ABSTRACT

Surface acoustic waves (SAW) can be generated along the surface of a piezoelectric material and efficiently transferred into fluid deposited on the surface to induce its nebulization. Recently, we demonstrated that surface acoustic wave nebulization (SAWN) can produce ions that are readily detectable by mass spectrometry (MS). Here we present the detailed fabrication process of a SAWN chip and demonstrate the ability of SAWN to generate ions from a variety of analytes ranging from small molecules (MW~100Da) to whole protein (MW>10kDa). The development of this new soft-ionization process holds the potential to bridge the gap between microfluidics and mass spectrometry.

KEYWORDS: Surface Acoustic Wave, Nebulization, Mass spectrometry, Ionization source.

INTRODUCTION

Mass spectrometry (MS) is an essential analytical method that measures mass/charge from which molecular weight and chemical structures may be derived. Importantly, MS requires that the sample of interest be transferred to the gas phase and ionized for subsequent manipulation by electric and magnetic fields. While a large variety of ionization techniques exist, this area is still an intensive field of research, particularly for the analysis of large, thermally labile bio-molecules.

Piezoelectric materials provide a convenient method to nebulize liquid samples by SAW and have been widely employed to deliver medicine in aerosol form. Recently, we demonstrated that SAW could also produce ions during nebulization, and that these ions were readily detectable by MS [1]. We termed this new ionization method Surface Acoustic Wave Nebulization (SAWN). We later observed that SAWN generates ions either with or without an added voltage on the chip's surface. Liquid samples, whether aqueous or pure organic, are simply pipetted onto a lithium niobate chip and nebulized following the activation of the SAW transducer. Ions in the generated plume are drawn into the mass spectrometer by a pressure gradient.

The development of SAWN as a soft-ionization method holds the potential to bridge the gap between microfluidics and mass spectrometry. Specifically, the ability to directly couple ionization from a chip to MS without any added matrix provides for low chemical noise mass spectra. In addition, the lack of need for a nozzle or cone of any sort circumvents common engineering problems with ESI. Finally, nebulizing directly from the surface minimizes sample precipitation problems that can occur when the sample is nebulized through a sharp tip (e.g. ESI). Since SAWN performs well with just a droplet of liquid sample, it also simplifies the sample preparation process [2].

DESIGN AND FABRICATION

The SAWN interdigitated electrodes (IDT) were fabricated on 128° Y-cut X-propagating LiNbO₃ wafers. The LiNbO₃ wafer was first cleaned with acetone, isopropanol and deionized water, then dried using nitrogen gas. The wafer was covered in positive photoresist AZ1512 and spun at 4000 rpm for 30 seconds using a vacuum spinner. It was then baked at 115°C for 90 seconds to cure the resist. The wafer was then placed in a mask aligner and moved into position in contact mode under a chrome mask with the desired pattern. After exposure with UV light for 4.5 seconds the wafer was placed in a developer solution of 1:1 water to developer for 60 seconds before being placed in DI water to stop the development. During the
development step the areas of resist that had been exposed to UV light become more soluble to the developer than unexposed areas and so are washed away, leaving behind a patterned layer of resist. A 20 nm of Cr layer was used as an adhesion layer for the 60 nm Au layer which created the metal contacts for the interdigitated (IDT) electrodes. The wafer was then soaked in acetone for 2 hours to remove the excess resist and metal layers revealing the final SAWN device.

EXPERIMENTAL
The SAWN chip was positioned 5-10 mm in front of the inlet port of the mass spectrometer. The SAWN Device was powered using an Agilent MXG Analog Signal Generator N5181A 250KHz - 1GHz (Santa Clara, United States) and Mini Circuits ZHL-5W-1, 5-500MHz amplifier (New York, United States). Copper contacts were made for the SAWN interdigitated electrode pads. These were attached to the signal amplifier that was activated by the power supply. The SAW IDT used here operated at 9.56 MHz. An additional electrode was constructed on the chip to allow a high voltage to be applied to the droplet prior to nebulisation (see Figure 1D). All experiments were performed on linear ion traps (LTQ, Thermo Fisher Scientific Inc).

The chemicals used in this experiment were Angiotensin II, Myoglobin, Retinoic Acid (Sigma-Aldrich Co, St. Louis, MO), substituted Benzylpyridinium ions (synthesized, reactants were from Sigma-Aldrich Co, St. Louis, MO) and lipid A (Avanti Polar Lipids, INC, Alabaster, AL). Except where mentioned, 2ul of sample solution was used in each experiment.

RESULTS AND DISCUSSION
We have demonstrated the ability of SAWN to generate ions of a variety of compounds from various solvents in both positive and negative ion modes. For example, peptides and proteins were dissolved in mixtures of water and organic solvent (methanol or acetonitrile), whereas Lipid A and retinoic acid were dissolved in pure organic solvents mixtures. The samples solution was either deposited by pipetting a droplet directly on the chip surface for quick analysis or transferred by a capillary and syringe for controlled flow. Figure 2 shows mass spectra obtained using SAWN for a variety of chemical compounds.

Angiotensin II, a standard peptide for mass spectrometry, was dissolved in H₂O:Acetonitrile 94.9:5 with 0.1% Formic Acid (see Figure 2a). Angiotensin II displayed the same ions as those produced by ESI, indicating that SAWN can ionize peptides and produce multiply charged ions. Fragmentation of the multiply charged peptides is typically used in proteomics analysis to determine peptides sequence and identify protein. This procedure could be carried out following SAWN ionization to produce Angiotensin II fragmentation spectra (data not shown). Myoglobin, a 17 kDa protein, was injected
continuously and the signal averaged over 30sec. The spectrum in Figure 2b shows a series of peaks corresponding to multiple charge states of myoglobin ranging from 9+ to 22+. Study from substituted benzylpyridinium ions (Figure 2c) indicated that SAWN ionization is soft and minimizes unwanted analyte fragmentation.

An important aspect of SAWN is its planar nature that requires no moving parts to generate ions. This simplicity and robustness allows samples that are prone to precipitation in capillaries, e.g. in ESI, to be analysed with minimal difficulty. Lipids are such a class of biomolecules challenging to analyse by mass spectrometry due to their frequent clogging of capillaries required for electrospray. Specifically, lipid A has been studied intensively by MS for its role in toxicity of gram-negative bacteria. While lipid A is challenging to work with in ESI it is readily ionized by SAWN. Figure 2d shows the MS1 spectra of Salmonella Minnesota R595. In Figure 2e, m/z 1506 ions were captured and fragmented (MS2) with collision induced dissociation (CID). Figure 2f shows a subsequent fragmentation (MS3) of the m/z 1262 ions from the previous spectra.

As a final example retinoic acid was analysed by SAWN. Retinoic acid is a metabolite related to vitamin A that mediates the functions of vitamin A required for growth and development. Retinoic acid is highly reactive and can be easily oxidized in air or with light exposure. From the spectrum in Figure 2 we can see that both positive (Figure 2h) and negative (Figure 2g) ions are generated with SAWN and can be detected by the mass spectrometer without excess fragmentation or unnecessary oxidation.

CONCLUSION

SAWN coupling with MS provides a powerful analytical tool for the detection of molecules from microfluidic chips. A wide variety of molecules can be nebulized and detected with this method. In the future, this might act as an interface from lab on a chip to the standard detection method. More research about improving the nebulization efficiency and signal intensity, digitally monitor the movement of droplets on the surface are in progress.

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