Quantitative Study of Solvent and Surface Effects on Analyte Ionization in Desorption Ionization on Silicon (DIOS) Mass Spectrometry

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Deuterated solvents and DIOS surfaces derivatized with different functional groups are used to investigate impacts of local chemical environment on analyte ionization. Both solvent molecules and surface functional groups are found to directly participate in analyte protonation in the condensed phase. The corresponding protonation effectiveness is quantitatively estimated based on the relative MS peak intensities of $[M + 2]^+/[M + 1]^+$. A direct correlation between ionization of triethylamine and the relative acidities of the surface and the solvent is evident. In addition, the proton donating effectiveness of a solvent is found to be related to its vapor pressure. Improved MS detection of small molecules via proper surface treatment and solvent selection is demonstrated. (J Am Soc Mass Spectrom 2008, 19, 8–13) © 2008 American Society for Mass Spectrometry

Extensive research in metabolite profiling in recent years has revived interest in desorption ionization on porous silicon mass spectrometry (DIOS-MS). Elimination of matrix molecules in DIOS-MS reduces background noise in the low-mass range and enables direct detection of biologically significant small molecules [1–4]. It also overcomes matrix-induced analyte redistribution on a sample surface, which allows two-dimensional imaging of tissue samples with good spatial accuracy [5]. Recent studies have shown that the local chemical properties of a DIOS substrate, including the residual solvent on the surface and the surface functional groups, are critical in analyte ionization [6–9]. Quantitative investigation of these factors, however, is lacking. In this report, we semiquantitatively assessed contributions of various proton sources in DIOS-MS and the feasibility of improving MS detection of lipids and peptides by proper sample treatment.

Experimental

Materials

Phosphorus-doped (100) single-crystalline silicon wafers at 0.005 to 0.02 Ω/cm were purchased from Silicon Sense, Inc. (Nashua, NH). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Triethylamine (TEA), pentanedione, and deuterium oxide (D_2O) were purchased from Sigma Aldrich (St. Louis, MO). 3-Aminopropyltrimethoxysilane (APTMS) was purchased from Fluka (Milwaukee,k WI). Acetone-d_6, toluene-d_8, and methanol-d_4 were purchased from Cambridge Isotope Laboratories (Andover, MA). Deuterium peroxide (D_2O_2, 30% in D_2O) was purchased from Icon Isotopes (Summit, NJ). Hydrofluoric acid (HF, 49%) and H_2O_2 (30%) were purchased from Fisher Scientific (Pittsburgh, PA). CH_3CH_2OH (EtOH) was purchased from Aaper Alcohol (Shelbyville, KY). DI H_2O of 18 MΩ (Millipore, PO) was used throughout the experiments.

DIOS Substrate Preparation

DIOS substrates were prepared by dipping the wafer into a solution of 5% HF/EtOH for 1 min to remove the oxidized layer, followed by a 1.5-min anodic etching in 25% HF/EtOH at 5 mA/cm^2 [10, 11]. Before MS experiments, the DIOS substrates were dipped in 5% HF/EtOH to regenerate the H-terminated surface. For OH-terminated surfaces, the DIOS substrates were dipped in a solution of 15% H_2O_2/MeOH (or 15% D_2O_2/MeOH-d_4) for 30 min. For RNH_2-terminated surfaces, the same OH-terminated surfaces were refluxed in a solution of 10% APTMS/toluene under N_2 for 4 h. To remove residual solvent molecules, the DIOS or chemically modified DIOS substrates were dried under high vacuum overnight, unless specified otherwise.

DIOS-MS Measurements

An Applied Biosystems Voyager DE-STR MALDI-TOF mass spectrometer (Framingham, MA) was operated at an accelerating voltage of 20 kV in a linear mode. The delay time was varied from 100 to 250 ns to achieve optimal MS performance. For each data point, 30 spectra were collected from different locations on the same substrate and were accumulated to yield the final

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spectrum. Three replicate substrates were used in each experiment to calculate measurement variations.

TEA was dissolved in various deuterated solvents at 20 pmol/μL. In most studies, after an overnight storage under vacuum the DIOS or chemically modified DIOS substrates were immersed in TEA solutions. The exception was made in the study of surface acidity where 20 pmol/μL of TEA/D_2O was drop-coated on the H-, OH-, and RNH_2-terminated DIOS substrates to minimize surface hydrolysis. DPPC was dissolved in 2,4-pentanedione/methanol (1:1) or methanol at 100 pmol/μL. DPPC in the 2,4-pentanedione/methanol solution was then drop-coated on a OH-terminated DIOS substrate and the one in the methanol solution was drop-coated on a freshly prepared H-terminated DIOS substrate for comparison.

Surface Characterization

Surfaces of H-terminated and OH-terminated DIOS substrates were quantitatively analyzed using a Physical Electronics TRIFT I TOF-SIMS instrument with a 25 keV Gallium primary beam (Eden Prairie, MN). For each experiment, the spectra were collected from multiple locations for averaging. All spectra were normalized by the dominant $[Si]^+$ peak.

Results and Discussion

Tracking hydrogen migration with a deuterated reagent has been well established in physicochemical studies [12]. Given in DIOS-MS most analytes are detected as protonated species, the same methodology was used in this report by deliberately replacing protons with deuteria in the surroundings to isolate and quantify the source(s) of analyte protonation. Triethylamine (TEA, $C_6H_{15}N$) was used as the MS model molecule for its high basicity and good thermal stability.

Figure 1a shows the natural isotopic distribution of TEA with the molecular ion, $[M + H]^+$, at m/z = 102.13 and an isotopic peak at m/z = 103.13 ($[M + 2]^+$). This peak is mainly due to the presence of isotopes ^{13}C (1.08%), ^{15}N (0.37%), and ^{2}D (0.24%), and the natural abundance of these isotopes yield a relative ion intensity (RI) of 7.3 ± 0.6%. In this study, the RIs of $[M + 2]^+$ beyond 7.3% was used to estimate the incorporation of deuteria during protonation (i.e., $[M + D]^+$). The monoisotopic mass peak of protonated TEA was labeled as $[M + H]^+$ and was used as the base peak in intensity normalization. A small peak at m/z = 100.11 was also noticed throughout the collected MS spectra. It was assigned as $[M - H]^+$ (Supplemental Data, which can be found in the electronic version of this article).

Note that the absence of $[M - H]^-$ ions during negative-ion detection reduced the likelihood of proton exchange among analyte molecules themselves. The previous findings on the aqueous-phase basicity of an analyte contributing more to ionization than the gas-phase basicity of the same analyte suggested that proton transferring probably occurred in the condensed phase [6]. Consequently, this investigation focused on protonation between the analyte and its surrounding molecules in the condensed phase, including proton exchange with (1) the solvent where the analytes were dissolved in, (2) the solid surface where they were deposited on, and (3) other chemical species co-adsorbed on the surface. In addition, several assumptions were made in the following discussions: (1) the ionization cross-sections of the deuterated and non-deuterated species were considered relatively similar; (2) within the concentration range studied, the ion intensities were linearly dependent on the amount of the species in the system, i.e., the $[M + H]^+$ or $[M + D]^+$ peak intensities were proportional to the amount of $[M + H]^+$ or $[M + D]^+$ ions formed; and (3) the absolute amount of protons donated from the same solvent was relatively constant, independent of changes in surface functional groups, and vice versa.

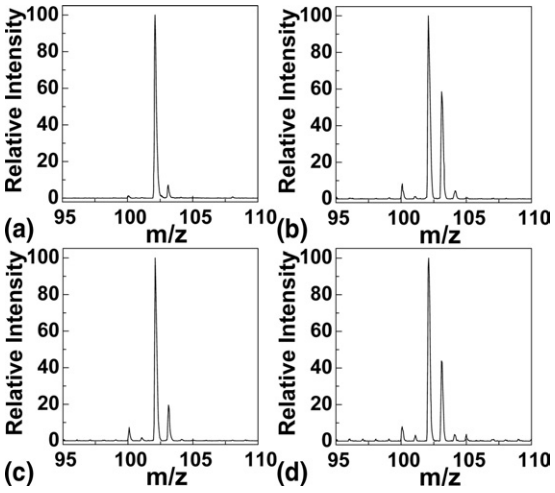

Figure 1. Mass spectra of TEA collected from (**a**) a DIOS substrate immersed in a 20 pmol/μL TEA/H_2O solution, (**b**) a DIOS substrate immersed in a 20 pmol/μL TEA/D_2O solution, (**c**) the same DIOS substrate reloaded with 10 μL of ethanol, and (**d**) the same DIOS substrate reloaded with 10 μL of D_2O again. The $[M + 1]^+$ peak was the base peak that set at 100% RI. For detailed experimental conditions see the text.

Analyte Protonation by Solvent

A freshly prepared DIOS substrate was dried under high vacuum overnight to remove solvent molecules adsorbed during substrate preparation and storage. After taking the substrate out of the vacuum chamber, it was immediately immersed in a 20 pmol/μL solution of TEA/D_2O for 3 h, followed by MS measurements. A MS peak of $[M + 2]^+$ was clearly observed with a calculated RI of 54 ± 7% (Figure 1b). It was significantly more intense than that from the natural isotopes, which confirmed direct analyte protonation from the solvent. Additional evidence of solvent contribution was obtained when 10 μL of ethanol was later added to the same substrate to displace residual D_2O molecules

Table 1. Calculated proton donating effectiveness of the solvents

Solvent	pKa	Vapor pressure[19] (25 °C mm Hg)	MS RI of $[M + 2]^+$	ΔRI = measured RI – 7.3%	Calculated protonation effectiveness
d_8-Toluene	41[16]	26	11 ± 1%	4 ± 1%	4 ± 1%
D_2O	15.74[17]	23.74	54 ± 7%	47 ± 7%	32 ± 5%
d_4-Methanol	15.54[17]	127.05	29.6 ± 0.5%	22.3 ± 0.8%	18.2 ± 0.6%
d_6-Acetone	19.3[18]	229.52	14.8 ± 0.3%	7.5 ± 0.7%	7.0 ± 0.6%

adsorbed on the surface. A visible decrease of the $[M + 2]^+$ signal to a RI of 22 ± 7% was observed (Figure 1c). Note that this $[M + 2]^+$ peak was still more intense than that from the natural isotope abundance (7.3%) due to incomplete displacement of D_2O molecules. The $[M + 2]^+$ peak intensity was recovered by reloading the substrate with 10 μL of D_2O (Figure 1d).

In the condensed phase, residual solvent molecules left on the surface directly participate in analyte protonation as in [6, 12]:

$$[\text{solvent}] + [\text{TEA}] \rightarrow [\text{solvent-H}]^- + [\text{TEA} + \text{H}]^+$$

Thus, the proton affinity (pKa) and the amount of the selected solvent in the condense phase are expected to play important roles in analyte protonation. This notion was supported by two parallel experiments: in the first experiment, TEA was dissolved in the solvents of similar vapor pressures but different proton affinities. The measured $[M + 2]^+$ peaks had the RIs of 54 ± 7% or 11 ± 1% for TEA dissolved in D_2O or d_8-toluene, respectively, consistent with water being more acidic than toluene (i.e., pK_a = 15.7 versus 41) (Table 1). In the second set of experiments, three predried DIOS substrates were dipped in the TEA solutions where D_2O, d_4-methanol, and d_6-acetone were used as the solvents to study the impacts from the solvents of similar proton affinities but drastically different vapor pressures (i.e., different amounts of solvent residuals left on the surface). D_2O has the lowest vapor pressure due to strong hydrogen bonding; therefore the highest amount of solvent molecules was retained on the surface, and subsequently the most intense $[M + 2]^+$ peak was observed (Table 1). The use of acetone with the highest vapor pressure among the three resulted in the smallest RI of the $[M + 2]^+$ peak due to fast evaporation. Continuous removal of solvent molecules through constant pumping at $<2 \times 10^{-7}$ torr led to reduction of RIs in all three solvents, in spite that the MS spectra were collected from different spots on the same substrate to avoid analyte depletion (Figure 2). The decreasing rate of $[M + D]^+$ peak intensities slowed down over time with fewer solvent molecules to be removed from the surface. The RIs of the $[M + D]^+$ peaks later became relatively stable and the plots leveled off when only a few layers of solvent molecules were left on the surface.

A semiquantitative calculation of the proton donating effectiveness from the solvent was carried out by comparing the RI values of the $[M + 2]^+$ peaks collected with and without the use of deuterated solvents. For example, the $[M + 2]^+/[M + 1]^+$ in Figure 1a and b can be written as the following:

$$\frac{[^{13}CC_5H_{15}N + H]^+}{[C_6H_{15}N + H]^+} \times 100\% = 7.3 \pm 0.6\%$$

$$\frac{[C_6H_{15}N + D]^+ + [^{13}CC_5H_{15}N + H]^+}{[C_6H_{15}N + H]^+} \times 100\%$$

$$= \frac{[C_6H_{15}N + D]^+}{[C_6H_{15}N + H]^+} \times 100\% + (7.3 \pm 0.6)\% = 54 \pm 7\%$$

$$\text{or}: \frac{[M + D_{solvent}]^+}{[M + H_{others}]^+} \times 100\% + (7.3 \pm 0.6)\% = 54 \pm 7\%$$

where $[M + D_{solvent}]^+$ refers to D_2O contribution and $[M + H_{others}]^+$ refers to contributions from other factors (for detailed calculation see Supplemental Data). Mathematic rearrangement of these equations shows that the fraction of the protons donated by water (i.e., proton donating effectiveness) in overall analyte protonation was estimated to be 32 ± 5%. Applying the same calculation to TEA dissolved in methanol and acetone showed that the fraction of protons from the solvent were at ~18.2% and ~7.0%, respectively (Table 1). Note that these calculated numbers were expected to be smaller than the actual values, mainly due to fast adsorption of H_2O molecules from the air during the

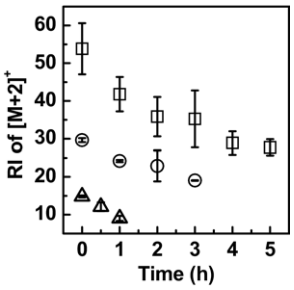

Figure 2. The calculated RIs of the $[M + 2]^+$ peaks plotted as a function of storage time of the substrates s under 2×10^{-7} Torr. The substrates were pretreated with 20 pmol/μL TEA in D_2O (open square), methanol-d_4 (open circle) or acetone-d_6 (open triangle). All mass spectra were collected under the same instrument parameters. For other experimental conditions see the text.

short period of air exposure when the substrate was taken out of the vacuum but before its immediate immersion in the TEA/D_2O solution. It is interesting to point out that the calculated proton donating effectiveness from different solvents of similar acidity was in linear reciprocal to their vapor pressures (Supplemental Data F1).

Additional consideration was given to potential indirect proton-transferring from solvent-induced surface modification since silicon is H_2O-reactive [13–15]. MS spectra were collected from the same DIOS surface at different time points to monitor surface oxidation. If there was any, a slow but steady increase in the $[M + 2]^+$ peak intensity would be expected due to more and more surface groups being oxidized/converted to the Si-OD groups over time. Yet, no increase in $[M + 2]^+/[M + 1]^+$ was observed in this study, suggesting that this indirect proton transfer pathway played a minor role, if any, in analyte protonation.

Analyte Protonation by Porous Si Surface

Surface functional groups of DIOS substrates are decided by substrate preparation and storage [11, 20, 21]. For example, surface oxidation occurs immediately after a brief exposure of the substrate in air and has been found to be the culprit for performance degradation of DIOS-MS [22]. In this study, two DIOS substrates were carefully oxidized in H_2O_2/MeOH or D_2O_2/MeOH-d_4. The use of deuterated peroxide introduced deuteria to the surface as in Si-OD, and possibly other deuterated functional groups through isotopic exchange. After drying both substrates under vacuum, they were dipped in a TEA/D_2O solution before MS measurements. As expected, the calculated RI of the $[M + 2]^+$ peak increased from 34 ± 6% from the H_2O_2-treated surface to 80 ± 9% from the D_2O_2-treated one.

The fraction of protonation from the surface can be similarly estimated by comparing the RI values of the $[M + 2]^+$ peaks collected from the H_2O_2 or D_2O_2-oxidized substrates (Table 2). For example, $[M + 2]^+/[M + 1]^+$ can be written as:

$$\frac{[M + D_{solvent}]^+}{[M + H_{others}]^+ + [M + H_{Si\text{-}OH}]^+} \times 100\% + (7.3 \pm 0.6)\% = 34 \pm 6\% \ (H_2O_2\text{−treated substrate})$$

$$\frac{[M + D_{solvent}]^+ + [M + H_{Si\text{-}OD}]^+}{[M + H_{others}]^+} \times 100\% + (7.3 \pm 0.6)\% = 80 \pm 9\% \ (D_2O_2\text{−treated substrate})$$

where $[M + D_{solvent}]^+$ refers to D_2O contribution, $[M + D_{Si\text{-}OH}]^+$, or $[M + D_{Si\text{-}OD}]^+$ refers to the contribution of the oxidized −OH/OD groups, and $[M + H_{others}]^+$ refers to the contributions from other factors, including residual H_2O, unoxidized Si-H groups, and other co-adsorbents (for detailed calculation see Supplemental Data). Mathematic rearrangement of the equations shows that the fraction of protonation from SiOD/H was 21 ± 5% and the solvent contribution was reduced to 21 ± 4%.

The pKa value of a surface functional group is also found to affect its ability of donating protons. A set of porous silicon substrates were modified separately to introduce three different functional groups: Si-RNH_2, Si-H, and Si-OH with increasing acidity. While a 100% surface conversion to any particular surface functional group was unlikely, a more acidic surface was expected from the one with a higher density of Si-OH, and a more basic one for the substrate with Si-RNH_2 groups that may compete with the analyte for protons. Due to the lack of deuterated surface modification reagents, the changes in $[M + H]^+$ peak intensities were used to evaluate the relative contribution of various surface functional groups, where $[M + D]^+$ was used as the internal calibrator (from the use of deuterated water as the solvent). This experiment design was based on the findings that the proton-donating capability of a solvent was largely decided by the chemical nature of the solvent itself. As shown in Table 2, the relative intensities of the $[M + 2]^+$ peaks increase as the acidity of the corresponding surface function group decreases, correlated well with the reduced protonation contribution of the surface (i.e., reduced formation of $[M + H]^+$ ions) (Supplemental Data F2). Note that in this set of experiments, the modified porous silicon substrates were drop-coated with a 20 pmol/uL solution of TEA/D_2O to minimize surface hydrolysis by reducing incubation time. Thus, the results were not directly comparable to those from the dipping experiments discussed in the previous section due to the difference in the amounts of the residual solvent, i.e., D_2O, present in the system.

Table 2. Calculated proton donating effectiveness of the surfaces

	Surface functional groups	MS RI of $[M + 2]^+$	ΔRI = measured RI – 7.3%	Calculated protonation effectiveness of surface
Dip coating	Si-OH	34 ± 6%	27 ± 6%	21 ± 5%
	Si-OD	80 ± 9%	73 ± 9%	
Drop coating	Si-OH	17 ± 6%	10 ± 6%	N/A
	Si-H	33 ± 9%	26 ± 9%	N/A
	Si-NH_2	70 ± 11%	63 ± 11%	N/A

Analyte Protonation by Other Environmental Factors

Despite of the efforts to replace hydrogen in the solvent and on the surface with deuterium, a strong $[M + H]^+$ peak was observed throughout the MS spectra collected. The residual Si-H groups on the porous Si surface were believed to be the main culprit to this remaining $[M + H]^+$ peak. The speculation was supported by surface characterization using secondary ion mass spectrometry (SIMS), in which the relative intensity of $[Si\text{-}OH]^+$ was found to increase from 9 ± 2% to 23 ± 2% after oxidation while the relative intensity of Si-H reduced from 97 ± 2% to 51 ± 2% (Supplemental Data F3). An absolute calculation on the conversion efficiency of the surface functional groups was difficult without extensive surface calibration, mainly due to the varying ionization probabilities of $[SiOH]^+/[SiOD]^+$, $[SiH]^+$, and $[Si]^+$. However, this experimental observation suggests that a significant amount of surface functional groups was not converted to hydroxyl groups during surface oxidation [23]. Additional proton source(s) in the analyte local chemical environment in the forms of adsorbed hydrocarbons and H_2O vapor in the sample chamber also contributed to the remaining presence of $[M + H]^+$, grouped together in this report as the "other" factors.

Improvement in DIOS-MS Performance

The findings that analyte protonation is directly related to the properties of the DIOS surface and the solvent suggest that DIOS-MS detection can be enhanced by purposely modifying the substrate surface with low-pKa functional groups and by selecting solvents of low-pKa values and low vapor pressures. Figure 3 shows detection of dipalmitoylphosphatidyl-choline (DPPC) that was dissolved in 2,4-pentanedione/methanol and the mixture was drop-coated on an oxidized DIOS substrate. 2,4-Pentanedione was selected as the appropriate solvent for its low acidity, low vapor pressure, and good biocompatibility (pKa = 9.8, vapor pressure = 6 mm Hg). In comparison, DPPC was dissolved in methanol at the same concentration and was loaded on a control DIOS piece where the surface was freshly regenerated (i.e., Si-H-rich). A remarkable enhancement of 17-fold in the molecular ion intensity was obtained under the Si-OH/pentanedione treatment, in comparison with that of the control experiment. Similar result was also observed in detection of small peptides, such as des-Arg^1-bradykinin and angiotensin I (data not shown).

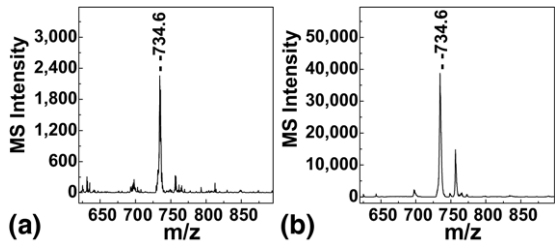

Figure 3. Mass spectra of (**a**) a DPPC/Methanol solution drop-coated on an unmodified DIOS substrate, and (**b**) a DPPC/2,4-pentadione/methanol mixture at the same concentration drop-coated on an oxidized DIOS substrate. The detected analyte were labeled by their molecular ion peaks.

Conclusions

The local chemical environment of an analyte, including the residual solvent and the surface function groups, were identified as the major proton sources in DIOS-MS. Their relative contributions were semiquantified. The experimental conclusions allowed further optimization of sample treatment for improved DIOS-MS performance, as demonstrated in lipid and peptide detection. It is important to note, however, that chemical modification of the Si surface changes not only the functional groups but also the physical properties of the porous surface. For example, moderate oxidation leads to formation of acidic Si-OH groups that benefit analyte ionization. However, the formation of an overoxidized silicon oxide layer slows down vertical thermal dissipation and reduces overall ionization efficiency. As a result, the degree of surface modification needs to be carefully monitored to achieve optimal MS results.

Acknowledgments

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Uncited References

This section contains references that occur in the reference list but are not cited in the text. Please position each reference in the text or delete it. Reference not dealt with will be retained in this section: [16, 17, 18, 19].

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