix solution was deposited by means of Gilson pipetter (type P2, Gilson, Villiers-le-Bel, France) onto the target surface and dried at RT.

2.2.3. Sample preparation of the intact protein BSA

An aliquot of the protein solution (ACN/0.1% aqueous TFA 1:9 v/v) and an aliquot of the matrix (SA or THAP) solution (7 mg/ml, ACN/0.1% aqueous TFA 1:9 v/v) were mixed in the ratio 1:2 (v/v) in a polypropylene tube. An aliquot (50 nl, one single nanodrop) of this mixture was then deposited by means of Nanozyme™ Pipetter onto the two different target surfaces and dried at RT in a gentle stream of air.

2.3. Off-line CZE/MALDI mass spectrometry

CZE separations of the tryptic digest of cytochrome *C* were carried out on the ^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) and fractionated as well as deposited by the robotic system Probot™ (Dionex, Sunnyvale, CA, USA). The lyophilized tryptic peptide samples were dissolved in background-electrolyte prior to CZE injection. An uncoated fused silica capillary (80 cm length, 50 μm I.D.) was used and the CZE voltage was set to 30 kV. The background-electrolyte as well as necessary sheath-liquid was an aqueous solution consisting of 200 mM HCOOH and 20 mM NH_3. First, numerous 1 μl droplets of CHCA matrix solution in acetone (10 mg/ml) were deposited onto the hydrophobic DropStop™ foil or stainless steel MALDI target surface (microtiter plate format) by means of the Probot™ apparatus and dried at RT. Subsequently, 0.5 μl fractions containing the separated tryptic peptides, derived from the *on-line* CZE/Probot™ combination, were placed on top of the CHCA spots. The following MALDI RTOF measurements were carried out on the instrument described above under the mentioned conditions.

2.4. Electron microscopy

The electron micrographs were obtained on a digital scanning microscope DSM 940 (Zeiss, Goettingen, Germany) with an acceleration voltage of 15 kV.

3. Results and discussion

3.1. Matrix suppression effects

Applying the multilayered hydrophobic DropStop™ foil as target surface we could observe a dramatic low-mass matrix ion suppression in case of the positive-ion MALDI mode for a tryptic digest of BSA (low-femtomole range amounts deposited) using

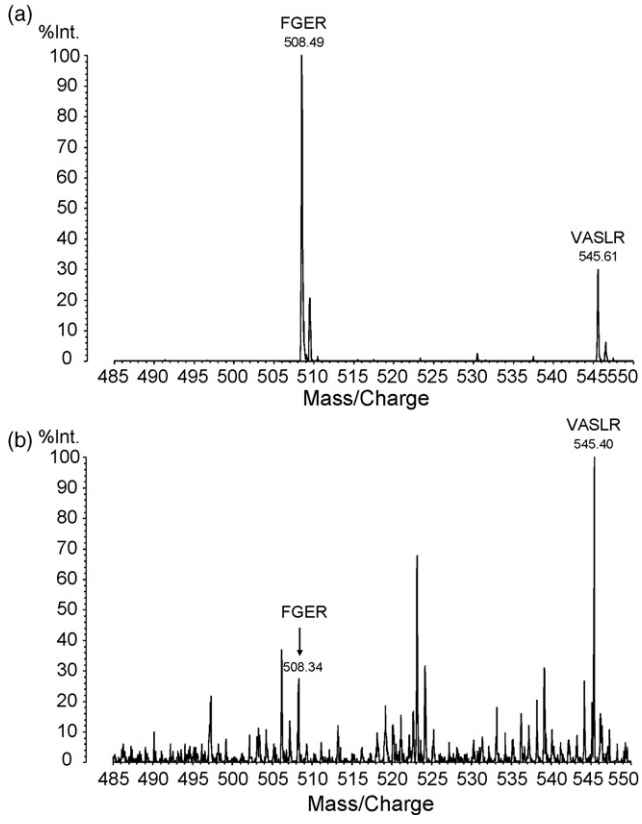

Fig. 2. Low-mass sectors of the positive-ion MALDI mass spectra of an tryptic BSA digest deposited on the hydrophobic DropStop™ foil (a) and on the stainless steel target surface (b) applying constant instrumental parameters and the thin layer sample preparation technique.

the thin layer sample preparation technique (matrix: CHCA), as shown in Fig. 2(a). In this example, the tryptic peptides with amino acid sequence FGER and VASLR could be easily identified as protonated molecular ions due to the absence CHCA-related chemical noise (e.g., CHCA matrix cluster ions). In Fig. 2(b), however, where a conventional stainless steel surface was used as a sample support, we could not reliably differentiate between the protonated molecular ions of peptide mass fingerprint (PMF) relevant peptides and chemical background noise. The obtainable signal-to-noise ratio in this *m/z* range is much lower for the stainless steel surface compared to the DropStop^{TM} surface. This matrix suppression effect is in accordance to the already described effect for standard peptides (e.g., substance P [25] and angiotensin III) in the low-femtomole range. In all experiments, no protonated and/or sodiated matrix ions, directly matrix-related fragment ions as well as cluster ions were detected using the hydrophobic sample support.

The observed matrix suppression effects related to the DropStop^{TM} foil turned out to be also very useful for the molecular mass determination of other compound classes such as a synthetic difuroquinone derivative DF (Fig. 1(a)), the diaminoacetyl mannuronic acid DM (Fig. 1(b)), and a modified disaccharide "D III Zemplen" (Fig. 1(c)). The DF derivative was analyzed in combination of CHCA matrix and thin layer technique as well as 2,5-dihydroxybenzoic acid (DHB) [29] matrix applying the dried droplet technique. In both cases, the mass spectrometric performance achieved, using the hydrophobic foil was significantly better than using stainless steel surface. Fig. 3(a) shows as dominating $[M+Na]^+$ ion signal of the DF derivative at *m/z* 371.2 and complete suppression of unwanted chemical noise. This facilitates considerably the ease of molecular mass determination of such a small molecule. On the other hand, the same sample preparation conditions and the same sample amount deposited on the stainless steel surface led to a very noisy MALDI mass spectrum in the mass range of interest (Fig. 3(b)). The unambiguously determination of the molecular mass was not possible in this case. To evaluate the dependence of the matrix suppression effects on the type of matrix, the positive-ion MALDI mass spectrum of the DF derivative with the DropStop^{TM} foil as target surface in combination with DHB as matrix was recorded and is shown in Fig. 4(a). Again, no matrix-related ions are observed at all, whereas the mass spectrum obtained from the stainless steel target reveals a considerable number of DHB matrix-related ions. However, the sodiated molecular ion of the analyte could be detected as the base peak (Fig. 4(b)).

For the derivatized monosaccharide diaminoacetyl mannuronic acid similar results were obtained as illustrated in Fig. 5. The positive-ion MALDI mass spectrum of DM exhibits as base peak a protonated molecular ion at *m/z* 291.0 and a $[M+Na]^+$ ion with still high abundance using the hydrophobic foil in connection with DHB as matrix. A further example related to synthetic

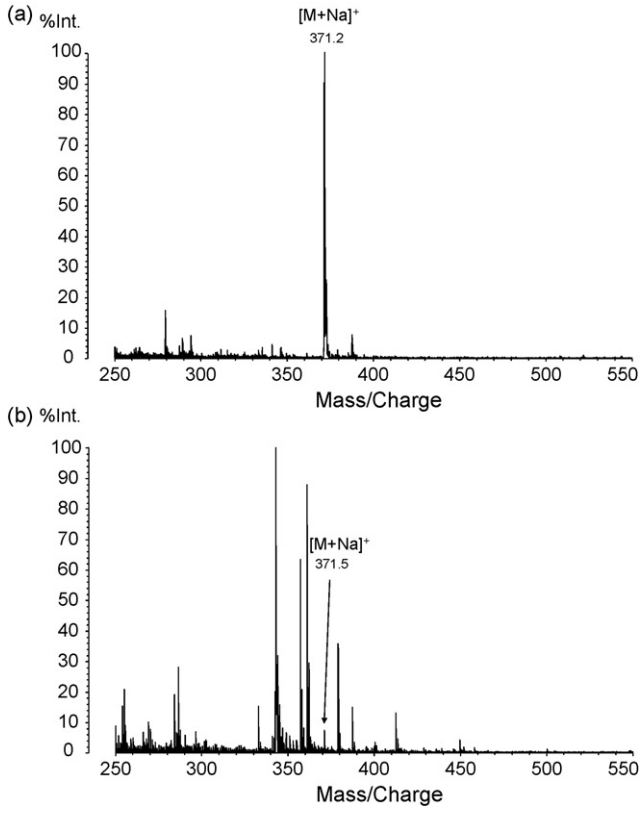

Fig. 3. Positive-ion MALDI mass spectra of a synthetic difuroquinone derivative prepared on the hydrophobic DropStop^{TM} foil (a) and the stainless steel target surface (b) (thin layer sample preparation technique with CHCA matrix).

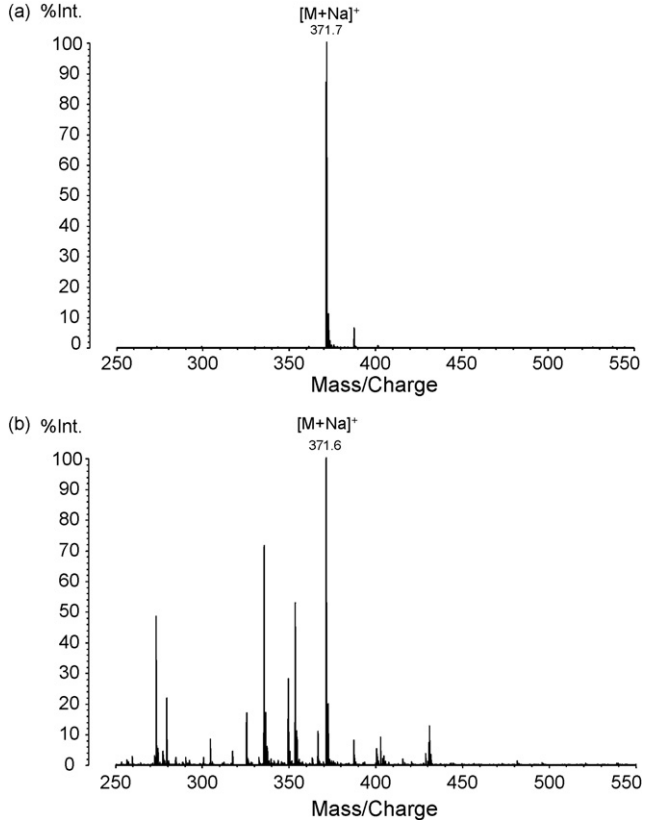

Fig. 4. Positive-ion MALDI mass spectra of the difuroquinone derivative deposited by means of the dried droplet technique with DHB as MALDI matrix on the hydrophobic DropStop^{TM} foil (a) and stainless steel target surface (b).

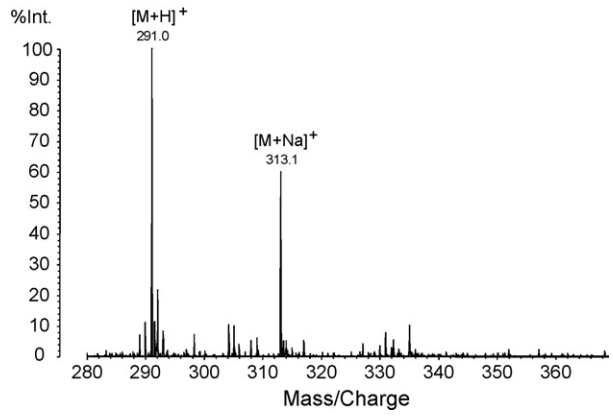

Fig. 5. Positive-ion MALDI mass spectrum of diaminoacetyl mannuronic acid deposited by means of the dried droplet technique with DHB as MALDI matrix on the hydrophobic DropStop™ foil.

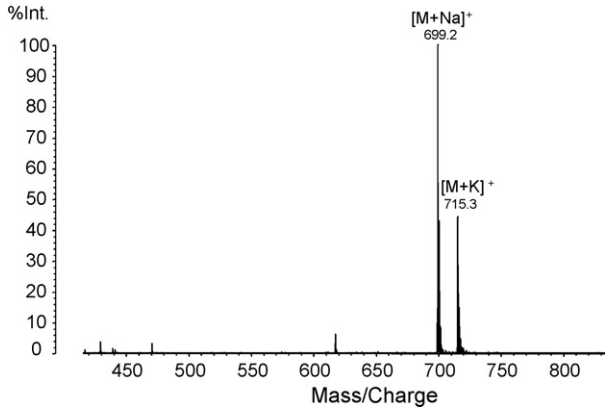

Fig. 6. Positive-ion MALDI mass spectrum of the disaccharide "D III Zemplen" deposited by means of the thin layer sample preparation technique with CHCA as MALDI matrix on the hydrophobic DropStop™ foil.

carbohydrate chemistry is shown in Fig. 6. The MALDI mass spectrum of the very labile disaccharide DIII Zemplen is characterized by a very abundant $[M+Na]^+$ ion at m/z 699.2 and some fragment ions.

In general, the matrix suppression effects related to the DropStop™ foil could be observed in the positive-ion MALDI mass spectra dealing with various types of low-mass compounds combined with different, popular MALDI matrices and solvents. The unambiguous determination of the exact molecular masses of unknown low-mass compounds and tryptic peptides is by means of the sample deposition onto the one-way DropStop™ foil easily possible. Furthermore, cross-contamination could be avoided due to the low price of a piece of the one-way foil.

3.2. Crystallization and spot area reduction effects

The electron micrographs of a tryptic BSA digest prepared with the matrix CHCA according to the thin layer technique on a stainless steel target surface and on the DropStop™ foil are shown in Fig. 7. As can be seen from the images, the crystals formed on the hydrophobic foil (Fig. 7(a)) are resulting in a homogenous spot of high crystal coverage (especially advantageous when performing automated analyses), whereas the identical sample preparation on the stainless steel target surface (Fig. 7(b)) led to much smaller crystals of less even crystal distribution and completely different morphological structure. Additionally, there was no regularity of spot geometry and position on the stainless steel target due to the high migration of

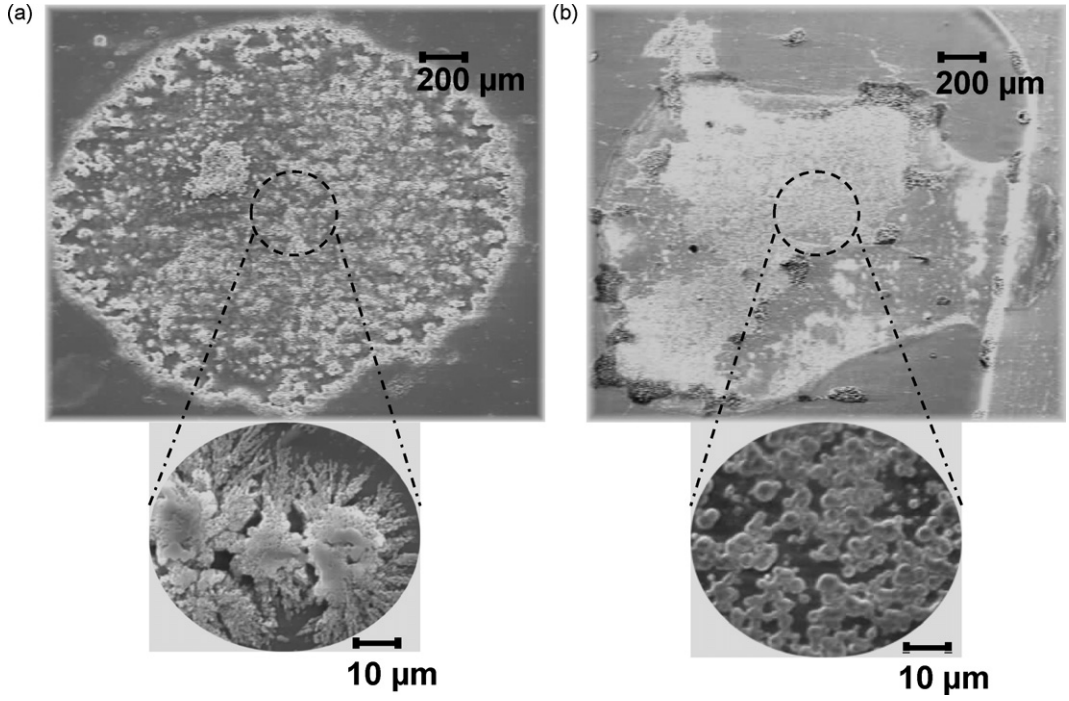

Fig. 7. Electron micrographs of a sample spot containing a tryptic BSA digest prepared on two different surfaces: hydrophobic DropStop™ surface (a) and stainless steel target surface (b). The thin layer sample preparation technique with CHCA as matrix was applied.

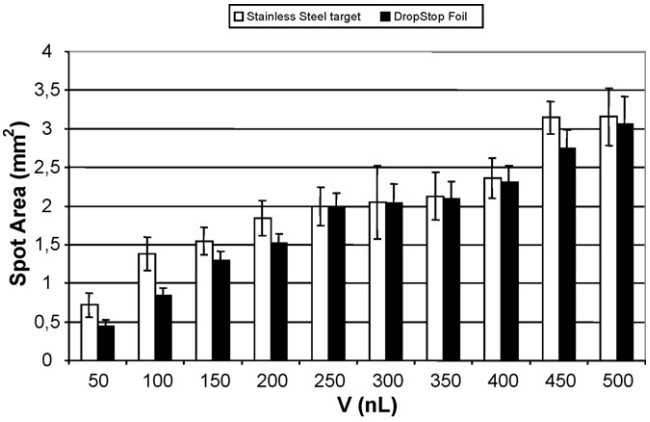

Fig. 8. Comparison of the measured average spot areas ($n = 10$ [mm^2]) obtained from 10 different nl-volumes (50–500 nl) of the CHCA/standard peptides mixture in ACN/0.1% aqueous TFA (1:9 v/v) and deposited by means of hand-held Nanozyme™ pipetter on the stainless steel target surface as well as the DropStop™ foil.

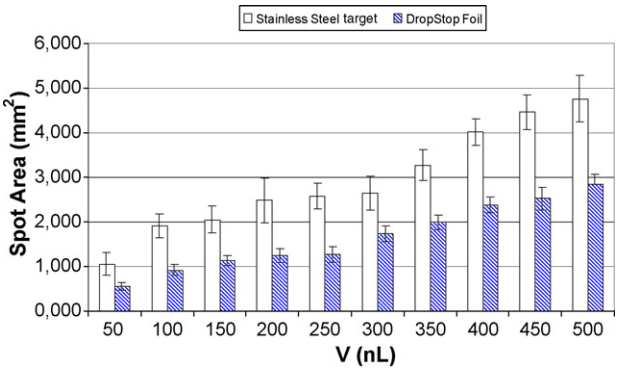

Fig. 9. Comparison of the measured average spot areas ($n = 10$ [mm^2]) obtained from 10 different nl-volumes (50–500 nl) of the DHB/standard peptides mixture in ACN/0.1% aqueous TFA (1:9 v/v) and deposited by means of hand-held Nanozyme™ pipetter on the stainless steel target surface as well as the DropStop™ foil.

the acetone droplet (containing the matrix CHCA) upon the deposition.

A further important effect observed with the DropStop™ foil was a significant reduction of spot areas after the crystallization process, which was particularly found for various ACN/0.1% aqueous TFA solvent compositions of various sample/matrix mixtures (dried droplet technique). Similar crystallization behaviours and spot reduction effects have been reported by other research groups dealing with hydrophobic coated MALDI sample plates [30,31]. For comparison of the obtained average spot areas, various nl-volumes (50–500 nl) of CHCA/standard peptide mixture solutions (in ACN/0.1% aqueous TFA 1:9 (v/v)) were prepared and deposited by means of the Nanozyme™ Pipetter on the stainless steel as well as the hydrophobic foil surface. The found spot areas for the different nl-volumes are shown in Fig. 8 (detailed data see Table 1). The spot areas observed on the DropStop™ foil are significant smaller, practically across the whole examined nl-volume range, compared to those on the steel target. The spot area reduction on the hydrophobic foil is more pronounced for the DHB/standard peptide mixtures with the same solvent composition (Fig. 9, Table 2). The improvements in crystal homogeneity and reduced spot area with the DropStop™ foil improves the sweet spot localization, if necessary, and the sample concentration per area. Due to reduced spot area (higher sample concentration per area) and higher spot homogeneity gained by the DropStop™ foil we could achieve higher sensitivity dealing with, e.g., tryptic peptides applying both the CHCA and the DHB matrix.

Additional work was performed using THAP as well as SA as matrices, which are better suited for high molecular weight biomolecule analysis as for example CHCA, for MALDI linear TOF MS analysis of BSA (66 kDa) in order to demonstrate the universal applicability of the hydrophobic foil. THAP in combination with the DropStop™ foil turned out to be the MALDI matrix of choice for high mass proteins. In terms of sensitivity an approximately two fold increase compared to the results obtained on the stainless steel target was achieved (sample solution was deposited by a P2 Gilson Pipetter). Depositing a 50 nl-droplet by means of the hand-held Nanozyme™ Pipetter approximately a four fold increase of the singly charged molecular ion sensitivity compared to the stainless steel target surface

Table 1
Average spot area ($n = 10$ [mm^2]) and standard deviations of spot areas measured for 10 different nl-volumes (50–500 nl) of the CHCA/standard peptides mixture in ACN/0.1% aqueous TFA 1:9 (v/v) and deposited by means of hand-held Nanozyme™ Pipetter on the stainless steel target surface as well as the DropStop™ foil

Spot volume (nl)	Stainless steel target	DropStop™ foil
50	0.72 ± 0.16	0.45 ± 0.08
100	1.38 ± 0.22	0.84 ± 0.10
150	1.54 ± 0.18	1.29 ± 0.12
200	1.84 ± 0.23	1.52 ± 0.12
250	1.99 ± 0.24	1.99 ± 0.18
300	2.05 ± 0.47	2.04 ± 0.24
350	2.13 ± 0.31	2.09 ± 0.23
400	2.36 ± 0.26	2.30 ± 0.22
450	3.15 ± 0.21	2.75 ± 0.23
500	3.16 ± 0.37	3.06 ± 0.36

Table 2
Average spot area ($n = 10$ [mm^2]) and standard deviations of spot areas measured for 10 different nl-volumes (50–500 nl) of the DHB/standard peptides mixture in ACN/0.1% aqueous TFA 1:9 (v/v) and deposited by means of hand-held Nanozyme™ Pipetter on the stainless steel target surface as well as the DropStop™ foil

Spot volume (nl)	Stainless steel target	DropStop ™ foil
50	1.05 ± 0.26	0.56 ± 0.09
100	1.92 ± 0.27	0.92 ± 0.13
150	2.05 ± 0.30	1.13 ± 0.12
200	2.48 ± 0.50	1.25 ± 0.16
250	2.58 ± 0.29	1.27 ± 0.18
300	2.64 ± 0.38	1.72 ± 0.18
350	3.27 ± 0.35	1.99 ± 0.18
400	4.02 ± 0.30	2.38 ± 0.18
450	4.46 ± 0.38	2.52 ± 0.26
500	4.77 ± 0.52	2.84 ± 0.23

Table 3
Comparison of the sequence coverages (% cov) for cytochrome *C* based on the detected tryptic peptides: two right columns, CZE separation of the tryptic peptide mixture prior to MALDI (*off-line* CZE/Probot^{TM}/MALDI)

$[M+H]^+$ *m/z* (monoisotopic) theoretical value	Position	Number of MC	Peptide sequence	CZE–MALDI	
				Found peptides on DropStop ^{TM} (**81%** cov)	Found peptides on Stainless steel (**46%** cov)
779.45	80–86	0	MIFAGIK	×(779.46)	
795.44	80–86; MSO: 80	0	MIFAGIK	×(795.43)	
860.48	1–8	2	GDVEKGKK	×(860.48)	
964.53	92–99	0	EDLIAYLK	×(964.53)	×(964.33)
1168.62	28–38	0	TGPNLHGLFGR	×(1168.62)	×(1168.21)
1260.58	14–25	1	CAQCHTVEKGGK	×(1260.43)	
1306.70	89–99	1	GEREDLIAYLK	×(1305.85)	
1456.67	40–53	0	TGQAPGFSYTDANK	×(1456.67)	×(1456,29)
1584.77	39–53	1	KTGQAPGFSYTDANK		×(1584.37)
1633.89	9–22	1	IFVQKCAQCHTVEK	×(1633.87)	×(1633.81)
2009.95	56–72	0	GITWGEETLMEYLENPK	×(2009.95)	
2025.94	56–72 MSO: 65	0	GITWGEETLMEYLENPK	×(2025.97)	

In case of CZE/MALDI coupling, matrix (CHCA) and peptides were spotted by means of Probot^{TM} onto the hydrophobic DropStop^{TM} foil as well as onto the conventional stainless steel target surface (in the thin layer sample preparation mode). MC, missed cleavage; MSO, oxidized to form methionine sulfoxide containing peptides; ×, detected tryptic peptides.

could be obtained. These improvements in sensitivity are an additional positive effect of the hydrophobic surface.

3.3. Off-line CZE/MALDI mass spectrometric combination

To the main benefits of combining separation methods such as micro-HPLC [32] or CZE with the MALDI MS technique belongs without doubt the possibility of the "frozen-in-time" analysis. A major issue is the optimal deposition of the separated compounds (present in the liquid phase) on the MALDI target. We have now evaluated the DropStop^{TM} foil as a MALDI sample support for fraction collection/deposition of tryptic peptides in a CZE/Probot^{TM}/MALDI MS system due to the described helpful features. It turned out, as shown in Table 3, that the combination of the CZE (with an uncoated fused silica capillary) with MALDI MS using the robotic system Probot^{TM} as high-accuracy sample fraction/deposition device and the DropStop^{TM} foil as a sample support (prespotted CHCA matrix) led to the best results regarding sequence coverage of a cytochrome *C* digest (81%) digest compared to results yielded on stainless steel probe (46%). This improvement can be mostly attributed to the small spot diameter, increased sensitivity and to the better shot-to-shot reproducibility. The use of this DropStop^{TM} foil, which can be purchased also as large-scale foil, will improve in combination with the other devices the future operation of a CZE/Probot^{TM}/MALDI MS/MS system for the analysis of complex peptide maps.

4. Conclusion

We have demonstrated the usefulness of a simple device for improved MALDI MS sample preparation. With this one-way hydrophobic DropStop^{TM} foil as sample support, avoiding cross-contaminations, highly reproducible MALDI mass spectra of low-mass molecules (e.g., tryptic peptides with just a few amino acids, quinones, mono- as well as disaccharide) can be obtained with significant matrix-related background ion reduction applying both CHCA as well as DHB matrix, which facilitates considerably the molecular mass determination and PMF. Additionally, the on this surface formed sample layer promotes a uniform matrix/analyte ratio across the whole spot, and eliminates the need to search for "sweet spots". In case of high mass proteins as well as peptides, the combination of the hydrophobic foil with the Nanozyme^{TM} Pipetter, led additionally to significant enhancement of the sensitivity.

The DropStop^{TM} foil is also attractive for the *off-line* coupling of the CZE/Probot^{TM} with MALDI MS due to the fact that improved analyte sensitivity is given and a higher homogeneity of the sample spot is obtained. All these features make automation more feasible in the near future. The enhanced sequence coverage of proteins obtainable by means of the CZE/MALDI MS approach is also of general importance in proteomics.

Acknowledgments

This work has been supported by the Austrian Science Foundation (grant 14181 to G.A.). We thank P. Messner (University of Natural Resources and Applied Life Sciences) for assistance with the electron microscopy and A. Plematl (University of Vienna) for the help with the *off-line* CZE/Probot^{TM} device as well as A. Hedenetz (University of Vienna) and P. Kosma (University of Natural Resources and Applied Life Sciences) for providing us with samples of the synthetic compounds. Furthermore we thank R. Blum (Eppendorf Instrumente) for the gift of the prototype Nanozyme^{TM} Pipetter.

References

[1] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Proc. 78 (1987) 53.
[2] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[3] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151.

[4] M. Karas, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Proc. 92 (1989) 231.
[5] M. Karas, M. Glückmann, J. Schäfer, J. Mass Spectrom. 35 (2000) 1.
[6] T.W.D. Chan, A.W. Colburn, P.J. Derrick, D.J. Gardiner, M. Bowden, Org. Mass Spectrom. 27 (1992) 188.
[7] R. Knochenmuss, F. Dubois, M.J. Dale, R. Zenobi, Rapid Commun. Mass Spectrom. 10 (1996) 871.
[8] R. Knochenmuss, V. Karbach, U. Wiesli, K. Breuker, R. Zenobi, Rapid Commun. Mass Spectrom. 12 (1998) 529.
[9] E. Stimson, O. Truong, W.J. Richter, M.D. Waterfield, A.L. Burlingname, Int. J. Mass Spectrom. Ion Proc. 167/170 (1997) 231.
[10] H. Zhang, P.E. Andren, R.M. Caprioli, J. Mass Spectrom. 30 (1995) 1768.
[11] G. Allmaier, Rapid Commun. Mass Spectrom. 11 (1997) 1567.
[12] D.P. Little, T.J. Cornish, M.J. O'Donnell, A. Braun, R.J. Cotter, H. Köster, Anal. Chem. 69 (1997) 4540.
[13] P. Onnerffjord, J. Nilsson, L. Wallmann, T. Laurell, G. Marko-Varga, Anal. Chem. 70 (1998) 4755.
[14] M.E. McComb, R.D. Oleschuk, D.M. Manley, L. Donald, A. Chow, J.D.J. O'Neil, W. Ens, K.G. Standing, H. Perreault, Rapid Commun. Mass Spectrom. 11 (1997) 1716.
[15] K. Strupat, M. Karas, F. Hillenkamp, C. Eckerskorn, F. Lottspeich, Anal. Chem. 66 (1994) 464.
[16] T.A. Worrall, R.J. Cotter, A.S. Woods, Anal. Chem. 70 (1998) 750.
[17] J. Bai, Y.H. Liu, T.C. Cain, D.M. Lubman, Anal. Chem. 66 (1994) 3423.
[18] I.P. Smirnov, L.R. Hall, P.L. Ross, L.A. Haff, Rapid Commun. Mass Spectrom. 15 (2001) 1427.
[19] H. Zhang, R.M. Caprioli, J. Mass Spectrom. 31 (1996) 690.
[20] K.C. Hung, H. Ding, B. Guo, Anal. Chem. 71 (1999) 518.
[21] K.C. Hung, Y. Rashidzadeh, Y. Wang, B. Guo, Anal. Chem. 70 (1998) 3088.
[22] M. Schürenberg, C. Lübbert, H. Eickhoff, M. Kalkum, H. Lehrach, E. Nordhoff, Anal. Chem. 72 (2000) 3436.
[23] Y. Kim, G.B. Hurst, M.J. Doktycz, M.V. Buchanan, Anal. Chem. 73 (2001) 2617.
[24] J. Gobom, M. Schürenberg, M. Müller, D. Theiss, H. Lehrach, E. Nordhoff, Anal. Chem. 73 (2001) 434.
[25] J. Rechthaler, G. Allmaier, Rapid Commun. Mass Spectrom. 16 (2002) 899.
[26] R.C. Beavis, T. Chaudry, B.T. Chait, Org. Mass Spectrom. 27 (1992) 156.
[27] O. Vorm, P. Roepstorff, M. Mann, Anal. Chem. 66 (1994) 3281.
[28] A. Hedenetz, PhD Thesis, University of Vienna, Vienna, Austria, 2003.
[29] K. Strupat, M. Karas, F. Hillenkamp, Int. J. Mass Spectrom. Ion Proc. 111 (1991) 89.
[30] S.J. Owen, F.S. Meier, S. Brombacher, D.A. Volmer, Rapid Commun. Mass Spectrom. 17 (2003) 2439.
[31] S. Xiong, Q. Ding, Z. Zhao, W. Chen, G. Wang, S. Liu, Proteomics 3 (2003) 265.
[32] O. Krokhin, Y. Qian, J.R. McNabb, V. Spicer, K.G. Standing, Proceedings of the 50th ASMS Conference, 2002, WPP, p. 233.