e-MALDI: An Electrowetting-Enhanced Drop Drying Method for MALDI Mass Spectrometry

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ABSTRACT: The performance of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) is frequently compromised by the heterogeneous distribution of matrix and analyte deposits on the target plate arising during the conventional drop-drying sample preparation procedure. It was recently shown that this so-called coffee stain effect can be suppressed by exciting evaporating complex fluids throughout the drying process using AC-electrowetting. Here, we demonstrate that electrowetting-assisted drying of solutions of common MALDI matrix materials and a variety of common low molecular weight pharmaceutical molecules indeed leads to substantially smaller and more homogeneous sample spots on special electrowetting-functionalized e-MALDI target plates. The improved spot quality enables 2–30× enhanced MALDI-MS signals along with substantial reductions of the typical lateral variations of the MALDI-MS. The latter largely eliminates the time-consuming need to search for “sweet spots”.

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has become one of the most widely used and rapidly growing analytical tools for the identification of natural and synthetic organic molecules in many areas including proteins, carbohydrates, polymers, small molecules, and pathogens. MALDI-MS combines gentle ionization of frequently fragile compounds, ease-of-use, decent sensitivity, and also high throughput capability. Despite the fact that MALDI-MS has been around since the 1980s, the understanding of important aspects determining sensitivity, resolution, and reproducibility is still incomplete. Fundamentally, this is caused by the complexity of the ionization and vaporization process that involves the absorption of laser light by the matrix material, the subsequent evaporation and ablation of matrix and analyte(s), and a variety of competing charge generation and transfer processes.

Substantial efforts are devoted to a better understanding of these fundamental processes and to the improvements of specific matrix materials, optimization of the laser wavelength, beam size, and beam profile, and combinations of multiple laser pulses (see, e.g., ref 14 for a recent review); one of the most important practical challenges in MALDI-MS is still the identification of the optimum sample preparation method. Extensive databases are nowadays available for the best choice of matrices and solvents for many applications. Various sample deposition methods have been developed, including various spraying techniques, inkjet printing, solvent-free systems, and ionic liquids. Some of them are specifically optimized for MALDI imaging, as recently reviewed in ref 19. Other approaches involve acoustic nebulizers and target plates with specific wettability patterns.

Yet, the vast majority of the subsequent preparation protocols for MALDI MS spectroscopy are still based on the traditional drop drying method, in which a drop of dissolved matrix, analyte, and additives is pipetted manually or automatically onto a target plate where the solvent is left to evaporate. The problem of this method is that it typically results in a rather heterogeneous distribution of matrix material and analyte on the target plate (Figure 1). This heterogeneity poses a major problem to routine MALDI-MS analysis because it implies that the intensity of the MALDI signal varies dramatically depending on the precise impact position of the laser spot (see, e.g., ref 23). As a consequence, operators typically have to search for “sweet spots” on the target plate where a “good” signal is obtained. This procedure is time-consuming and error-prone, and it introduces subjectivity to the analysis. While a variety of alternative preparation methods were developed to address this problem, none of them found wide acceptance, potentially due to the additional complexity of the protocols and/or additionally required equipment.

In physical chemistry and wetting science, the formation of heterogeneous deposits upon evaporation of drops of complex
fluids, solutions and suspensions, is known as the "coffee stain effect". Intensive research in recent years (see ref 24 for a recent overview) has shown that coffee stain formation arises from a combination of an evaporation-driven flux within the evaporating drop and pinning of the three phase contact line of the drop at microscopic heterogeneities on the substrate.25,26 This process depends on many details including the wettability of the substrate, contact angle hysteresis, temperature, and the nature of the solute.24 Motivated by a variety of applications, several methods have been developed to suppress coffee stain formation, including the addition of surfactants, controlled variations of temperature gradients that induce Marangoni flows,27,28 the addition of gelators,29 and the use of specifically shaped particles that form solid layers at the drop surface.30 More recently, it was shown that electrowetting (EW)31,32 provides a versatile alternative to these processes enabling coffee stain suppression without requiring any additives. Extensive experimental and numerical studies demonstrated that this effect is based on a combination of depinning of the contact line33 and the generation of intensive internal flow fields that supersede the evaporation-driven flux.31,34,35 First tests even demonstrated that the technique enables improved MALDI-MS signals for the solutions of polyethylene glycol (PEG).32

In this communication, we apply the EW-enhanced MALDI-MS sample preparation technique e-MALDI to a variety of small pharmaceutical molecules. We demonstrate that this technology leads to substantially smaller and more homogeneous sample deposits on the target plates for all samples investigated. Subsequent MALDI-MS analysis displays 2−30× enhanced signal strength along with a substantially improved lateral homogeneity. e-MALDI technology is simple to use and compatible with sample preparation protocols and does not require any additives that might interfere with the signal of interest. In particular, the technology is fully compatible with advanced microfluidic sample pretreatment assays based on electrowetting.36−38

**EXPERIMENTAL SECTION**

**Materials.** For coating of eMALDI chips, 0.5% (w/w) of Teflon TM AF (DuPont) in Fluorinet FC-40 (Sigma-Aldrich, ≥97.0%) was used. The selected analyzed molecules, including paracetamol (Sigma-Aldrich, pharmaceutical secondary standard) and commercial paracetamol (Kruidvat), quinine (Sigma-Aldrich, ≥98.0%), penicillin V (Sigma-Aldrich, analytical standard), aspirin (Sigma-Aldrich, USP), ibuprofen (Sigma-Aldrich, ≥98.0%), polyethylene glycol (PEG MW 650 and 1000, Sigma-Aldrich), griseofulvin (from Penicillium griseofulvum, Sigma-Aldrich, 97.0−102.0%), and fenofibrate (Sigma-Aldrich, ≥99%), were used as received. MALDI matrixes, including 2,5-dihydrobenzoic acid (DHB, Sigma-Aldrich, >99.0%), α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich, >99.0%), sinapic acid (SA, Sigma-Aldrich, >99.0%), and dithranol (DIT, Sigma-Aldrich, >99.0%), were likewise used as received. Trifluoroacetic acid (TFA, Sigma-Aldrich, 99%) and acetonitrile (Sigma-Aldrich, LC-MS Ultra CHROMASOLV) were used to prepare solutions without further purification.

**e-MALDI Target Plates.** The EW effect describes the reduction of the contact angle of a partially wetting conductive liquid on a thin insulating surface covering an electrode upon applying a voltage between the drop and the electrode on the substrate.39 In conventional EW, the voltage is applied to the

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![Figure 1. Illustration of the drop drying process. (a) Conventional drop drying process without electrowetting illustrating evaporation-driven flux within the drop (top figure; green arrows) and the resulting coffee stain pattern (middle). Bottom: optical micrograph of dried DHB-quinine sample. (b) electrowetting-enhanced e-MALDI drying process with recirculating internal flow (top figure: red arrows) leading to a smaller and more homogeneous precipitate (middle). Bottom image: same DHB-quinine sample (10:1 molar ratio) as in (a) dried by e-MALDI. Inset top right: illustration of interdigitated electrode pattern (not to scale; final dried sample spot covers several electrodes). See text for details.](image-url)
drop by immersing a wire into it. To circumvent the latter, we fabricated dedicated e-MALDI target plates using sets of so-called coplanar electrodes that consist of arrays of interdigitated stripe electrodes made of indium tin oxide (ITO) on a conventional microscope slide from glass. Both the width and the spacing of the electrodes are 20 μm (see Figure 1). The ITO electrodes on the glass surface are covered by a composite dielectric layer consisting of a 4.3 ± 0.2 μm thick layer of Parylene C deposited by a plasma CVD process (Parylene coater PDS 2010) and an ≈50 nm thick top coating of Teflon AF (DuPont). The latter was deposited by dip-coating from a 0.5% w/w solution in Fluorinert FC-40. The entire sample is cured at 210 °C for 120 min after the dipcoating process.

Sample Preparation Procedure. Materials and matrixes were obtained from Sigma-Aldrich (see above) and used without further purification. Mixtures (1:1 v/v) of water and acetonitrile (ACN; Sigma-Aldrich) were used as a standard solvent throughout. Matrix (2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CHCA)) was dissolved together with the analyte in a 10:1 matrix/analyte molar ratio with an initial analyte concentration of ≈1% w/w in the fluid. Sample drops with a volume of 7 μL were pipetted onto the e-MALDI target plates and left to evaporate. Control experiments with EW-assisted drying and conventional (non-EW) drying were carried out side by side on the same target plate. EW-assisted drying was carried out at an alternating AC voltage of \( U_0 = 90 \text{ V} \) (amplitude) and an excitation frequency \( f = 100 \text{ Hz} \). For the pure drop solvent mixture, these conditions lead to a contact angle reduction from Young’s angle \( \theta_Y = 91 \pm 0.5^\circ \) (on average) at zero voltage to \( \theta (90 \text{ V}) = 80 \pm 0.6^\circ \), as confirmed by contact angle goniometry. Note that these operating conditions of EW are very mild in the sense that (i) the applied voltage is only a fraction of the critical voltage for undesirable effects such as the emission of satellite drops and (ii) the frequency is well below the transition frequency to dielectrophoresis (several tens of kHz). As numerical solutions of the full Nernst-Planck-Boltzmann heat convection problem show, the latter implies that heating induced by the AC voltage is negligible in the present experiments.

The drying process was carried out at an ambient temperature of 22 °C and ambient humidity of 85–87% R.H. The evaporation process took approximately 20–25 min. After the drying is completed, the samples are disconnected from the AC voltage and the e-MALDI target plate is inserted into the sample lock, as usual.

Sample Characterization and MALDI-MS Measurements. Dried samples were characterized by optical microscopy and scanning electron microscopy (SEM) prior to MALDI-MS analysis. Subsequent MALDI-MS analysis was carried out in a Waters MALDI SYNAPT High Definition Mass Spectrometer equipped with a 200 Hz solid state high repetition rate laser system. Except for a few control measurements in negative mode, the instrument was operated in positive ion mode. The laser was focused to a spot size of approximately 120 μm. 1000 laser pulses were accumulated per location on the sample. MALDI MS images were acquired using the same instrument in positive mode at 150 Hz frequency. Approximately 1000 laser shots were accumulated per image with 150 pulses per shot. The reduction, analysis, and plotting of imaging was done using MSiReader software.

RESULTS AND DISCUSSION

Drop Evaporation, Precipitation, and Dried Spot Characterization. Figure 1 illustrates the drop drying process and solid residue for conventional (a) and for EW-enhanced e-MALDI (b) drop drying. The mass flux due to the evaporation of the solvent causes the drop surface to recede. In conventional drop drying, however, the contact line is pinned and can not recede. As a consequence, the drop diameter...
remains constant and the contact angle decreases during evaporation (see Figure 1a). The evaporation-induced mass flux along the pinned contact line is compensated by a flux from the center of the drop toward the contact line (blue arrows in Figure 1a, top). This flux carries nonvolatile solute molecules and particles along with it, leading to an increase of their concentration along the contact line until precipitation sets in. Matrix crystals hence preferentially nucleate along the contact line and grow from there toward the center of the drop. The micrograph in the bottom of Figure 1a shows the characteristic needle-like DHB crystals formed in the presence of analyte (quinine).

In the presence of AC-EW, a strong recirculating flow is generated within the drop that continuously mixes the contents of the drop. This prevents the accumulation of solute and premature nucleation of precipitates. At the same time, the contact line is mobilized due to the excitation by the periodically oscillating electrostatic force \( f_{el} = \varepsilon U_0^2 \cos^2(2\pi f t)/2 \). (Here, \( \varepsilon \approx 10^{-5} \) F/m² is the capacitance per unit area between the drop and the electrodes.) As a consequence, the diameter of the drop shrinks during the evaporation while the contact angle remains approximately constant. The micrograph in the bottom of Figure 1b shows a typical dried residue for the same type of sample as in (a).

While the optical micrographs already demonstrate the much smaller diameter for the dried sample, scanning electron microscopy (SEM) images provide an even clearer view of the much more compact structure of the e-MALDI-dried samples, Figure 2. While the conventionally dried samples consist of rather large isolated crystallites that are sometimes partially freely distributed, the e-MALDI-dried spots typically display a smaller average size of the matrix/analyte crystallites. All these crystallites are tightly packed together in a single object that reflects that spherical cap shape of the solution drop in the final stages of its evaporation. The images suggest that the moment of precipitation has been delayed until only very little solvent was left.

MALDI-MS Characterization. To characterize the efficiency of the preparation technique for MALDI-MS, we first recorded the total ion current along cross sections of equidistantly spaced points through the dried sample spots. Consistent with the heterogeneous distribution of sample seen in the optical and SEM images, the conventionally dried samples display regions of decent total current next to regions of almost vanishing current. As expected, the signal of the e-MALDI-dried samples was much more localized than the signal from conventionally dried samples, Figure 3. Moreover, the total ion current is found to be much stronger than even that for the best “sweet spots” from the conventionally dried samples. In addition, the lateral variations of the current within the spot are substantially reduced for e-MALDI-dried samples.

The observed increase in signal intensity and homogeneity were both confirmed upon recording actual MALDI spectra. In Figure 4, we compare the few sample MALDI spectra obtained at an arbitrary location within the dried spot within an e-MALDI sample (red curves) to the spectra obtained at a sweet spot of a conventionally dried sample for common pharmaceutical molecules of low molecular weight, including mixtures of several species, Figure 4. (See the Supporting Information for detailed peak assignment as well as control experiments using metallic target plates and negative mode measurements.)

### Figure 3
Typical profile of the total ion current along a cross section through a conventionally dried sample spot (blue squares) and an e-MALDI-dried spot (quinine/DHB 1:10 sample).

### Figure 4
MALDI mass spectra for various samples comparing conventionally dried samples (blue; plotted in downward direction) and e-MALDI-dried samples (red). (A) Quinine. (B) Ibuprofen. (C) Mixture of paracetamol, penicillin V, and quinine. (See the Supporting Information for detailed peak assignment as well as control experiments using metallic target plates and negative mode measurements.)
plates are not relevant under the conditions of the present measurement.

To demonstrate the homogeneity of the material distribution and the homogeneity of the corresponding signal, we collected MALDI images for the mixture of paracetamol, quinine, and penicilline V dried with e-MALDI and conventionally, Figure 5. As it is clearly seen from the images, the signal from e-MALDI samples is approximately $10^\times$ stronger and much more homogeneous than in the case of conventionally dried samples. Note in particular that there is no noticeable sample left behind on the surface outside the well-defined dried spot of the e-MALDI samples.

Figure 5. MALDI MS image spectra for a mixture of paracetamol (A, D), quinine (B, E), and penicillin V (C, F) comparing e-MALDI-dried samples (A, B, C) and conventionally dried samples (D, E, F).

Figure 6 conﬁrms the improved level of homogeneity of the MALDI signal for a series of spectra obtained at random locations within the spot of a conventionally dried spot (left) and an e-MALDI-dried spot on the same vertical scale. As the graphs show, the peak height varies by less than 10% between adjacent regions on e-MALDI samples, whereas relative variations of 90% are not uncommon for conventionally dried samples. Interestingly, an analysis of the peak height shows that the relative intensity of the various individual peaks hardly depends on the position. This suggests that the primary effect of the e-MALDI process is simply to enhance the signal and to make it laterally more homogenous while preserving all the chemical information in the spectra.

Similar results were obtained for a wide range of samples. Table 1 summarizes the results obtained for optimum matrix and solvent composition, including a characterization of the statistical properties of the signal, as evaluated on the basis of the spectra. Here, we define the e-MALDI signal increase as the ratio between the laterally averaged height of the main peak for e-MALDI samples divided by the same quantity for conventionally dried samples. More extensive data sets for other (nonoptimal) matrices and solvent composition as well as more detailed information about the analysis of the spectra can be found in the Supporting Information. Table 1 also shows that
the signal-to-noise ratio as calculated by the operating software of the instrument is typically enhanced by a factor of 2–3. For hydrophobic drugs, however, the enhancement is found to be somewhat less pronounced.

Table 1 shows that e-MALDI is a rather versatile tool to enhance the quality of the classical drop drying sample preparation procedure. The gain in signal intensity and signal-to-noise ratio should enable a lower overall detection limit as compared to conventional drop drying. The improved lateral homogeneity leads to a better region-to-region consistency of the measurement. We expect that this enhanced consistency will eventually translate into improved reproducibility and quantitiveness of the overall MALDI-MS analysis. Systematic studies of the benefits in these respects are ongoing. Obviously, such studies should include a broader class of materials, in particular, proteins, peptides, glycans, bacteria/pathogens, etc. While initial tests are positive, this will require the development of specific protocols for each kind of analyte. In particular, the interaction of partly hydrophobic molecules with the hydrophobic dielectric surface of the e-MALDI target plate requires specific attention to prevent early unspecific adsorption that can hinder the e-MALDI process. Compared to other advanced MALDI target plate designs such as the Bruker Anchor Chip and NALDI technology, not only does e-MALDI exploit the benefits of surface hydrophobicity to enable improved contact line mobility and more compact spots, but also the additional internal flows within the evaporating drop guarantee continuous mixing of the drop content throughout the evaporation phase and thereby allow additional control over the precipitation process. Further optimizations of electrical waveforms (e.g., higher frequencies) and electrode geometries are expected to lead to even stronger signal enhancements.

Overall, the technology is easy to use and fully compatible with standard workflows. Moreover, e-MALDI target plates can be fabricated in arbitrary geometries fitting the requirements of all standard MALDI instruments. Likewise, it is possible to integrate the technique into automated sample handling and pipetting systems.

A particularly interesting perspective for future developments would be to combine the e-MALDI technology with existing drop-based “digital” microfluidic systems (DMF). Substantial efforts have been devoted in recent years to develop combined systems that harvest the synergy between microfluidic sample preparation and MALDI peak assignment for Figure 4. (PDF)

**CONCLUSIONS**

The electrowetting-based e-MALDI sample preparation technology for drop drying MALDI sample preparation provides smaller sample spots and a more homogeneous distribution with generally smaller crystallites. Enhanced signals and improved signal homogeneity were demonstrated for a variety of small pharmaceutical molecules. The technology is easy-to-use and ready to be exploited and evaluated by users.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b04283.

Technical information on electrowetting sample preparation and MALDI peak assignment for Figure 4. (PDF)

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**Notes**

The authors declare no competing financial interest.

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**Table 1. Signal Increase by e-MALDI**

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<th>p-value regular MALDI</th>
<th>signal-to-noise eMALDI</th>
<th>signal-to-noise regular MALDI</th>
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<td>0.02</td>
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“Standard deviation of signal intensity for samples did not exceed 7%.”
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(43) Users interested in testing the methods are invited to contact the authors.