An Impulse-Driven Liquid-Droplet Deposition Interface for Combining LC with MALDI MS and MS/MS

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A simple and robust impulse-driven droplet deposition system was developed for off-line liquid chromatography matrix-assisted laser desorption ionization mass spectrometry (LC-MALDI MS). The system uses a solenoid operated with a pulsed voltage power supply to generate impulses that dislodge the hanging droplets from the LC outlet directly to a MALDI plate via a momentum transfer process. There is no contact between the LC outlet and the collection surface. The system is compatible with solvents of varying polarity and viscosity, and accommodates the use of hydrophobic and hydrophilic MALDI matrices. MALDI spots are produced on-line with the separation, and do not require further processing before MS analysis. It is shown that high quality MALDI spectra from 5 fmol of pyro-Glu-fibrinopeptide deposition after LC separation could be obtained using the device, indicating that there was no sample loss in the interface. To demonstrate the analytical performance of the system as a proteome analysis tool, a range of BSA digest concentrations covering about 3 orders of magnitude, from 5 fmol to 1 pmol, were analyzed by LC-MALDI quadrupole time-of-flight MS, yielding 6 and 57% amino acid sequence coverage, respectively. In addition, a complex protein mixture of an *E. coli* cell extract was tryptically digested and analyzed by LC-MALDI MS, resulting in the detection of a total of 409 unique peptides from 100 fractions of 15-s intervals. (J Am Soc Mass Spectrom 2006, 17, 325–334) © 2006 American Society for Mass Spectrometry

Proteomic analysis of complex samples is routinely carried out by gel electrophoresis [1] followed by mass spectrometric analysis. However, gel-based proteomic methods tend to be a slow, manual process [2–4] unless automated by sophisticated robotic spot-pickers. Alternatives to gel separation include techniques such as multidimensional liquid chromatography (LC) [5] or capillary electrophoresis (CE) [6]. Separation of proteins or peptides can be performed by LC and CE, although protein identification is usually carried out on peptides generated from protein digestion (i.e., bottom-up proteomics approach). Analysis platforms often couple LC or CE separation using an electrospray ionization (ESI) interface with tandem mass spectrometry (MS/MS) [5, 6]. For very complex biological samples, ion suppression and limited spectral recording duty cycle of a mass spectrometer may yield an incomplete analysis of the mixture. Techniques such as peak parking [7, 8] attempt to address the latter issue by pausing the chromatographic run to afford more time for the MS analysis.

It is well known that matrix-assisted laser desorption ionization (MALDI) is complementary to ESI in producing biomolecular ions for MS analysis. MALDI offers greater tolerance to sample contaminants such as salts, buffers, and surfactants. In addition, MALDI MS consumes less sample per analysis. Although not represented in any commercial systems, online LC-MS systems based on MALDI have been reported [9–19]. However, these systems have not yet emerged as viable and routine technologies for proteomics applications.

Many off-line LC-MALDI approaches have been reported, and commercial platforms have also recently emerged [20]. The simplest approach to the interface involves simple fraction collection into collection tubes or well plates, followed by concentration and transfer onto a MALDI target plate for analysis. More recent advances in the interface involve depositing the LC eluate directly from the separation onto the MALDI target, usually requiring a novel technique for the physical process of the deposition. Two modes of deposition have been represented, and are characterized by either a continuous stream, or a series of discrete spots. Continuous stream approaches typically involve devices that spray the eluate on the MALDI plate [21, 22]. A novel vacuum deposition interface [23] has been reported to deposit LC eluate onto a MALDI plate continuously [24], which retains the best chromatographic resolution. Techniques that produce discrete spots include

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blotting methods which involve contact between the eluate droplet and the MALDI plate [25–29], piezoelectric dispensers which dislodge a series of tiny droplets at a high-frequency [30–32], pulsed electric field depositors that use a pulsed electric field to eject droplets [33, 34], and heated droplet interfaces that use thermal energy to evaporate solvents in the hanging droplets to concentrate the eluate and produce smaller droplets for deposition [35].

At present, commercially available LC-MALDI interfaces and most reported interfaces can only deposit low flow, capillary LC fractions (i.e., less than a few microliters per min). Our previously reported heated droplet interface [35] is unique in that a relatively high flow LC separation can be used. For example, a microbore column separation using a 1-mm column operated at a flow rate of 40 μL/min has been routinely used for a number of applications with demonstrated advantages, including high sample loading capability, which for example, facilitate relative proteome quantification [36–38] and characterization of posttranslational modifications of proteins [39]. However, this interface makes use of passive droplet deposition. For capillary LC at a flow rate of 1 or 2 μL/min, passive droplet depositions occur at ~1 min intervals. Thus, the droplet deposition frequency of this interface is inadequate to maintain good chromatographic resolution from the analysis of the sample spots deposited on a MALDI plate. To increase the droplet deposition frequency, an active mechanism of deposition is needed. The ideal droplet deposition technique would also be compatible with the heated droplet interface which requires the heating of a short segment of the LC eluate exit tube and the MALDI plate to different temperatures.

In this paper, we describe a new impulse-driven deposition system for non-contact deposition of the liquid eluate of an LC separation in discrete spots on a MALDI plate using an electric solenoid. The device is very simple to set up and is capable of depositing spots of varying volumes at a variety of frequencies. The device is also capable of co-deposition of MALDI matrix with the eluate. It can deposit both aqueous and organic solvents, making it compatible with a wide range of chromatographic separation modes. The device can be used as a stand-alone system for depositing low flow, capillary LC fractions to MALDI plates, thus complementing the heated droplet interface technique. While the ultimate goal of our research is to integrate the two interfaces into one unit, the goal of this paper is to describe the operational principle of this novel impulse-driven device and the experimental setup to implement this deposition system in detail. The performance of this system for off-line LC-MALDI MS and MS/MS is demonstrated using standard peptides and a tryptic digest of a model protein. Analysis of a tryptic digest of an *E. coli* extract using this device is also demonstrated.

Experimental

Materials and Reagents

HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Chemicals (Fairlawn, NJ), and filtered before use. Water (H_2O) was deionized with a Millipore deionizer to 18 MOhm and filtered through a 22 μm filter before use. Dithiothreitol (DTT), iodoacetamide (IAA), trifluroacetic acid (TFA), bovine serum albumin (BSA), trypsin enzyme, and pyro-Glu-fibrinopeptide (pyro-GFP) were obtained from Sigma Aldrich (St. Louis, MO) at the highest available purity. 2,5-dihydroxybenzoic acid (DHB) was obtained from Sigma Aldrich at 98% purity, and recrystallized before use three times in ethanol, and once in water. DHB was dissolved in methanol to a final concentration of 100 mg/mL before use.

E. coli Protein Extraction

E. coli extracts were prepared by a solvent suspension method [35]. Lyophilized *E. coli* cells (6 mg) were suspended in 2 mL of 10 mM Tris-HCl buffer (pH 7.90) in a centrifuge tube and sonicated for 1 min with a probe tip sonicator (Branson Sonifier 450, Branson Ultrasonics, Danbury, CT) while the centrifuge tube was immersed in an ice bath. The suspension was centrifuged at 11,750 $\times$ g for 10 min. The supernatant was transferred in 500 μL aliquots to Microcon-3 3000 Da molecular weight cutoff filters (Millipore, Billerica, MA) and centrifuged at 13,000 $\times$ g for 30 min. The filtrate was collected, pooled, and the protein content quantified by Bradford assay, using γ-globulin as a protein standard.

Enzymatic Digestion

Five μL of protein in solution was added to 5 μL of 0.1 M NH_4HCO_3 buffer. One μL of 45 mM DTT was added to the solution, and the solution was incubated for 20 min at 37 °C. One μL of 100 mM iodoacetamide was added, and the solution was stored in the dark for 15 min at room-temperature. 0.5 μL of 1 μg/μL trypsin solution was added to this mixture, and incubated for 18 h at 37 °C. The digestion was stopped with the addition of 0.1% TFA.

Chromatographic System

An Agilent 1100 Capillary HPLC system (Palo Alto, CA) was used to perform the reversed-phase separation on a 150 μm $\times$ 15 cm C18 column (Grace Vydac, Hesperia, CA). A reproducible flow rate of 1 μL/min through the column was achieved by using a chromatographic splitter, installed upstream of the sample injector. The pump was operated at a flow rate of 100 μL/min, and the split ratio was adjusted to ~100:1. The flow rate was subsequently tested using volumetric

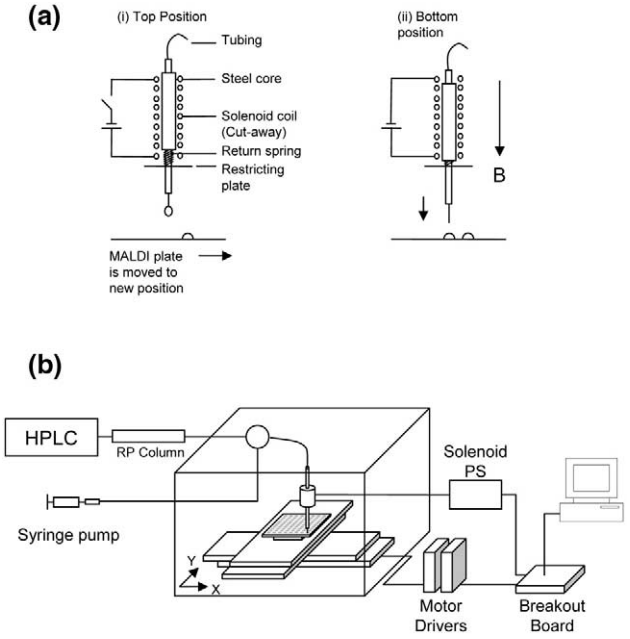

Figure 1. (**a**) Schematic of an impulse-driven momentum transfer droplet deposition device. (**b**) Schematic of the LC-MALDI interface.

glass capillaries. The mobile phase gradient was generated using a binary mixture of A: 0.1% TFA in 4% ACN/H_2O, and B: 0.1% TFA in ACN. The gradient program used was 0% B for 5 min, 0–15% B from 5 to 7.5 min, 15–25% B from 7.5 to 25 min, 25–35% B from 25 to 35 min, and 35–80% B from 35 to 55 min.

Impulse-Driven Deposition Device

A diagram of the deposition system is shown in Figure 1a. The micro-depositor consists of a small solenoid coil (TP6X12-I-24D, Guardian Electric, Woodstock, IL), which is mounted in place with a homebuilt cradle/motion control system. The solenoid core is a homebuilt hollow ferromagnetic steel dowel, with a custom fitted steel capillary. A fused silica capillary (i.d. 50 μm and o.d. 180 μm) is threaded through the i.d. of the steel capillary (i.d. 200 μm and o.d. 1/16 in.) and is fixed in place at the top of the solenoid core assembly with standard fittings and a union (VICI/Valco, Houston, TX).

The solenoid assembly is mounted on a x,y,z micro-adjustment stage (1.75 in. three axis center drive positioning stage, Edmund Optics, Barrington, NJ). The assembly is positioned above motorized translation tables, as shown in Figure 1b (MX80S, Parker Hannifin, Rohnert Park, CA). The motor drivers, computer control card, breakout board, and cables required for the motion tables were acquired from the manufacturer. The solenoid is operated by a homebuilt power supply with an internal function generator which produces a square wave. The power supply can be remotely toggled via a gated logic signal, which is triggered through the break-out board. The motion tables and solenoid power supply are controlled by a program that was written using the supplied software development tools (ACR-View, Parker Hannifin Compumotor Division, Rohnert Park, CA).

Matrix Addition

Matrix was added during the deposition via a post-column T-connection (VICI/Valco, Houston, TX). DHB solution was infused into the T-connection using a syringe pump (Cole-Parmer, Vernon Hills, IL) at a flow rate of 1 μL/min. The LC fractions were deposited on a 100-spot MALDI target (Applied Biosystems, Foster City, CA) that was fixed into position on the motion tables by a homebuilt mounting adapter.

Mass Spectrometry

MALDI MS data were acquired either using a MALDI time-of-flight (TOF) mass spectrometer (Bruker Reflex, Bremen, Germany) in a reflectron mode, or using the Sciex Qstar Pulsar-i Quadrupole-TOF (QqTOF) mass spectrometer (Applied Biosystems, Concord, ON). MS/MS spectra were collected using the Qstar instrument. The Information Dependent Acquisition (IDA) system was used to automatically and independently acquire MS/MS data. The orthogonal MALDI source came equipped with sampling algorithms that were used to automatically target the laser on the samples deposited on the MALDI plate.

Results and Discussion

Principle of Operation

Droplet deposition from a continuous flow stream can be done passively or actively: the former involves allowing the weight of a droplet to overcome the fluid surface tension, and the latter usually involves blotting a droplet to a contact surface, such as a MALDI plate. The passive droplet deposition often results in a large sample spot because it requires the formation of a relatively large droplet, particularly at room-temperature, at the tip of a capillary tube to overcome the adhesion forces between the droplet and the tube. The blotting method is capable of depositing smaller droplets, and these methods include techniques that involve either touching the capillary tip to the collection surface, or by applying an external stimulus to the hanging droplets such that the droplets travel between the capillary to the collection surface. The latter technique, known as non-contact deposition, may improve the robustness of the system by reducing the likelihood of damage to both the spotting capillary and the collection surface. One reported non-contact deposition technique involves the use of an electric field to drive the droplet deposition [32]. However, this technique requires the plate to be an electrical conductor, and requires the use of relatively high voltages. Special care must be

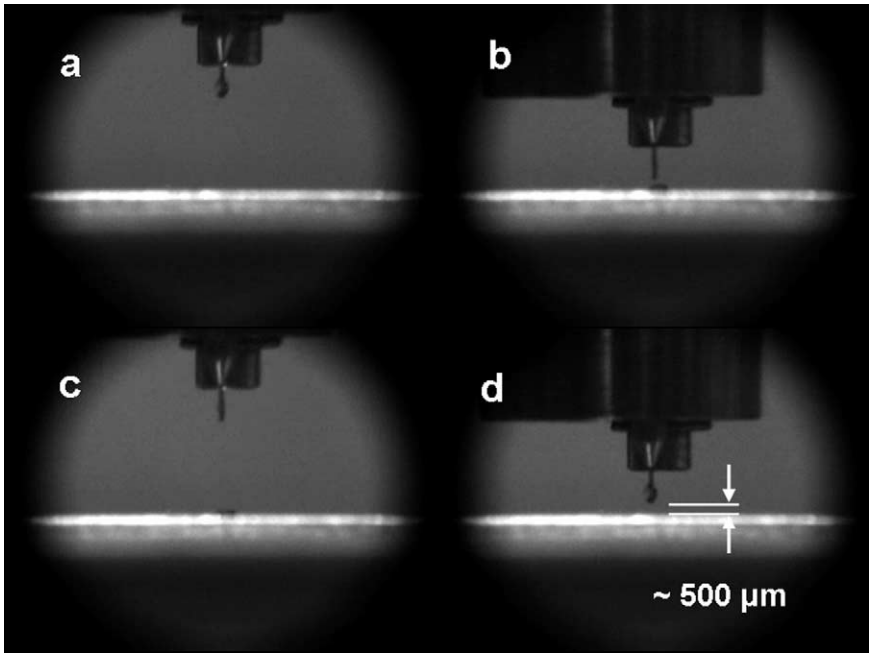

Figure 2. Deposition of a droplet of 50 nL. (**a**) The tip and droplet ready for deposition. (**b**) Deposition of the droplet on the MALDI plate upon activation of the solenoid depositor. (**c**) Droplet after deposition where the solenoid is switched off and the capillary tip is returned to the starting position. (**d**) A droplet suspended at the tip of a capillary at the bottom of the travel for the micro-depositor. The droplet and capillary are separated from the MALDI plate by approximate 500 μm.

taken to avoid electrochemical reactions and to ensure safety in operating a high voltage system.

The idea behind the impulse-driven droplet deposition method is to quickly halt the motion of a moving capillary tube with a droplet hanging at the tip to produce an impulse, and thus provide the droplet with a momentum which can cause the deformation and elongation of the droplet. Droplets of sufficient mass gain enough momentum to deform and significantly reduce its contact area with the tip, causing the droplet to fall to the collection surface. If the droplet is very small, the supplied impulse may not provide enough momentum to the droplet to cause its separation from the tip. However, the capillary tip can be placed very close to the plate so that as the impulse is applied, the droplet elongates and spans the distance between the tip and the collection surface. Wicking action at the collection surface aids in dislodging the droplet from the capillary tip. In both cases, large or small droplet, the capillary tip does not touch the plate. The perils presented by contact with the surface during deposition in the present device include scratching the collection surface and, in an extreme case, damaging the capillary tip to the point that the capillary breaks. Surface scratching can cause an increase in the surface roughness, which in turn causes strong adsorption of analyte to the MALDI surface, making it difficult to clean the surface for re-use. When scratched plates are re-used in MALDI experiments, cross contamination of analytes is often observed. This is particularly apparent when trace sample amounts are analyzed using MALDI plates that were previously used for experiments involving large sample amounts. We note that the present device also supports the use of surface-modified plates, such as those with hydrophobic coatings for analyte concentration. These coatings can be disrupted by strong contact achievable with the present device. Thus it is important to underscore that the present device be used in the non-contact mode.

Figure 1a shows the schematic of the impulse-driven depositor. Impulses are generated by a solenoid driven by a pulsed voltage. The solenoid is used to control the motion of the capillary tube which can be part of or merely attached to a solenoid core. The solenoid is oriented such that the solenoid core travels from top to bottom. The solenoid core is supported in the top-most "rest" position by a return spring. When a voltage is applied to the solenoid coil, the core is induced to accelerate in the direction of the generated magnetic field. The travel is limited by a restricting plate, which arrests the motion of the accelerating solenoid core at the bottom-most position.

The post-column effluent from an HPLC separation travels through a length of fused-silica tubing, which is in turn fixed inside the inner tube of the solenoid core assembly. Hanging droplets are dislodged from the capillary tip at the bottom-most position. Figure 2 illustrates the process for dislodging a droplet. In Figure 2b, a hanging droplet is formed at a given time interval defined by the droplet deposition rate used. The capil-

lary and the droplet are accelerated downwards when a voltage is applied to the solenoid (Figure 2c). The motion is arrested at the bottom-most position by the restricting plate, producing an impulse, which becomes a momentum with respect to the droplet. The momentum of the droplet continues its motion downward. If the droplet is of sufficient mass it will impart a downward force great enough to exceed the adhesion forces and the droplet will separate from the tip, falling to the MALDI plate. This is the case for droplets greater than ~100 nL (up to ~2 μL, the volume at which droplets passively drop from the tip), with this deposition strategy. Droplets less than 100 nL do not have sufficient mass to dislodge from the tip. Although it was possible to dislodge these small droplets by increasing the applied force (this was accomplished by adjusting the displacement of the solenoid core) the accuracy of droplet placement was greatly reduced. The greater applied force caused droplets to take on erratic trajectories from the capillary tip, and deviate several millimeters away from the target location.

A workable method of depositing small droplets (20 to 100 nL) involves placing the collection plate to within a droplet diameter of the capillary tip, typically less than 500 μm (Figure 2d). In this strategy, the droplet deforms and elongates to span the distance between the capillary tip and the plate surface. Wicking action at the collection surface draws the droplet away from the capillary tip, completing the deposition.

It is important to note that the quality of the impulse directly affects the nature of the deposition. For example, operating the solenoid without the restricting plate spreads the impulse over a longer time period, effectively decreasing the force applied to the hanging droplet. Thus, without the restricting plate to supply a near-instantaneous halt to the motion of the solenoid core, droplets do not gain enough momentum to overcome their own surface tension. To reduce droplet adhesion to the capillary, hydrophobic coatings on the capillary wall and modified tip geometries may be used. However, for most LC-MALDI experiments, deposition of 20 nL or greater on each spot is desirable. The use of smaller droplets would result in spots containing very little analyte from which the sample would be consumed in a few laser shots. In the case of MALDI MS/MS, many more laser shots are required to generate a database-searchable product ion spectrum from a few fmol of peptide. The present device is capable of depositing >20 nL/spot, which is adequate for most LC-MALDI applications. Applying hydrophobic coatings to the capillary wall proved difficult to demonstrate reproducibly, although droplets less than 50 nL have been deposited without using the wicking mode. Likely, commercial hydrophobic coatings would alleviate many of the problems with reproducibility, and we will report our progress with this research soon.

It should be noted that the hanging droplets have a tendency to "climb" the capillary o.d., as illustrated in Figure 2. As the droplet size decreases, this can become so severe that the bottom-most position of the droplet climbs to the level of the capillary tip. In this situation, simple blotting methods would fail to achieve droplet deposition, or deposit partial droplets. Figure 2b demonstrates how the impulse-driven deposition overcomes the surface tension of the droplet, and achieves complete droplet deposition.

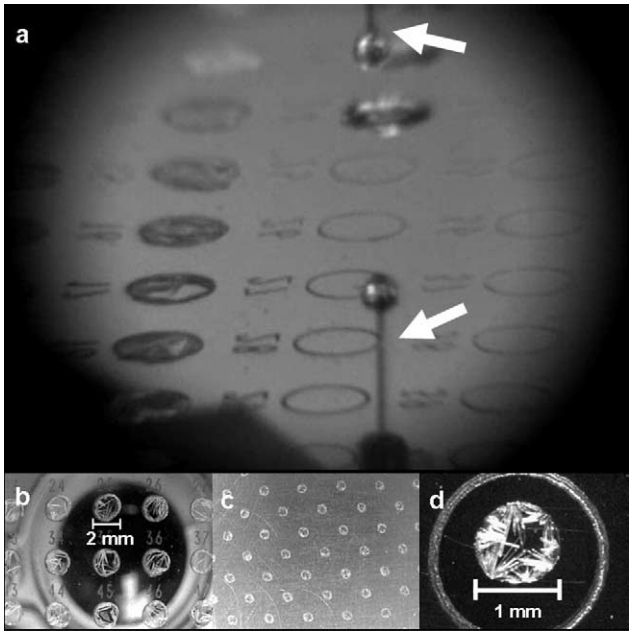

Figure 3. (**a**) Photograph of the automated deposition of eluent droplets onto a 100-target MALDI plate. The bottom of the picture features a reflection of the hanging droplet. (**b**) 2 μL spots at 10X magnification. (**c**) Photograph of 160 nL matrix spots on a MALDI plate. (**d**) 160 nL spot at 60× magnification.

Matrix/Sample Spot Size and Placement

As discussed above, the impulse-driven momentum transfer depositor is capable of depositing droplets of both small and large volumes. For a peptide separation on a 150 μm i.d. capillary column, the typical flow rate is 1 μL/min. In this example, the matrix solution is infused post-column at 1 μL/min, or 1:1 with the HPLC. At these flow rates, droplet depositions are typically made every 5 to 60 s. At a total flow rate of 2 μL/min, the volume of the deposited droplets ranges between 160 nL to 2 μL. Figure 3a and b illustrate the latter end of this range, showing the deposition of LC fractions post-column, with each fraction ~2 μL. On an unmodified plate surface, a 2 μL droplet typically dries to ~2 mm in diameter. Since in this example the matrix is infused post-column, matrix spots crystallize on the target once the carrier solvents evaporate. Droplets of smaller volume in the droplet range between 160 nL to 2 μL are advantageous in this case, since the droplets will evaporate more quickly, leading to smaller overall sample spots. For example, Figure 3c and d show matrix spots produced from 160 nL droplets, which dry to ~1 mm in diameter. Generating smaller spots tends

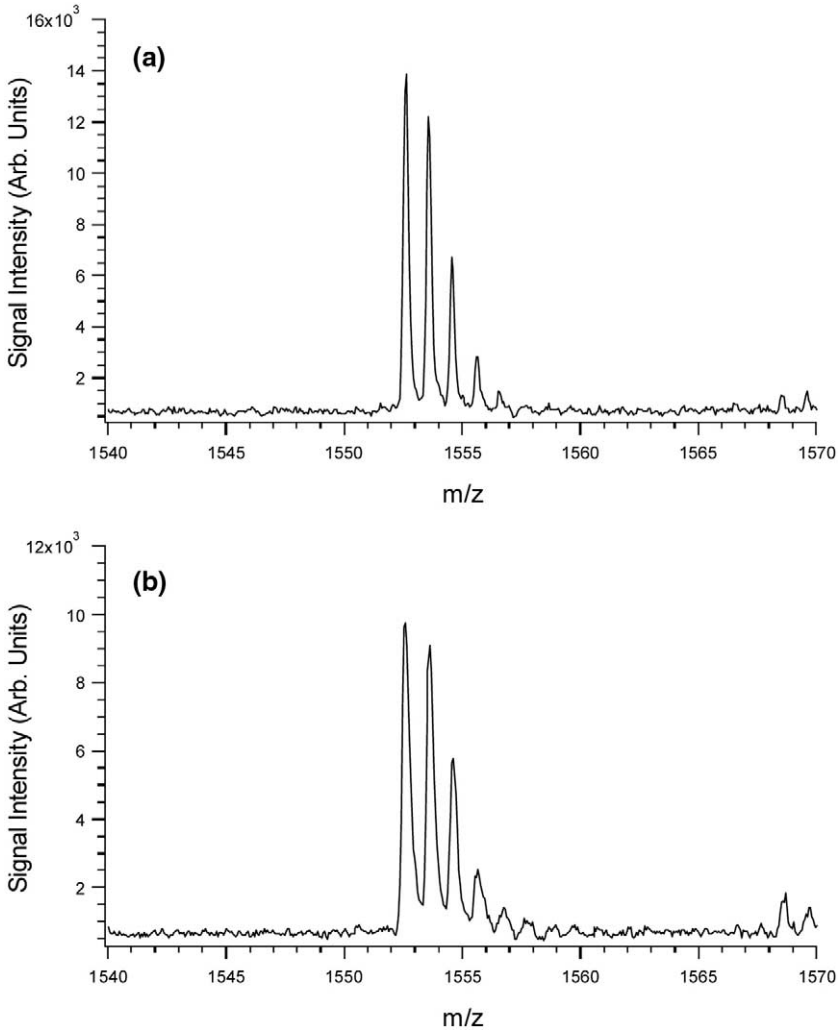

Figure 4. Mass spectra of 5 fmol pyro-GFP generated by (**a**) LC-MALDI MS and (**b**) direct MALDI-TOF MS.

to produce fewer "hot spots" of signal intensity during MS analysis, thus facilitating the automation of data acquisition. We note that different matrices can be used to alleviate the occurrence of hot-spots, most notably with α-cyano-4-hydroxycinnaminic acid (CHCA). While the use of CHCA can produce good results, this particular matrix must be used carefully, as it exhibits low solubility in aqueous conditions. In addition, CHCA is a "hot" matrix, which can preclude its use in certain MALDI experiments, such as in the analysis of phosphopeptides, due to in-source fragmentation and metastable ion formation.

As it was pointed out earlier, in interfacing LC to MALDI using off-line fraction collection, the chromatographic separation can be represented by a continuous stream deposited on the collection plate or as discrete spots. A continuous stream would ideally provide superior chromatographic resolution, although in practice, migration of sample components along the continuous stream may broaden chromatographic peaks. Collecting the eluent as discrete spots at a sufficient frequency can preserve the chromatographic integrity of the separation, and serve to concentrate minor components to small areas for improved detection. Thus, producing smaller fractions at greater frequency better preserves the chromatographic separation of components. Larger fractions produced at lower frequency sacrifices separation resolution in favor of greater sensitivity, providing that ion suppression is not severe in both cases. As it is illustrated below, each of these scenarios presents unique advantages, and must be chosen to reflect the needs of the experiment.

Analytical Performance

In evaluating the impulse depositor, it is important to establish that the technology does not hinder the MS sensitivity, nor greatly diminish the LC separation power. To this end, standard samples of pyro-GFP and BSA digests were used to benchmark the sensitivity and performance of the technique as a proteomic analysis tool.

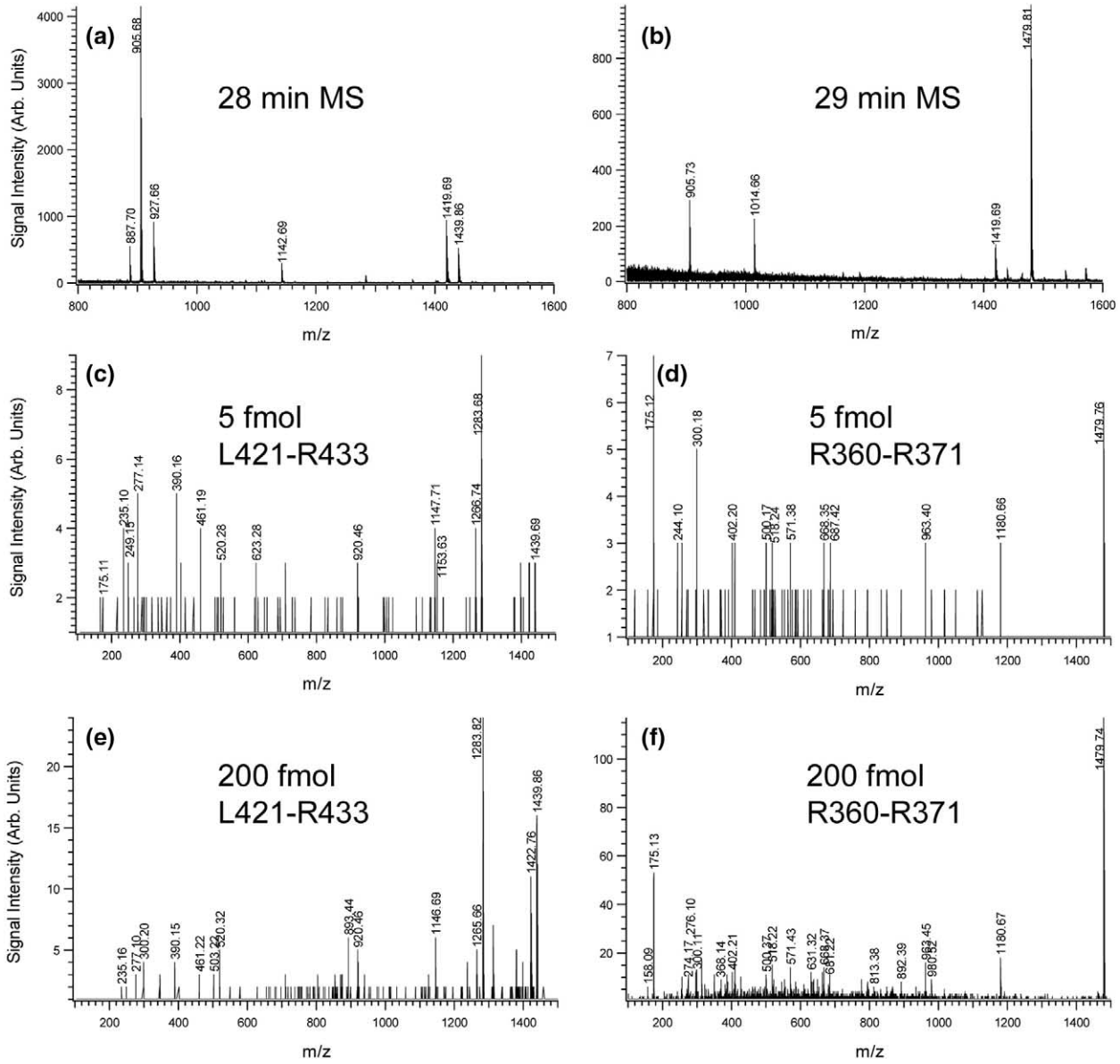

Figure 5. LC-MALDI mass spectra of (**a**) fraction 28 and (**b**) fraction 29 of a 200-fmol BSA digest. LC-MALDI MS/MS spectra of (**c**, **e**) L421-R433 and (**d**, **f**) R360-R371 from 5 fmol and 200 fmol BSA digest.

Five fmol of pyro-GFP was injected into the HPLC for reversed-phase separation, and the corresponding chromatographic peak (~50 s wide at the peak base) was collected in one fraction. DHB in methanol was infused post-column, 1:1 with the LC flow rate. The resulting spot was analyzed by MALDI-TOF MS and the result is shown in Figure 4a. For comparison, a manually spotted sample of the same amount was analyzed using the same instrument and the resulting spectrum is shown in Figure 4b. As Figure 4 shows, the intensity and quality of the mass spectrum for the spot produced by the impulse depositor is comparable to that obtained from a spot prepared manually. This indicates that sample loss is not an issue, and that the matrix crystallization process is not adversely affected by the deposition process.

The ability of the impulse-driven depositor to act as an LC-MALDI interface was demonstrated by fractionating tryptic digests of BSA. Different amounts of a BSA digest were analyzed to gauge the sequence coverage and detection sensitivity. The results are shown in Figures 5 and 6. In all cases, chromatographic base-peak widths for peptides from the reversed-phase separation were ~40 s. For the best sensitivity of detection, fractions of the separation were collected every 60 s to reduce the incidence of peak splitting between fractions. Each separation was collected over the entire course of the 50 min gradient in one minute fractions, giving 50 discrete fractions. The fractions were each analyzed by MALDI MS/MS. The analysis was automated using the MALDI spot raster and data-dependent subroutines supplied with the instrument. This was done to both reduce the level of user intervention,

Figure 6. Sequence coverage of BSA by LC-MALDI MS/MS of the digests using different amounts of sample loading.

and to better reflect the current state-of-the art for MALDI MS/MS using the QqTOF instrument.

Figure 5a and b show MS spectra obtained from two adjacent, representative fractions, 28 min and 29 min, in an LC-MALDI experiment using 200 fmol of the BSA digest. Fractions 28 min and 29 min are representative of the fractions collected for this BSA digest separation, showing only a handful of unique masses in each fraction. The masses identified in each fraction were subjected to MS/MS analysis. Figure 5c–f show MS/MS spectra from two different BSA digest sample loading amounts, 5 and 200 fmol. Two peptides, L421-R433 and R360-R371, are represented in both samples. The spectra illustrate that even in the 5 fmol sample, the MS/MS data contain enough product fragment ions to identify the peptide using a database search.

The BSA tryptic digest was fractionated and deposited at several sample loading amounts, between 5 fmol and 1 pmol. The resultant MS/MS data were searched using the MASCOT algorithm on the Swissprot database. Characteristic peptides of BSA were identified at all sample loading amounts, and sequence maps were produced to illustrate the change in sequence coverage relative to peptide concentration (Figure 6). Using the smallest sample amount, 5 fmol, 6% of the sequence is accounted for, with 3 characteristic peptides identified. Thus, the LC-MALDI MS/MS technique using the impulse depositor appears to be applicable to analyze proteome samples containing low fmol of proteins. As expected, to increase sequence coverage, a larger amount of sample is required. Using 1 pmol of BSA digest, sequence coverage of 57% can be achieved, as shown in Figure 6.

Figure 6 also illustrates that the collection time for each fraction is sufficient for samples of this complexity. Two experiments were performed at the 200 fmol level: BSA digest was fractionated using 30 s intervals, and again at 60 s intervals. The sequence coverage is approximately equal between the two, indicating that, for this simple protein digest, the analyses of the fractionated peptides are not significantly affected by signal suppression and the spectral recording duty cycle of the mass spectrometer.

To demonstrate the application of the impulse-deposition device for analyzing more complex proteomic samples, a mixture of peptides was generated from a trypsin digestion of an *E. coli* protein extract. Approximately 0.17 μg of the *E. coli* protein digest was separated by HPLC, followed by fraction collection with the impulse-deposition device. An experiment was first carried out using 60 s fraction intervals, and the fractions were analyzed by MALDI MS. Analysis of the fractions corresponding to the retention time from 25 to 50 min of the separation yielded

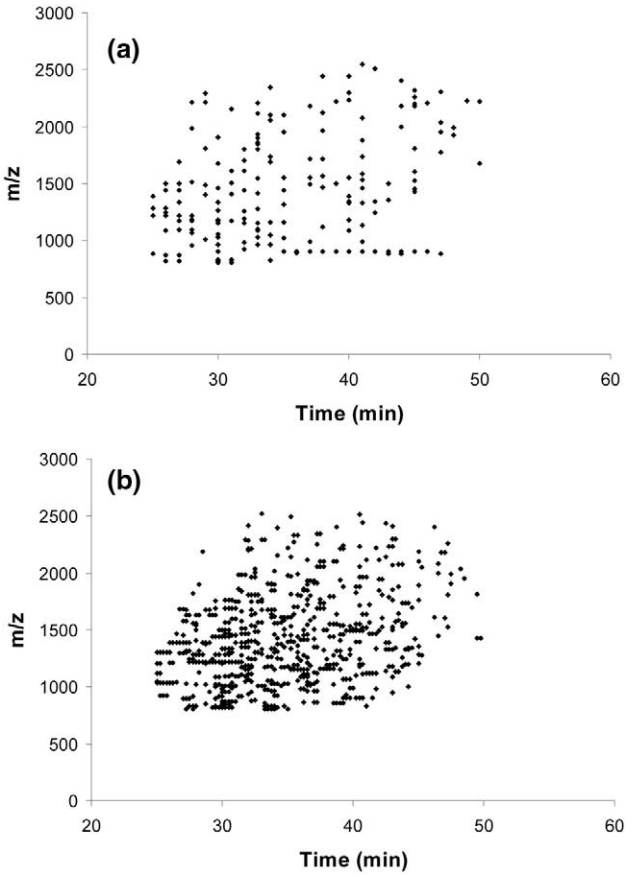

Figure 7. LC-MALDI mass analysis of an *E. coli* protein extract digest. (**a**) 25 fractions collected at 60 s intervals result in the detection of 145 unique peptides. (**b**) 100 fractions collected at 15 s intervals result in the detection of 409 unique peptides.

145 unique peptides. A second LC fractionation experiment was then carried out using 15 s fraction intervals. MALDI MS analysis of the 15 s fractions corresponding to 25 to 50 min of the separation yielded 409 unique peptides. Figure 7 presents the data as *m/z* versus time plots, illustrating the difference in the number of detected peptides. It can be seen from Figure 7 that, for very complex samples such as this *E. coli* protein extract, shorter fraction intervals allow for better MS detection, yielding a more complete analysis of the components within the sample. For most peptides detected using the 60 s fraction collection, they were observed in a single fraction. In the case of 15 s fraction collection, some peptides were detected in multiple spots. This is due to the splitting of the analyte peak over several spots. As the width at the base of the peak of a peptide was generally less than 50 s under the LC separation conditions used in this experiment, most peptides detected did not go over 5 spots. This result also confirmed that the impulse deposition interface did not introduce chromatographic peak broadening.

This *E. coli* protein digest example, along with the analysis of a simple BSA digest, demonstrates that the optimal interval of time for LC-MALDI fractions is dependent on the complexity of the sample. The LC separation efficiency should also play an important role. One would expect that, with the reduction of peptide sample complexity using multidimensional separation, the selection of fractionation interval time may become less important. Clearly, the issues of sample complexity, separation efficiency, fractionation intervals, mass spectrometric detection capability, and the required proteome analysis results (i.e., quantitative information, posttranslational modification information, etc.) are intertwined and need to be properly considered in designing a proteome analysis strategy for a given proteomics application. Nevertheless, the results shown in this work illustrate that the impulse deposition device provides the flexibility that allows the researcher to optimize the deposition conditions that suits a particular application.

In conclusion, we have developed a novel impulse-driven droplet deposition technique for LC-MALDI MS. This technique can be used to deposit small droplets ranging from 20 nL to 2 μL, making it particularly useful for depositing the eluate from capillary column LC separations that typically operate at flow rates of up to 2 μl/min. It complements our previously reported heated droplet interface, which was designed and optimized for operation in relatively higher flow experiments such as those involving 1-mm microbore column LC separations with a flow rate of 40 μL/min. While the impulse-driven deposition device described in this work can be used as a stand-alone unit for capillary LC-MALDI experiments, we are in the process of further developing this technique by integrating it with the heated droplet interface. We envisage a LC-MALDI interface capable of depositing the eluate from LC separations at a broad range of flow rates, which can be tailored to a wide range of proteomics applications. Work in this direction, including comparisons of low-flow LC-MALDI versus high-flow LC-MALDI, will be reported soon.

Acknowledgments

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