Journal of Chromatography A, 1439 (2016) 161–166

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Screen-printed digital microfluidics combined with surface acoustic wave nebulization for hydrogen-deuterium exchange measurements

Lucas Monkkonen[a], J. Scott Edgar[a], Daniel Winters[b], Scott R. Heron[c,d], C. Logan Mackay[e], Christophe D. Masselon[f,g,h], Adam A. Stokes[e], Patrick R.R. Langridge-Smith[e], David R. Goodlett[c,d,*]

[a] University of Washington, Seattle, WA, USA
[b] Trioptics GmbH, Wedel, Germany
[c] Deurion, LLC, Seattle, WA, USA
[d] University of Maryland, Baltimore, MD, USA
[e] University of Edinburgh, Edinburgh, UK
[f] University Grenoble-Alpes, Grenoble, France
[g] CEA, iRTSV, Biologie à Grande Echelle, Grenoble, France
[h] INSERM, U1038 Grenoble, France

ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form
16 November 2015
Accepted 17 December 2015
Available online 21 December 2015

Keywords:
Screen-printed digital microfluidics
Surface acoustic wave nebulization
Hydrogen-deuterium exchange
Mass spectrometry

ABSTRACT

An inexpensive digital microfluidic (DMF) chip was fabricated by screen-printing electrodes on a sheet of polyimide. This device was manually integrated with surface acoustic wave nebulization (SAWN) MS to conduct hydrogen/deuterium exchange (HDX) of peptides. The HDX experiment was performed by DMF mixing of one aqueous droplet of angiotensin II with a second containing various concentrations of D_2O. Subsequently, the degree of HDX was measured immediately by SAWN-MS. As expected for a small peptide, the isotopically resolved mass spectrum for angiotensin revealed that maximum deuterium exchange was achieved using 50% D_2O. Additionally, using SAWN-MS alone, the global HDX kinetics of ubiquitin were found to be similar to published NMR data and back exchange rates for the uncooled apparatus using high inlet capillary temperatures was less than 6%.

1. Introduction

Hydrogen deuterium exchange (HDX) is a powerful technique for studying protein structure [1]. As evidenced by the rapidly growing body of HDX literature, which shows steady growth in the technique. For the most part, HDX workflows have deviated little from HPLC or a direct infusion apparatus coupled to electrospray ionization (ESI) MS. In this study, we investigate the use of two fundamental changes to the HDX workflow. First, we examine the use of an alternative ionization method called surface acoustic wave nebulization (SAWN) and second, we explore the use of microfluidics as an alternative to manual or an automated LC-type unit for sample preparation.

While ESI has been critical to HDX success and many other MS based assays [2,3], it is not without limitations. For instance, while ESI is very sensitive, it can lead to in-source fragmentation and/or the oxidation of small molecules and proteins [4–6], and ESI requires its own charged, continuous flow apparatus. To address the analytical short-comings of ESI, many alternative ionization techniques have been developed, such as desorption ionization on silicon [7], desorption-ESI [8] and Laser Ablation-ESI [9].

In this study, we employed surface acoustic wave nebulization (SAWN) for HDX analysis. SAWN generates ions from a planar piezoelectric surface and delivers them to the inlet of a mass spectrometer [10]. To accomplish this, an alternating current is applied to interdigitated transducers (IDT, interlocking electrodes) on a piezoelectric $LiNbO_3$ wafer to generate a high frequency surface acoustic wave [11]. When the SAW reaches an area on the surface of the chip where an aqueous droplet of sample is located reflection of the wave within the droplet results in nebulization of the liquid sample within seconds. To date, a range of analytes of small molecules have been analyzed by SAWN-MS [5,6,10].

SAWN has several advantages over ESI and other ionization techniques for HDX. First, SAWN has been found to generate ions of lower energy than ESI [6], which has the potential to maintain

* Corresponding author at: University of Maryland, Baltimore, MD, USA.
E-mail addresses: lucasm7@uw.edu (L. Monkkonen), dgoodlett@rx.umaryland.edu (D.R. Goodlett).

http://dx.doi.org/10.1016/j.chroma.2015.12.048
0021-9673/

the structural integrity of more analytes during the ionization process [5]. Additionally, given its relative "softness" compared to ESI, there is the potential advantage of reduced back exchange during HDX, which we test here. Second, in contrast to ESI, SAWN is very simple to operate with no possibility of clogging since it is a planar substrate. Specifically, SAWN operation only involves transferring a droplet of sample directly onto the chip and activation of the chip, which leads to the immediate nebulization of the sample. Here we validated the use of HDX via SAWN-MS using ubiquitin, a well-characterized protein [12–14].

In addition to the ionization source, we explored the use of a sophisticated fluid handling platform called digital microfluidics (DMF) for sample manipulation within the HDX workflow. The concept of DMF is representative of a class of techniques using the relatively weak interactions between electric fields and polarizable droplets of liquid on a planar surface. A typical DMF device consists of a flat substrate covered in a microelectrode pattern which is covered by subsequent layers of a suitable dielectric and a hydrophobic layer [15]. This technology has been referred to by many names during its development including; metal-insulator-solution-transport [16], digital microfluidic system [17], electrowetting on insulator coated electrodes [18], electrowetting on dielectric [19] and DMF [20], which we prefer.

Due to the promise of automated sample handling, small sample volumes, loss-less sample preparation, and miniaturized devices that can forgo large and costly LC systems, much effort has been placed into connecting microfluidics with ESI-MS as seen in the publication data presented in these review articles [21,22]. However, coupling ESI-MS to microfluidic systems remains a challenge due to the requirement of maintaining constant fluid flow for steady electrospray [23]. The most popular means for coupling microfluidics with electrospray is simply attaching a micro-electrospray emitter [21], such as the microfluidic bottom-up HDX device manufactured by Rob et al. [24]. This microfluidic device has successfully characterized HDX on very short timescales with low back exchange [25,26]. However, this device has several potential disadvantages such as time-consuming fabrication (due to attachment of a sprayer and incorporation of microchannels) and susceptibility to clogs (especially when using native buffer). Microfluidics has also been coupled with matrix assisted laser desorption ionization (MALDI) MS to circumvent these problems, but MALDI is susceptible to matrix effects [27]. Alternatively, integrating SAWN and DMF is simple and conserves sample since both are planar platforms which manipulate discrete droplets as low as half a microliter [20]. Other studies utilizing a combined DMF and SAWN approach for non-MS applications further supports the simplicity and minimal sample use of these two techniques [28–36].

Here, we demonstrate the ease of use of DMF-SAWN to perform HDX on an inexpensive DMF device. Briefly, the DMF device was made by screen-printing conductive ink on top of a flexible polyimide substrate and coating the device with hydrophobic materials. The motivation for utilizing an inexpensive DMF design was to minimize biofouling by making the device disposable. We report results obtained from fusing droplets of D_2O and angiotensin II on such a disposable DMF device and analyzing the sample immediately by SAWN-MS.

The significance of this report is three-fold. Firstly, it represents the first use of SAWN to ionize HDX samples for MS analysis. Secondly, we demonstrated that SAWN-MS can yield high-resolution, reproducible data for whole proteins with low back exchange. Thirdly, SAWN was successfully coupled with a disposable, screen-printed DMF device to provide a means for sample preparation at the mass spectrometer. This combination points the way toward monitoring more complex chemical reactions in real time by DMF-SAWN-MS with many different types of analytes for a new, sample conserving "lab on a chip" or micro total analysis system [22,37].

2. Experimental

2.1. Fabrication and operation of SAWN chips

Fabrication of SAWN chips has been reported in detail previously [6,10]. In summary, a SAW transducer consisting of 20 pairs of 100 μm interdigitated (IDT) electrodes (40 in total) with 100 μm spacing and 10 mm aperture, along with a secondary electrode to apply external electrical potential, were patterned onto the surface of 128 Y-cut X-propagating 3 in $LiNbO_3$ wafers purchased from Crystal Technology, Inc. (Palo Alto, Ca). The SAWN configuration was first designed in AutoCAD before being written into a chrome mask by a Heidelberg μPG 101 Laser Pattern Generator (Heidelberg Instruments Mikrotechnik GmbH Tullastrasse 2, D-69126Heidelberg, Germany) at the University of Washington Nanotech User Facility (https://depts.washington.edu/ntuf/). Wafers were then coated using AZ 1512 positive photoresist (AZ Electronic Materials, Somerville, NJ) spun at 4000 rpm for 30 secondscreating a 1–1.2 μm thick resist layer. Exposure of the photo resist was done for 5 secondsusing an Oriel mask aligner (Newport Corporation, CA). The exposed wafers were placed in a development bath for 60 secondsin AZ 351 (AZ Electronic Materials, Somerville, NJ). To pattern conductive IDT electrodes a 20 nm chrome adhesion layer was deposited by heated vapor deposition followed by a 60 nm layer of gold, followed by lift-off in acetone for 30 min. The resulting SAWN IDT has a resonance frequency of 9.56 MHz. To operate the SAWN chip a MXG analog signal generator (Agilent N5181A, Santa Clara, CA) and a Mini Circuits ZHL-5W-1, 5–500 MHz RF amplifier (GwInstek GPS-2303, New York, NY) were used to generate and amplify the RF signal.

2.2. Fabrication of screen-printed DMF chips

Flexible, disposable, DMF chips were produced in a proprietary low-cost printing process using carbon-containing conductive ink on 50 μm thick flexible polyimide foils (DuPont Kapton) to pattern electrodes with 100 μm spacing, connection leads and external contact pads, followed by a 7 μm layer of ink acting as dielectric layer. After the printing process, a fluoropolymer solution, either Teflon-AF (DuPont) or Cytop (Asahi Glass) solution was spin-coated onto the devices and left to dry in ambient condition to create a 150 nm thick hydrophobic layer. The finished devices could be stored in normal laboratory conditions over the time period of several months without influence on performance.

2.3. Operation of screen-printed DMF chips

A schematic showing the operation of the DMF is shown in Fig. 5. Droplets were moved, merged and transferred to the SAWN chip in AC mode [28,29,37,38] with a driving voltage of 500 Vpp and a frequency of 20 kHz. The devices performed flawlessly and without any noticeable degradation over several experimental cycles. The electrode geometry used was an arrangement of two parallel columns of square electrodes. The droplet was held between two adjacent electrodes from the respective columns by applying the driving voltage between them. To move the droplet to the next electrode pair, the driving voltage was first applied to the new pair, pulling the droplet in between the pairs, and then switching off the voltage between the first pair. DMF operation was controlled using control software running on a PC that was connected to custom drive electronics. For this, the 2.5 Vpp AC sine output of a 20 kHz signal generator was amplified by a high-voltage amplifier (Trek)