

Digital Microfluidics: An Emerging Sample Preparation Platform for Mass Spectrometry

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ABSTRACT: Mass spectrometry (MS) has become an indispensable tool for laboratory science, but a drawback is the laborious sample processing required before MS analysis. Digital microfluidics (DMF), a microscale liquid handling technique characterized by the manipulation of fluid droplets on open electrode arrays, presents a potential solution to this problem. In DMF, discrete droplets can be made to merge, mix, split, and dispense from reservoirs. Since droplets are manipulated individually and act as discrete microreactors, DMF is well suited for microscale sample processing. Coupling the versatility of MS analysis with DMF sample handling has been beneficial for a number of DMF-based applications, including proteomics, chemical synthesis, and clinical diagnostics. In this review, we provide a summary of efforts to integrate these two technologies, focusing on examples of both off-line and in-line MS analysis for DMF sample processing.



Credit: Nelson Lafrenière

Since the coupling of electrospray ionization (ESI) with mass spectrometry (MS) and the development of matrix-assisted laser desorption/ionization (MALDI) in the 1980s, mass spectrometry has experienced a renaissance, becoming one of the most popular and powerful tools in laboratory science. Both of these ionization techniques have garnered success as a result of their capacity for “soft” ionization, allowing for gas-phase ionization of large biomolecules with little or no fragmentation. In ESI (and its counterpart nanoESI), liquid samples are sprayed through charged emitters that terminate in a small orifice, and ions generated in the resulting electrospray are attracted to the counter electrode MS inlet.¹ In MALDI, a laser is focused on a solid sample of analyte cocrystallized with a matrix; the matrix absorbs energy from the laser, ablating the surface and forming a plume containing gas-phase matrix and analyte ions.²

Despite the widespread use of MS, it is not an ideal method for all analyses; sample preparation before introduction into a mass spectrometer can be laborious and time-consuming, and many applications are limited by the inconvenience of off-line MS detection. Microfluidics presents a potential solution to these problems, as it offers the possibility of integrated, automated modules for sample handling and processing upstream of analysis by mass spectrometry. Microfluidics is most commonly implemented as networks of microchannels on planar substrates, and there has been widespread interest in coupling microchannel devices to mass spectrometry, both via ESI³ and MALDI.⁴ There are a number of excellent reviews on this subject;^{5–7} in this paper, we focus on a different mode of microfluidics, known as digital microfluidics (DMF).

Digital microfluidics is emerging as an alternative to microchannels that is particularly well equipped for sample processing. DMF is characterized by the manipulation of discrete droplets on hydrophobic insulated electrode arrays

with no channels; application of successive potentials to the electrodes facilitates droplet manipulations such as dispensing from reservoirs, merging, mixing, and splitting. Samples are individually addressable, and droplets can act as discrete microreactors. DMF can be operated in two formats: two-plate,^{8,9} in which droplets are confined between a bottom plate bearing the working electrodes and a top grounded counter electrode, and one-plate,^{10,11} in which droplets are free-standing on a single substrate bearing both the working and counter electrodes. The top plate in the former format is typically removable, such that samples processed on either type of platform are readily accessible for sample collection and downstream analysis. For a comprehensive discussion of DMF theory and applications, see a recent review by Choi et al.¹²

Digital microfluidics is less common than microchannels, and DMF has only recently become popular for applications involving mass spectrometry. Here, we review the state of the art of this emerging trend, describing the relative merits of digital microfluidic techniques developed for indirect off-line analysis, direct off-line analysis, and in-line analysis by mass spectrometry. We conclude with a look forward, proposing attractive areas for innovation for the future in this promising combination of technologies.

INDIRECT OFF-LINE ANALYSIS

The most straightforward combination of digital microfluidics and mass spectrometry is processing samples by DMF followed by analysis off-line, as there is no need to modify any of the instruments. The modularity of this format is attractive (i.e.,

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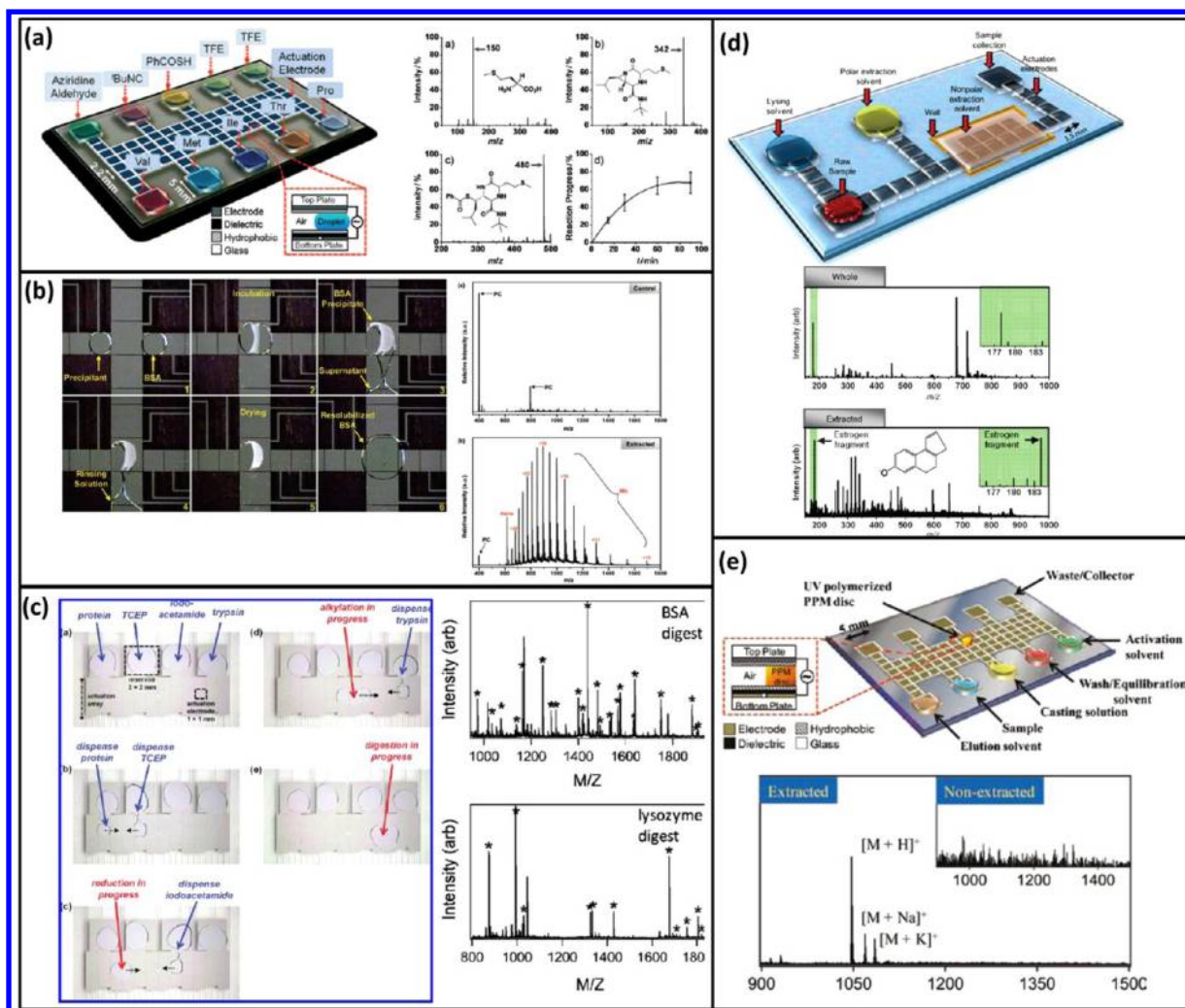


Figure 1. Digital microfluidics with indirect/off-line MS analysis. (a) (Left) Schematic of a DMF device used for parallel synthesis of five peptide macrocycles from five amino acid (AA) starting materials. AA droplets were dispensed and merged with aziridine aldehyde and *tert*-butyl isocyanide (tBuNC) and incubated for 1 h. (Right) Representative ESI mass spectra of the starting material methionine (m/z 150), the peptide macrocycle product (m/z 342), and the aziridine ring-opened derivative (m/z 480) synthesized on-device. The curve represents reaction progress over time, measured by ESI MS. Reprinted with permission from ref 14. Copyright 2010 Wiley. (b) (Left) Video sequence depicting the extraction and purification of proteins by precipitation using DMF. (Right) Representative mass spectra of a control and extracted sample of myoglobin (Mb) and model contaminant, PC. Reprinted from ref 16. Copyright 2009 American Chemical Society. (c) (Left) Video sequence showing a proteomic processing experiment using DMF. A protein droplet was dispensed, merged, mixed, and incubated with TCEP for reduction. This process was repeated with iodoacetamide for alkylation and trypsin for enzymatic digestion. (Right) Representative MALDI mass spectra of two proteins processed using DMF. Asterisks denote proteins identified by Mascot. Reprinted from ref 17. Copyright 2009 American Chemical Society. (d) (Top) Schematic of a DMF device used for extraction of estradiol from 1 μ L blood and tissue samples. (Bottom) Representative MS spectra of whole blood before and after DMF extraction. The insets (green) show the MS/MS of estradiol. Reprinted with permission from ref 22. Copyright 2009 AAAS. (e) (Top) Schematic of a DMF device used for in situ generation of porous polymer monolith (PPM) discs for solid phase extraction on DMF. The PPM disc was made by dispensing a droplet of the casting solution followed by UV exposure. Extraction steps included equilibration, sample loading, washing, and sample elution. (Bottom) Representative nanoESI mass spectra of a sample of angiotensin II (m/z 1047) with 100 mM NaCl before (inset) and after extraction. Reprinted from ref 23. Copyright 2011 American Chemical Society.

easy to reconfigure), but it also has some disadvantages, as it requires additional steps of sample transfer and processing (including dilution into appropriate ESI buffers or mixing and cocrystallization with MALDI matrices). These additional steps may lead to adsorption, contamination, and sample loss. Regardless, indirect off-line MS analysis has been an important and convenient confirmatory tool for DMF sample processing.

The discretized nature of sample handling makes DMF well suited for evaluating reaction progress for microscale chemical synthesis on-device. Dubois et al.¹³ used one-plate DMF to perform Grieco's reaction in ionic liquid droplets, a three

component reaction that produces a tetrahydroquinoline. Samples were pipetted off the device for reaction quenching and dilution in MS solvent before qualitative analysis of reaction progress by ESI MS. Jebrail et al.¹⁴ developed the first two-plate DMF platform capable of parallel multicomponent, multistep reactions, which was applied to the synthesis of peptide macrocycles and their corresponding aziridine ring-opened derivatives (Figure 1a). This strategy was further developed for the DMF synthesis of peptidomimetics¹⁵ using a multistep reaction catalyzed by solid Raney Nickel, in which four reactions were performed in a combinatorial fashion on a

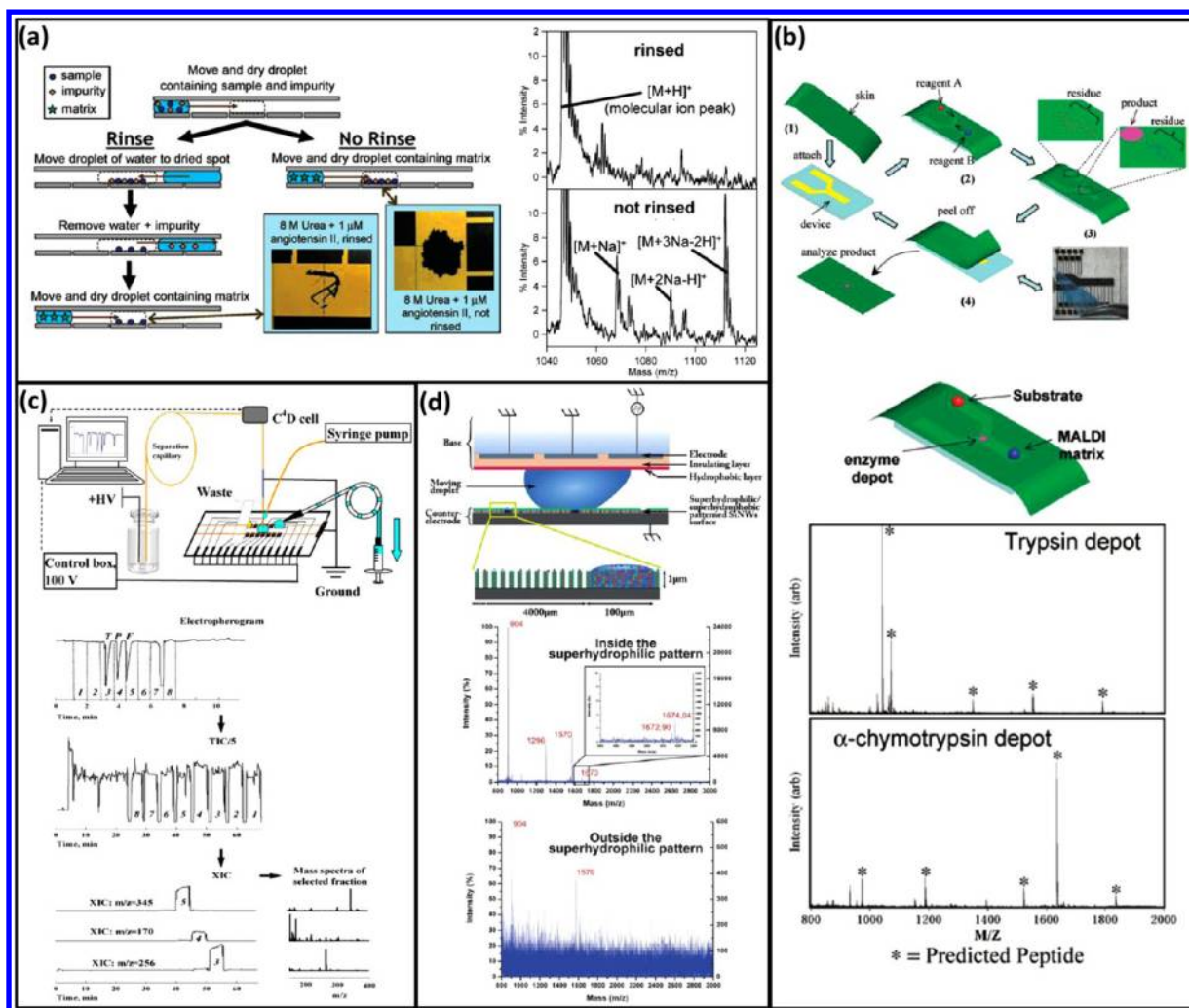


Figure 2. Digital microfluidics with direct/off-line MS analysis. (a) (Left) Schematic of DMF-based in-line protein purification. The photos show improved sample crystallization after rinsing. (Right) Representative MALDI mass spectra of rinsed and nonrinsed protein samples generated directly from a DMF device surface. Reprinted from ref 25. Copyright 2005 American Chemical Society. (b) (Top) Schematic of the removable film (or skin) strategy for DMF. A fresh dielectric and hydrophobic layer (skin) was affixed to the device; reagents were merged and mixed on the surface, and the skin was removed from the device and adhered to a MALDI plate. (Bottom) Schematic and representative MALDI mass spectra of analytes processed on a skin bearing dried spots ("depots") of trypsin or α -chymotrypsin for proteolytic digestion. Asterisks denote proteins identified by Mascot. Reprinted from ref 32. Copyright 2009 American Chemical Society. (c) (Top) Schematic of an instrumental setup used for coupling capillary electrophoresis (CE) with off-line ESI MS using a DMF sample collector. (Bottom) Representative electropherogram, total ion current (TIC) trace, and extracted ion chromatograms (XIC) for the separation, fractionation, and identification of a three vitamin mixture analyzed by the CE-DMF-ESI MS platform. Reprinted with permission from ref 33. Copyright 2012 Elsevier. (d) (Top) Schematic of the DMF device used for SALDI MS analysis. The superhydrophilic spots retained some liquid as droplets were actuated across the device and the spots were later used as targets for SALDI MS. (Bottom) Representative SALDI mass spectra of a peptide mixture actuated across a device surface in the superhydrophilic pattern and on the superhydrophobic surface. Reprinted with permission from ref 35. Copyright 2011 Royal Society of Chemistry.

single device. In both cases, processed samples were dried on the device before retrieval and resolubilization for nanoESI MS analysis.

There has been considerable interest in proteomics experiments on DMF, as automated microscale sample handling presents an attractive alternative to tedious manual protein processing. A DMF method was developed for protein extraction and purification from complex biological samples, by precipitation, rinsing, and resolubilization, with protein recoveries of $\sim 80\%$ (Figure 1b).¹⁶ A protein sample containing a large excess of phospholipid contaminant was processed using this method, and off-line ESI MS revealed the extraction was successful in purifying the protein by removing it from the contaminant. Luk and Wheeler¹⁷ developed a DMF-based

protocol for proteomic biochemical processing, including protein reduction, alkylation, and enzymatic digestion, followed by manual sample cleanup by solid phase extraction (SPE) and off-chip MALDI MS analysis (Figure 1c). Method effectiveness was verified by a Mascot database search that correctly identified all analytes at greater than 95% confidence. Protein extraction and biochemical processing were combined using an automated DMF platform for complete proteomic sample workup.¹⁸ Proteins extracted and processed using this platform were analyzed off-line by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and a Mascot search identified proteins with a sequence coverage of at least 30%. More recently, Luk and Fiddes et al.¹⁹ refined DMF proteomic processing methods by implementing hydrogel discs with

immobilized enzymes for proteolytic digestion. Off-line MALDI MS analysis revealed that hydrogel microreactors were more effective than solution-phase proteolytic digests in this format.

Off-line MS detection has been used for confirmatory analysis of a number of other DMF-based sample processing applications. Barbulovic-Nad et al.²⁰ used off-line MALDI MS to probe the effects of DMF actuation on cell biochemistry. Cell suspension droplets actuated by DMF were removed from a device for manual lysing, incubation, centrifugation, desalting, and protein elution before MS analysis, and data was compared with control samples of nonactuated cells. Aldelgawad et al.²¹ used DMF for sample cleanup by actuating aqueous droplets containing DNA and a histone contaminant through a water-immiscible phenolic pool. ESI MS analysis showed that the extraction was successful in removing the contaminant that suppressed ionization of the DNA analyte. A similar technique was employed by Mousa et al.,²² whereby a methanolic extract from a 1 μ L blood or serum sample was actuated through a pool of isooctane to remove lipids and other nonpolar contaminants (Figure 1d). Estradiol was quantified from purified sample extracts by off-line LC-MS/MS. Yang et al.²³ performed SPE using porous polymer monolith discs formed in situ on a DMF platform. Peptide samples contaminated with salt or surfactant were purified on-device using the SPE discs, and the extraction efficiency was probed by off-line nanoESI MS analysis.

■ DIRECT OFF-LINE ANALYSIS

DMF sample handling with MALDI MS analysis is a popular combination. We call this method “direct,” as samples are typically not handled after processing on the device (i.e., the device itself can be inserted into the mass spectrometer), but the method is inherently off-line, as the products of reactions (typically implemented in liquid phase at standard pressure) must be dried and then ionized in a vacuum chamber prior to analysis. DMF sample processing is a good match for MALDI MS analysis given the similarity in device substrates: both techniques require a planar device and rely on array geometries.

Assays for peptides and proteins were the first area of interest for DMF with in situ MALDI MS. Wheeler et al.²⁴ were the first to actuate peptide and protein droplets on a two-plate DMF device, followed by drying, addition of matrix, and the removal of the device top-plate before adhering the bottom substrate to a MALDI target plate for analysis. The method was compatible with a number of standard MALDI reagents, matrices, and recipes, with results comparable to samples prepared using conventional manual techniques. The same authors developed a DMF method for in-line sample purification of peptides and proteins from heterogeneous mixtures.²⁵ This process involved several steps, including drying the sample droplet, actuating a water droplet over the spot to dissolve impurities, and delivering a matrix droplet to the purified sample before drying and MALDI MS analysis (Figure 2a). This process was improved by redesigning the device to include reservoirs to store and dispense reagents on-demand, which permitted simultaneous purification of six samples.²⁶ Nichols and Gardeniers²⁷ developed a one-plate DMF platform to study pre-steady-state enzyme kinetics of tyrosine phosphatase, involving DMF-controlled mixing of enzyme and substrate, precisely timed addition of quenching agent, and addition of matrix. Rate constants obtained from MALDI MS compared well with published data, and the system was

successful in gathering kinetic data from a reaction that was previously too fast for MALDI MS analysis.

Complete integration of protein processing steps on a DMF platform with in situ MALDI MS analysis is also an area of interest. Chatterjee et al.²⁸ first developed a DMF platform for protein reduction, alkylation, enzymatic digestion, crystallization with matrix, and MALDI MS analysis directly from a device surface. The protein processing sequence was performed in less than 30 min. This method was improved by Nelson et al.²⁹ by integrating localized temperature control on-device to accelerate reduction and enzymatic digestion rates. A fluorinated liquid-aided method for protein processing, crystallization with matrix, and MALDI MS analysis was developed by Aijian et al.³⁰ Sample and reagent droplets were encapsulated in fluorinated liquid shells during processing to aid in smooth droplet actuation and minimize droplet evaporation and protein adsorption to the device surface.

There have been several other reported interfaces for DMF with direct off-line MS analysis. One of the first efforts to couple DMF and MALDI MS was a technique for stamping protein samples from a DMF device to a MALDI target plate.³¹ The top plate contained a hole to permit vertical passive transfer of protein droplets from the DMF device to a MALDI plate positioned on top. Yang et al.³² developed a creative method for reusing DMF devices by implementing a removable dielectric and hydrophobic layer. These “skin” coverings could be preloaded with dried enzyme spots and stored before use; once samples were processed and dried on a one-plate DMF device, the skin was removed from the device and placed on a MALDI target plate (Figure 2b). In the only report of a direct interface for DMF with off-line ESI MS, Gorbatoeva et al.³³ used DMF as an automated sample fraction collector to couple capillary electrophoresis (CE) separations with off-line ESI MS, whereby fractions exiting the CE capillary were merged with droplets of MS solvent near the capillary outlet and transported using DMF to a collection zone connected to a storage syringe (for droplets containing analyte) or to waste (for droplets with no analyte) (Figure 2c). Analysis was performed by interfacing the sample storage syringe to the mass spectrometer and infusing the contents directly to the ESI emitter, and results from the analysis of a three-component vitamin mixture showed good correlation between the contents of fractions identified in the electropherogram and in the mass spectrum. The same authors used a similar DMF-based strategy to couple CE with off-line MALDI MS analysis.³⁴ In this case, fractions collected from the outlet of the CE capillary in droplets containing MALDI matrix were transported via DMF to predetermined sampling locations on the device, and the removable skin strategy was employed for MALDI MS analysis. Lapierre et al.³⁵ reported the first example of DMF coupled to surface-assisted laser desorption/ionization (SALDI) MS, using a device top-plate composed of an etched silicon nanowire (SiNW) surface selectively patterned with superhydrophobic and superhydrophilic areas (Figure 2d). As droplets were actuated across the device, a small amount of the solution adsorbed onto the superhydrophilic areas, and the surface was subsequently used for the analysis of low molecular weight peptides with high sensitivity.

■ IN-LINE ANALYSIS

In-line analysis is desirable for many applications, as real-time evaluation allows determination of the progress of a reaction or assay as it is occurring. In-line analysis is particularly

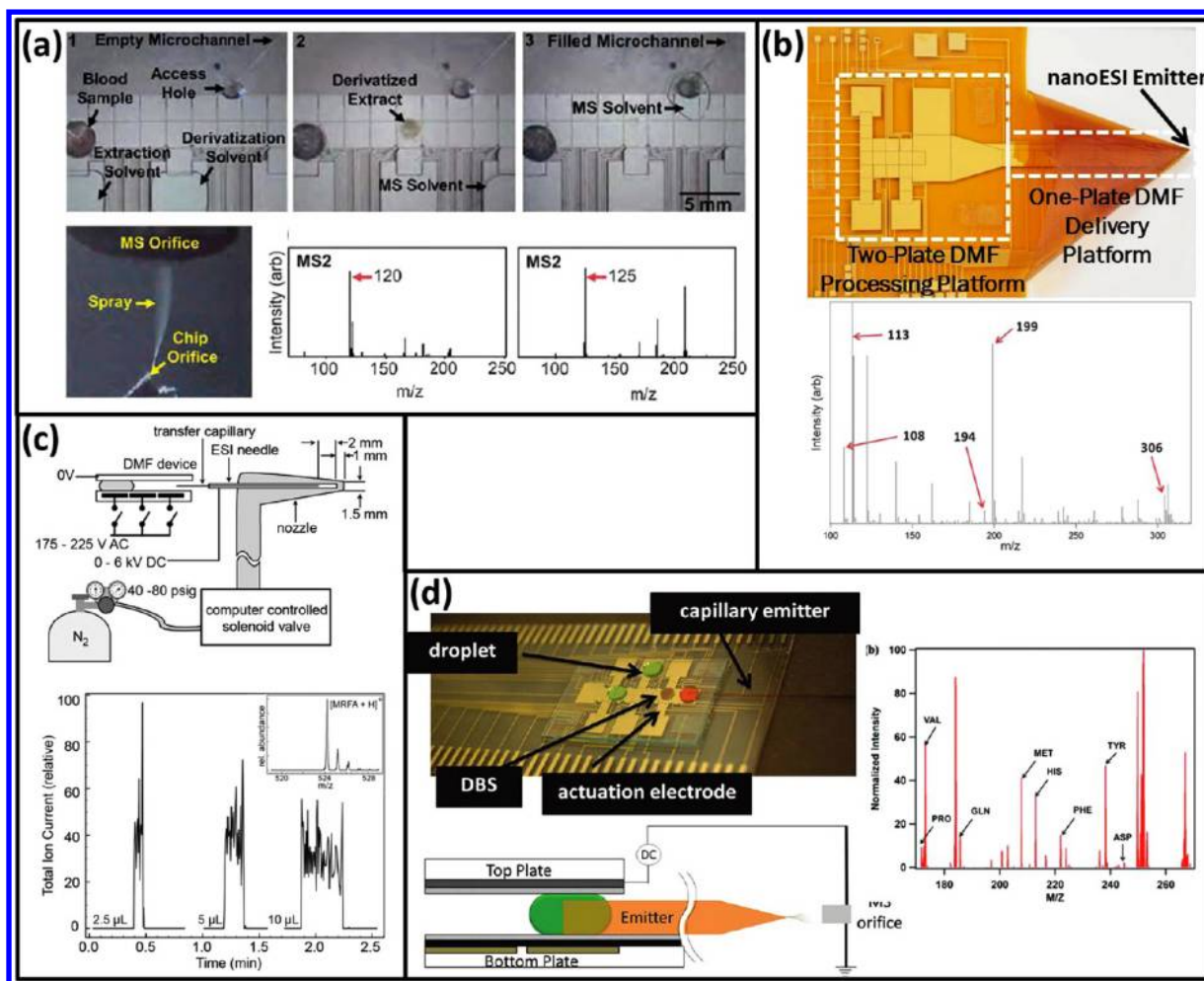


Figure 3. Digital microfluidics with in-line MS analysis. (a) (Top) Sequence of frames from a movie depicting the extraction and derivatization of amino acids from a dried blood spot (DBS) using DMF followed by in-line nanoESI MS analysis. (Bottom-left) Image of a sample spraying from the integrated nanoESI emitter. (Bottom-right) Product ion scans (MS2) of the amino acid Phe (m/z 120) and internal standard d5-Phe (m/z 125) extracted from DBS samples. Reprinted with permission from ref 36. Copyright 2011 Royal Society of Chemistry. (b) (Top) Image of the microfluidic origami device. The Morita-Baylis-Hillman (MBH) reaction was performed on the two-plate DMF processing platform, the reaction droplet was actuated to the one-plate DMF delivery platform and driven to the folded nanoESI emitter for in-line MS analysis. (Bottom) Representative mass spectrum of the MBH reaction. Labeled peaks include: starting material 2-pyridine carboxaldehyde (m/z 108), DABCO catalyst (m/z 113), intermediate 1 (m/z 199), intermediate 2 (m/z 306), and reaction product (m/z 194). Reprinted with permission from ref 39. Copyright 2013 Royal Society of Chemistry. (c) (Top) Schematic of the educator interface for DMF with in-line ESI MS analysis. (Bottom) Representative selected ion current traces when MRFA peptide droplets of increasing volume were transferred from a DMF device to the MS via the educator interface. Reprinted from ref 38. Copyright 2012 American Chemical Society. (d) (Left-top) Image of a DMF device mated to a pulled glass capillary emitter for processing DBS samples with in-line nanoESI MS analysis. (Left-bottom) Side-view schematic of the device. Processed samples were driven to the end of the emitter and filled to the tip by capillary action. (Right) Representative nanoESI mass spectrum showing several amino acids extracted from a DBS using DMF. Reprinted from ref 37. Copyright 2012 American Chemical Society.

advantageous for DMF-based applications, as sample droplets are not removed from the device, reducing manual sample handling and minimizing chances for contamination. However, in-line interfaces for DMF and MS can require custom device fabrication, the addition of extra hardware between the DMF device and mass spectrometer, or the use of specialized ionization techniques. Thus, there have been only a few reports of in-line interfaces for DMF and MS.

In-line coupling of DMF with ESI MS is a natural fit, as both techniques require liquid samples and compatible volumes (particularly when nanoESI MS is employed). The first in-line interface for DMF and nanoESI MS, reported by Jebrail et al.,³⁶ was a device composed of a DMF platform and a microchannel nanoESI emitter that was used for quantification of amino acids from dried blood spot (DBS) samples. Using DMF, samples

were extracted from DBS, mixed with internal standards, derivatized, dried, reconstituted, actuated to an access hole, filled the microchannel by capillary action, and subsequent MS/MS analysis gave sample recoveries of greater than 80% (Figure 3a). A second interface for DMF and nanoESI MS was developed by Shih et al.³⁷ and was also used for the analysis of metabolic disorder markers from DBS samples. This system was formed by sandwiching a pulled glass nanoESI capillary emitter between the two substrates of a two-plate DMF device, and processed samples actuated to the end of the emitter filled the emitter by capillary action (Figure 3d). An educator interface for DMF and ESI MS, developed by Baker and Roper,³⁸ was composed of a transfer capillary placed on a DMF device connected to a tapered gas nozzle and a metal ESI emitter (Figure 3c). When a gas pulse was applied to the nozzle, a

pressure differential generated at the emitter outlet pulled droplets from the DMF device past the ESI emitter and into the gas flow exiting the nozzle. A new device format, known as “microfluidic origami,”³⁹ was formed from a single flexible substrate comprising a DMF platform and a folded nanoESI emitter made by folding a portion of the device into a cone with a micrometer-sized orifice at the apex (Figure 3b). This integrated device was used to perform the Morita-Baylis-Hillman reaction followed by in-line nanoESI MS analysis of reaction progress.

Another means of in-line integration of DMF and MS is to employ a specialized ionization technique, such as surface acoustic wave nebulization (SAWN). SAWN exploits the acceleration of surface acoustic waves propagated on the surface of a piezoelectric substrate to produce an aerosol containing solvated ions from a liquid droplet on the surface.⁴⁰ Mating DMF and SAWN is logical, since both techniques require planar substrates and liquid droplets. Edgar et al.⁴¹ coupled DMF with in-line SAWN MS for rapid hydrogen/deuterium exchange experiments. A peptide sample and D₂O were mixed on a flexible DMF device, and the droplet was transferred to the piezoelectric LiNbO₃ substrate by positioning the DMF device perpendicular to the substrate and allowing the droplet to slide onto the SAWN electrodes. Dennison et al.⁴² employed a similar device strategy for top-down protein fragmentation studies, although in this case the SAWN and DMF electrodes were fabricated on the same piezoelectric substrate.

CONCLUSIONS AND OUTLOOK FOR THE FUTURE

As described herein, DMF is emerging as a powerful tool for sample processing upstream of analysis by mass spectrometry. Indirect off-line MS analysis (Figure 1) is the simplest mode, and to date, this method of analysis has been used for of the widest range of applications. Given the similarity in device substrates for DMF and MALDI MS, it is not surprising that the two techniques have been combined in several ways for direct off-line MS analysis (Figure 2). Finally, as the number and capacity of dedicated DMF-ESI interfaces increases, DMF with in-line MS analysis (Figure 3) is increasing in popularity. This mode is the most attractive for several reasons: most importantly, the “hands-off” nature of in-line analysis that enhances the ability to fully automate sample processing and analysis on a single platform.

Of course, DMF is not a panacea for all MS applications, and there are several limitations and challenges that must be overcome if DMF-MS techniques are to achieve widespread use going forward. For example, DMF device fabrication complexity currently limits its use to a small number of groups worldwide; this may change with the advent of low-cost and resource-limited fabrication.^{43,44} Biofouling is particularly problematic for DMF, which has led to significant innovation in the field, including the use of oils to encapsulate droplets,^{30,31} amphiphilic additives⁴⁵ or nanostructured superhydrophobic surfaces³⁵ to limit adsorption to surfaces, and removable films to prevent cross-contamination between experiments.³² Finally, the lack of access to multiplexed droplet control systems is a real challenge, but is one that may find relief in the future through open-access systems such as DropBot.⁴⁶

However, improvements in DMF are only part of the story for DMF-MS. For example, the recent surge in popularity of ambient ionization techniques⁴⁷ is likely to be important for

DMF-MS applications in the future. We described the development of techniques relying on SAWN here^{41,42} but propose that, in the future, adaptation of DMF for interfacing with desorption electrospray ionization (DESI), direct analysis in real time (DART), and other ambient ionization techniques may pave the way for exciting new possibilities. Finally, as DMF systems become more sophisticated, integrated, and compact, the potential for integration with miniature mass spectrometers⁴⁸ is an attractive idea for field-deployable applications. In summary, as DMF matures and MS develops as an ever more versatile technique, we speculate that DMF-MS will continue its current trajectory, becoming a practical, useful tool for myriad challenges in the laboratory and beyond.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37.
- (2) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299–2301.
- (3) Yin, H.; Killeen, K.; Brennen, R.; Sobek, D.; Werlich, M.; van de Goor, T. *Anal. Chem.* **2005**, *77*, 527.
- (4) Gustafsson, M.; Hirschberg, D.; Palmberg, C.; Jorvall, H.; Bergman, T. *Anal. Chem.* **2004**, *76*, 345–350.
- (5) DeVoe, D. L.; Lee, C. S. *Electrophoresis* **2006**, *27*, 3559–3568.
- (6) Koster, S.; Verpoorte, E. *Lab Chip* **2007**, *7*, 1394–1412.
- (7) Lee, J.; Soper, S. A.; Murray, K. K. *J. Mass Spectrom.* **2009**, *44*, 579–593.
- (8) Pollack, M. G.; Fair, R. B.; Shenderov, A. D. *Appl. Phys. Lett.* **2000**, *77*, 1725–1726.
- (9) Cho, S. K.; Moon, H.; Kim, C.-J. *J. MEMS* **2003**, *12*, 70–80.
- (10) Yi, U.-C.; Kim, C.-J. *J. Micromech. Microeng.* **2006**, *16*, 2053–2059.
- (11) Cooney, C. G.; Chen, C.-Y.; Emerling, M. R.; Nadim, A.; Sterling, J. D. *Microfluid. Nanofluid.* **2006**, *2*, 435–446.
- (12) Choi, K.; Ng, A. H. C.; Fobel, R.; Wheeler, A. R. *Annu. Rev. Anal. Chem.* **2012**, *5*, 413–430.
- (13) Dubois, P.; Marchand, G.; Fouillet, Y.; Berthier, J.; Douki, T.; Hassine, F.; Gmouh, S.; Vaultier, M. *Anal. Chem.* **2006**, *78*, 4909–4917.
- (14) Jebraïl, M. J.; Ng, A. H. C.; Rai, V.; Hili, R.; Yudin, A. K.; Wheeler, A. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 8625–8629.
- (15) Jebraïl, M. J.; Assem, N.; Mudrik, J. M.; Dryden, M. D. M.; Lin, K.; Yudin, A. Y.; Wheeler, A. R. *J. Flow Chem.* **2012**, *2* (3), 103–107.
- (16) Jebraïl, M. J.; Wheeler, A. R. *Anal. Chem.* **2009**, *81*, 330–335.
- (17) Luk, V. N.; Wheeler, A. R. *Anal. Chem.* **2009**, *81*, 4524–4530.
- (18) Jebraïl, M. J.; Luk, V. N.; Shih, S. C. C.; Fobel, R.; Ng, A.; Yang, H.; Freire, S. L. S.; Wheeler, A. R. *J. Visualized Exp.* **2009**, *33*, No. e1630.
- (19) Luk, V. N.; Fiddes, L. K.; Luk, V. M.; Kumacheva, E.; Wheeler, A. R. *Proteomics* **2012**, *12*, 1310–1318.
- (20) Barbulovic-Nad, I.; Yang, H.; Park, P. S.; Wheeler, A. R. *Lab Chip* **2008**, *8*, 519–526.
- (21) Abdelgawad, M.; Freire, S. L. S.; Yang, H.; Wheeler, A. R. *Lab Chip* **2008**, *8*, 672–677.

- (22) Mousa, N. A.; Jebrail, M. J.; Yang, H.; Abdelgawad, M.; Metalnikov, P.; Chen, J.; Wheeler, A. R.; Caspar, R. F. *Sci. Transl. Med.* **2009**, *1* (1), 1ra2.
- (23) Yang, H.; Mudrik, J. M.; Jebrail, M. J.; Wheeler, A. R. *Anal. Chem.* **2011**, *83*, 3824–3830.
- (24) Wheeler, A. R.; Moon, H.; Kim, C.-J.; Loo, J. A.; Garrell, R. L. *Anal. Chem.* **2004**, *76* (16), 4833–4838.
- (25) Wheeler, A. R.; Moon, H.; Bird, C. A.; Ogorzalek Loo, R. R.; Kim, C.-J.; Loo, J. A.; Garrell, R. L. *Anal. Chem.* **2005**, *77*, 534–540.
- (26) Moon, H.; Wheeler, A. R.; Garrell, R. L.; Loo, J. A.; Kim, C.-J. *Lab Chip* **2006**, *6*, 1213–1219.
- (27) Nichols, K. P.; Gardeniers, H. J. G. E. *Anal. Chem.* **2007**, *79*, 8699–8704.
- (28) Chatterjee, D.; Ytterberg, A. J.; Son, S. U.; Loo, J. A.; Garrell, R. L. *Anal. Chem.* **2010**, *82*, 2095–2101.
- (29) Nelson, W. C.; Peng, I.; Lee, G.-A.; Loo, J. A.; Garrell, R. L.; Kim, C.-J. *Anal. Chem.* **2010**, *82*, 9932–9937.
- (30) Aijian, A. P.; Chatterjee, D.; Garrell, R. L. *Lab Chip* **2012**, *12*, 2552–2559.
- (31) Srinivasan, V.; Pamula, V.; Paik, P.; Fair, R. Protein Stamping for MALDI Mass Spectrometry Using an Electrowetting-based Microfluidic Platform. In *Proceedings of SPIE-The International Society for Optical Engineering 5591*; International Society for Optics and Photonics: Bellingham, WA; 2004; pp 26–32.
- (32) Yang, H.; Luk, V. N.; Abdelgawad, M.; Barbulovic-Nad, I.; Wheeler, A. R. *Anal. Chem.* **2009**, *81*, 1061–1067.
- (33) Gorbatsova, J.; Borissova, M.; Kaljurand, M. *J. Chromatogr., A* **2012**, *1234*, 9–15.
- (34) Gorbatsova, J.; Borissova, M.; Kaljurand, M. *Electrophoresis* **2012**, *33*, 2682–2688.
- (35) Lapiere, F.; Piret, G.; Drobecq, H.; Melnyk, O.; Coffinier, Y.; Thomy, V.; Boukerroub, R. *Lab Chip* **2011**, *11*, 1620–1628.
- (36) Jebrail, M. J.; Yang, H.; Mudrik, J. M.; Lafreniere, N. M.; McRoberts, C.; Al-Dirbashi, O. Y.; Fisher, L.; Chakraborty, P.; Wheeler, A. R. *Lab Chip* **2011**, *11*, 3218–3224.
- (37) Shih, S. C. C.; Yang, H.; Jebrail, M. J.; Fobel, R.; McIntosh, N.; Al-Dirbashi, O. Y.; Chakraborty, P.; Wheeler, A. R. *Anal. Chem.* **2012**, *84*, 3731–3738.
- (38) Baker, C. A.; Roper, M. G. *Anal. Chem.* **2012**, *84*, 2955–2960.
- (39) Kirby, A. E.; Wheeler, A. R. *Lab Chip* **2013**, *13*, 2533–2540.
- (40) Ho, J.; Tan, M. K.; Go, D. B.; Yeo, L. Y.; Friend, J. R.; Chang, H.-C. *Anal. Chem.* **2011**, *83*, 3260–3266.
- (41) Edgar, J. S.; Winters, D.; Yoon, S. H.; Monkkonen, L.; Masselon, C. D.; Mackay, C. L.; Langridge-Smith, P.; Goodlett, D. R. Coupling Surface Acoustic Wave Nebulization with Digital Microfluidics for Rapid Hydrogen Deuterium Exchange. Presented at *60th ASMS Conference on Mass Spectrometry and Applied Topics*, Vancouver, Canada, 2012; MOG pm 3:10.
- (42) Dennison, A.; Edgar, J.; Winters, D.; Yoon, S. H.; Huang, Y.; Li, Y.; Walton, A.; Mackay, L.; Goodlett, D. R.; Langridge-Smith, P. Development and Application of Surface Acoustic Wave Nebulization for Top-Down Protein Fragmentation. Presented at *60th ASMS Conference on Mass Spectrometry and Allied Topics*, Vancouver, Canada, 2012; TOC pm 3:30.
- (43) Abdelgawad, M.; Wheeler, A. R. *Adv. Mater.* **2007**, *19*, 133–137.
- (44) Abdelgawad, M.; Wheeler, A. R. *Microfluid. Nanofluid.* **2008**, *4*, 349–355.
- (45) Luk, V. N.; Mo, G. C. H.; Wheeler, A. R. *Langmuir* **2008**, *24*, 6382–6389.
- (46) Fobel, R.; Fobel, C.; Wheeler, A. R. *Appl. Phys. Lett.* **2013**, *102*, 193513.
- (47) Harris, G. A.; Galhena, A. S.; Fernandez, F. M. *Anal. Chem.* **2011**, *83*, 4508–4538.
- (48) Xu, W.; Manicke, N. E.; Cooks, G. R.; Ouyang, Z. *JALA* **2010**, *15*, 433–439.