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Vibrating Sharp-edge Spray Ionization (VSSI) for Voltage-Free Direct Analysis of Samples using Mass Spectrometry

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Abstract

Rationale—The development of miniaturized and field portable mass spectrometers could not succeed without a simple, compact, and robust ionization source. Here we present a voltage-free ionization method, Vibrating Sharp-edge Spray Ionization (VSSI), which can generate a spray of liquid samples using only one standard microscope glass slide to which a piezoelectric transducer is attached. Compared to existing ambient ionization methods, VSSI eliminates the need of a high electric field ($\sim 5000 \text{ V}\cdot\text{cm}^{-1}$) for spray generation, while sharing a similar level of simplicity and flexibility with the simplest direct ionization techniques currently available such as paper spray ionization (PSI) and other solid substrate-based electrospray ionization methods.

Methods—VSSI device was fabricated by attaching a piezoelectric transducer onto a standard glass microscope slide using epoxy glue. Liquid sample was aerosolized by either placing a droplet onto the vibrating edge of the glass slide or touching a wet surface with the glass edge. Mass spectrometry detection was achieved by placing the VSSI device 0.5–1 cm to the inlet of the mass spectrometer (Q-Exactive, ThermoScientific).

Results—VSSI is demonstrated to ionize a diverse array of chemical species, including small organic molecules, carbohydrates, peptides, proteins, and nucleic acids. Preliminary sensitivity experiments show that high-quality mass spectra of acetaminophen can be obtained by consuming 100 femtomoles of the target. Dual spray of VSSI was also demonstrated by performing in-droplet denaturation of ubiquitin. Finally, due to the voltage-free nature and the direct-contact working mode of VSSI, it has been successfully applied for the detection of chemicals directly from human fingertips.

Conclusions—Overall, we report a compact ionization method based on vibrating sharp-edges. The simplicity and voltage-free nature of VSSI make it an attractive option for field portable applications or analyzing biological samples that are sensitive to high voltage or difficult to access by conventional ionization methods.

Introduction

Mass spectrometry is one of the most information-rich analytical techniques for characterizing a broad range of samples. The past decade witnessed explosive growth in the development of direct analysis and field portable mass spectrometers with the goal of bringing the analytical capability of mass spectrometry to various field applications

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including environmental monitoring, point of care diagnosis, chemical/biological warfare agent detection, and forensic investigation.^{1–3} A key component for portable mass spectrometers is an ionization source that can directly ionize the sample with minimum sample preparation and pretreatment. To date, numerous ambient ionization methods that allow direct sample ionization under atmospheric conditions have been reported.⁴ However, most of the existing ambient ionization methods including desorption electrospray ionization (DESI), easy ambient sonic spray ionization (EASI), plasma-assisted desorption ionization (PADI), and direct analysis in real time (DART) require dedicated and specialized instrumentation or auxiliary gas and solvents, making them less favorable options for many field-portable mass spectrometry applications.^{5, 6} Currently, the most compelling ionization sources for portable mass spectrometers are paper spray ionization (PSI)⁷ or solid substrate based electrospray ionization (ESI) (e.g., probe electrospray ionization (PESI)⁸) due to their simplicity, minimal sample preparation requirements, and wide range of suitable target molecules.^{6, 9, 10} These techniques have been utilized in many applications including biofluid analysis, food sample analysis, and chemical reaction monitoring.^{11–14}

A major limitation of several state-of-the-art ionization methods for field portable analysis is the requirement of a high-voltage (2–5 kv) to induce electrospray, which increases the external equipment demand for portable applications, and incurs safety concerns regarding both operators and living organisms as analytical samples.⁶ Here we report a voltage-free ionization method, Vibrating Sharp-edge Spray Ionization (VSSI), based on mechanical vibration induced nebulization of liquid samples. The new ionization source consists only of a microscope glass slide ($w \times l = 25 \times 60$ mm) and a piezoelectric transducer (diameter = 27 mm) with a power consumption as low as 150 mW (Fig. 1a & b). Compared to other voltage-free ionization methods, such as sonic spray¹⁵, EASI¹⁶, zero-voltage paper spray ionization¹⁷, ultrasonic ionization (UAI)¹⁸, and solvent assisted inlet ionization (SAII)¹⁹, VSSI is advantageous in terms of simplicity and flexibility, and it enables in situ analysis through direct contact-based nebulization that is not possible with other voltage-free methods. With the new source, we have demonstrated the successful ionization of various chemical species spanning a broad range of molecular classes including small molecules, carbohydrates, peptides, proteins, and nucleic acids. The experiments have been conducted in both positive and negative ionization modes. Additionally, experiments employing the simultaneous operation of two VSSI sources to study in-source protein denaturation through droplet mixing is demonstrated. Such experiments should become widely accessible because of the low cost, simplicity, and zero electrical field requirement of VSSI. Finally, we have demonstrated ionization of trace chemicals directly on wet human fingertips by leveraging the contact-spray working mode of VSSI (Fig. 1b), which could otherwise face regulatory obstacles for point of care (POC) applications if using PSI or other solid state ESI methods due to the safety concerns with the high voltage.

Experimental Section

Materials and Device Fabrication

All solvents used in this work are LC/MS grade. Water and Acetonitrile were purchased from Fisher Chemical (Optima). Acetaminophen, homocysteine, isoleucine, acetic acid, and

polyalanine peptide were purchased from Sigma-Aldrich. Ubiquitin was purchased from R&D systems.

The VSSI device was made by attaching a piezoelectric transducer (7BB-27-4L0, Murata) to one end of a No.1 microscope glass slide (VWR) using super glue (5-minute epoxy, Devcon). The RF signal was generated using a Tektronix function generator (AFG-1062) connected with an amplifier (Krohn-Hite 7500). The droplet images for size measurement were taken using an Olympus IX-73 inverted fluorescence microscope and analyzed using Image J (v1.51s).

Mass spectrometry Analysis

All mass spectrometry experiments were carried out using an Orbitrap mass spectrometer (Q-Exactive, ThermoScientific). For these experiments the resolving power was set at 7.0×10^4 for precursor ion analysis. The capillary inlet was maintained at 250 °C or 450 °C. Ion chromatograms were collected for varying times (~30 s to ~3 minutes). During the analysis, VSSI was either hand-held or placed on a stage that is closed to the mass spectrometer inlet. The distance between the tip of VSSI and MS inlet was ~0.5 – 1 cm. For continuous flow analysis, a syringe pump (KDS scientific) was used to pump (5 μ L/min or 20 μ L/min) solution through a fused silica capillary (polymicro) touching the edge of a VSSI source.

Results and Discussion

In VSSI, the spray of liquid samples can be generated by adding a drop of liquid to the edge of a vibrating microscope glass slide (Fig. 1c, see also supporting video S1). The vibration of the glass slide is induced by applying a RF signal to a piezoelectric transducer working at the natural frequency of the system (~97 kHz). It is not uncommon to observe mechanical vibration induced the aerosolization of liquids as demonstrated in many types of ultrasonic nebulizers.^{20–22} For ultrasonic nebulizers, the interaction between acoustic waves and liquid generates capillary wave and cavitation effects on the liquid-gas interface, which subsequently causes the detachment of liquid droplets from the body of the fluid. Unlike the classic phenomenon of ultrasonic nebulization, it has been determined that the liquid spray of VSSI is solely generated from the liquid touching the vibrating edge rather than the entire liquid-gas interface with a voltage input between 12.7 and 30.6 V_{pp} (corresponding to 150–1000 mW). In this power regime, no spray is generated when the water droplet is placed near - but not touching - the edge of the glass slide despite the observation of vibration of the water/air interface. When the power input is increased further to 32.0 V_{pp}, liquid aerosolization from the entire liquid-gas interface is observed, which matches the classic ultrasonic nebulization phenomenon.²³ These results convey the critical role of the vibrating sharp edge for the present nebulization phenomena at low power inputs. Due to the high ionization efficiency of the sharp-edge, the power consumption (~150 mW) in VSSI is lower to other ultrasonic based ionization methods such as the UAI (~5 W)¹⁸ and surface acoustic wave nebulization (~2–4 W)²⁴. Although the exact physical process by which the vibrating sharp-edge induces nebulization is still under investigation, it appears that the high frequency vibration at the edge of the glass slide causes the detachment of small liquid

droplets from the bulk fluid, resulting in a continuous spray of water only at the sharp edge site of the glass slide. It can be speculated that the relatively high amplitude vibration at the edge generates a strong streaming field in the thin layer of liquid at the edge thus shearing off droplets from the edge. While microcantilever based nanomechanical sensing has been used to detect the mass of molecules in mass spectrometry²⁵, VSSI demonstrates the use of cantilever vibration for ion generation.

Because of the unique sharp-edge based aerosolization mechanism, the VSSI also allows for direct analysis of wet surfaces using mass spectrometry. This working mode is especially useful to directly detect trace chemicals on sample surfaces, to aerosolize small amounts of fluid that are difficult to retrieve using common laboratory techniques, or to perform imaging mass spectrometry directly with a solid tip. For example, VSSI is shown to successfully induce liquid spray from wet cardboard (Fig. 1d). The new operational characteristics distinguish VSSI from previous ultrasonic nebulization based ionization methods, such as ultrasound ionization (USI) and surface acoustic wave nebulization (SAWN).^{18, 26} VSSI enables sampling of target analytes either on the device substrate or directly on the sample surface, while existing ultrasonic ionization methods require the placement of a liquid sample onto a pre-defined acoustic wave field. As the operation of VSSI does not rely on a well-aligned acoustic wave field, the technical format of VSSI is much more flexible in terms of substrate geometry, material and surface properties to suit different application scenarios.

The generation of ions by VSSI should follow an ESI-like process. Based on Dodd's theory²⁷, droplets with net charges can be created due to microscopic fluctuations of ion concentration. Therefore, in VSSI, a distribution of droplets ranging from net negative to net positive charge is thus generated. In positive ion mode, ionization proceeds more readily with desolvation (aided by the heated capillary) of the net positive charge droplets followed by droplet fission and finally either the charged residue or ion evaporation methods²⁸ of ion production. It should be noted that other ionization mechanisms for mechanical agitation-based ionization have also been reported. Chen *et al.* suggested a cavitation induced ion generation mechanism for their ultrasound ionization source.¹⁸ Because, for VSSI, bubbles are not observed in the bulk solvent at the time of droplet production from the sharp edge, it is unlikely that this mechanism is operative here. Usmanov *et al.* ascribed an ultrasonic cutter induced ionization mechanism to "the stripping of the thin surface layer enriched by the surface-active ions caused by the cavitation".²⁹ This is unlikely to be the case in VSSI as in Usmanov's work, sucrose, and large molecules like insulin (mw~5.7 kD) cannot be ionized, while, in VSSI, sucrose and the relatively large molecule ubiquitin (mw~8.5 kD) are readily detected, indicating a different ionization process. This is especially evident when considering the drastic difference in the power input required for the ultrasonic cutter induced ionization and VSSI (100 W vs. 0.15 W), respectively.

A brief comparison of VSSI with inlet ionization techniques is instructive. A number of innovative inlet ionization techniques are shown to utilize the pressure differential across the MS inlet as well as the heated inlet capillary itself to generate ions. Examples include matrix-assisted ionization inlet (MAII)³⁰, solvent-assisted ionization inlet (SAII)¹⁹, zero-volt paper spray ionization¹⁷ and droplet-assisted inlet ionization (DAII)³¹. VSSI is similar to

these techniques in that a heated inlet capillary is required to aid ion production and, because the overall process involves the desolvation of microdroplets, VSSI ion production is more similar to the latter two methods. A notable difference between VSSI and SAI is that, for VSSI, ions are not produced even when placing the sharp edge of the substrate right at the MS inlet; however, upon vibrating the glass slide, ions are readily detected. Additionally, unlike SAI but similar to DAI, VSSI can produce droplets for analyte ionization from a location that is removed from the MS inlet insofar as the droplets can be subsequently directed into the inlet. Here, the difference with DAI is the method of aerosolization where VSSI requires the vibration of a rigid substrate that contains a very sharp edge. Admittedly, VSSI is currently likely to be less sensitive than SAI as some of the analyte will be lost in droplets not entrained in the gas flow. Finally, it should be noted that, for VSSI, SAI and DAI, the final stages of ion production are thought to be ESI-like in nature³¹. That is, solvent evaporation and ion fission events ultimately result in the production of ions via the charge residue or ion evaporation models. Therefore, ion production for such techniques is similar to that experienced when employing ESI, PSI, SSI, and other spray/droplet-based methods.

Droplet size is an important parameter in spray-based ionization methods as it is important for both ionization efficiency and chemical processes occurring during ion production^{32–36}. We characterized the size distribution of droplets generated by VSSI and examined the factors that could influence the size of such droplets. Droplets were collected using a petri dish placed 20 mm away from the edge of a glass slide. A drop of mineral oil was immediately applied after liquid droplets reached the bottom of the petri dish to prevent further evaporation. The diameter of the droplets was obtained by examination under a microscope. Pure water was sampled first and the average droplet diameter was 18 μm with a standard deviation of 5 μm using the 12.7 V_{pp} power input (Fig. 2a). An increase in the average droplet size was observed with increasing voltage input. The average diameters for 19.8 V_{pp} and 26.8 V_{pp} were 22 ± 9 μm and 25 ± 6 μm , respectively (Fig. 2b and c). In addition to the voltage input, the solvent content also plays a role in determining the droplet size generated by VSSI. Upon replacing the water sample with a $\text{H}_2\text{O}:\text{MeCN}$ (1:1) mixture containing 1% HOAc, the average droplet size decreased from 18 ± 5 μm to 10 ± 4 μm (Fig. 2d) under the same power input conditions. The droplet size obtained using $\text{H}_2\text{O}/\text{MeCN}$ solvent is on the same order of those in ESI which are 1–20 μm ^{33, 37} but much larger than the droplets in nanoESI. These results indicate that droplet size in VSSI can be tuned by adjusting power input and solvent content, and thus optimized for specific applications and target samples.

After demonstrating the spray generation capability of VSSI, its performance as an ionization source for mass spectrometry analysis was investigated. For these experiments a series of 1 mM solutions were made consisting of homocysteine, sucrose, polyalanine peptides, and ubiquitin in $\text{H}_2\text{O}:\text{MeCN}$ (1:1) and 1% HOAc, respectively. For each solution, 20 μL of sample was added to the edge of a glass slide, and the spray was generated by applying a RF signal of 97.1 kHz and 14.4 V_{pp} to the piezoelectric transducer. The mass spectra were obtained using an orbitrap analyzer (Q-Exactive, ThermoScientific) under positive ion mode and with a capillary temperature of 250 $^{\circ}\text{C}$.

The mass spectra shown in Fig. 3 reveal the ESI-type ions produced by VSSI. Upon VSSI of the homocysteine sample, a dominant peak is observed at $m/z \sim 136$ (Fig. 3a). This peak corresponds to $[M+H]^+$ homocysteine ions. VSSI of sucrose ions produces a dominant feature at $m/z \sim 365$ (Fig. 3b) and corresponds with $[M+Na]^+$ precursor ions. The polyalanine solution produces a number of dominant features that are spaced apart by $m/z \sim 71$. These peaks correspond to $[Ala_n+H]^+$ ions (where n is 10 to 27). Upon VSSI of the ubiquitin sample, the +5 to +13 charge states are observed as dominant features (Fig. 3d). Such a spectrum is very similar to that obtained upon ESI of this protein. Finally, Fig. 3e shows the mass spectrum obtained upon VSSI of a mixture of DNA duplex (CAAATTG) with a DNA dye (Hoechst 33342) using negative ion mode analysis. Dominant features are observed at $m/z \sim 1203$ and ~ 1755 . These ions correspond to doubly-charged ssDNA and triply-charged DNA duplex complexed with the DNA dye – Hoechst 33342. Overall, as shown in Fig. 3, VSSI is able to efficiently ionize a number of diverse molecules in either positive or negative ion mode.

We further tested the sensitivity of VSSI using 1 μ M of acetaminophen in water and 1% formic acid solution. To better control the fluid delivery, we employed a syringe pump and a fused silica capillary (I.D. 75 μ m) to deliver fluid to the edge of the glass substrate. Fig. 4 shows the mass spectra averaged from ~ 1 s collection at flow rates of 5 and 20 μ L/min. Acetaminophen ($[M+H]^+ \sim 152.07$) was clearly detected at both flow rates. Under the 5 μ L/min flow rate, only ~ 100 femtomoles of acetaminophen is needed to generate a clear spectrum for detection. A full characterization of LOD, linear dynamic range, and the impacts of solvent composition and apparatus geometry on VSSI deserves a more thorough and systematic study in the future.

In addition to the diversity of suitable target molecules accessed by VSSI, another attractive feature of VSSI is the capability to operate multiple sources simultaneously. Such an approach would allow the probing of fast chemical processes by mixing the contents of different droplets within the ion source region^{38–41}. Compared to ESI-based spray, the zero-voltage characteristics of VSSI makes it convenient to operate multiple sources close to each other without worrying about the potential of electrical breakdown (in the case of opposite polarity needles) or problems of greater nanodroplet repulsion (in the case of similar polarity needles). To demonstrate the concept of droplet mixing, in-droplet denaturation of ubiquitin has been demonstrated using two VSSI sources. 40 μ L of a solution of ubiquitin in water was placed on one source and 40 μ L of MeCN (1% HOAc) was placed on the other source. The two sources were positioned near the mass analyzer inlet as shown in Fig. 5a. Source one was turned on first to generate ubiquitin droplets for a time period of 4 s. Next, the second source was turned on to generate MeCN droplets for which many are observed to combine with the droplets containing ubiquitin. This in-droplet denaturation process was recorded in the mass spectrum shown in Fig. 5b. Over the first 4 seconds of data acquisition, the major ubiquitin ions produced by VSSI are the +7 and +8 states (Fig. 5b). Overall the charge state distribution produced by VSSI extends from the +5 to the +11 ions. Immediately after turning on the MeCN source, a significant increase in higher (+9 to +13) charge states of ubiquitin ions is observed, indicating droplet denaturation of ubiquitin (Fig. 5c). The ability to mix droplets in such a manner may find utility in a number of studies

ranging from protein stability assessments to rapid, solution-phase hydrogen deuterium exchange analyses⁴².

Finally, we demonstrated the potential of VSSI for direct ionization of compounds within wet surfaces of living organisms. Although methods such as DESI and extractive electrospray ionization (EESI), have been applied to detect trace chemicals on human skin^{43, 44}, the requirement of sheath gas, solvent spray, and high voltage power supply makes it cumbersome for POC applications. In VSSI, trace surface chemicals can be detected simply by applying the tip of the glass slide to the wet surface of targets. It is also a safe sampling procedure to living organisms as there is no electric voltage applied to the tip. As shown in Fig 6 a and b, by touching the tip of a vibrating glass slide to a wet finger, a spray of the liquid can be generated (supporting information Video S2) in situ without the need of a sample transfer step. Direct analysis of trace chemicals on human finger tips was then performed using an orbitrap mass analyzer. First the amino acid, isoleucine, was used as the target molecule. The experimental finger first touched <5 mg isoleucine powder, and 10 μ L of water was then added to the fingertip. After 1 min, the vibrating glass tip was applied to the wet finger, and a mass spectrum was obtained under negative ion mode conditions (Fig. 6c). Compared to a blank analysis (separate finger as a negative control, Fig. 6d), and the on-substrate VSSI of isoleucine solution (positive control, Fig. 6e), the trace amount of isoleucine on the fingertip was clearly detected with direct VSSI analysis. In addition to this demonstration, a trace amount of red food coloring on a fingertip was detected by VSSI using the same setup. Here red food dye mixture (Gel Food Color, Wilton) was first diluted 100-fold, and a small drop was applied to the fingertip. After wiping off the liquid with a paper towel, a red trace was observed on the fingertip surface. 5 μ L of water was then applied to the red trace and ionized by direct VSSI. A peak having a nominal m/z of 835 was observed in the mass spectrum and corresponds with $[M-2Na+H]^-$ ions of the red dye (erythrosine) as shown in Fig. 6f. It should be noted that a well-defined droplet on the surface is not necessary for direct VSSI. As shown in Fig. 6b, a thin layer of liquid film is sufficient to generate liquid spray from the sample surface. This unique ionization setup could be especially useful in field applications that involve human as the analyzing targets such as explosive analysis, illicit drug detection, point of care diagnosis, and forensic analysis. By coupling with a position controller, VSSI could also be used as tissue imaging interface for mass spectrometry. Compared to existing mass spectrometry methods, VSSI could be advantageous for in vivo imaging applications due to its compactness, capability of sampling non-flat surface, and safety.

In summary, we reported a new ionization method based on the method of a vibrating sharp-edge of a glass slide termed VSSI. The method showed comparable characteristics to the widely used PSI technique in terms of simplicity, compactness, and ease of use. Additionally, like PSI, the materials used in VSSI are very affordable. The source consists only of a glass slide (~20 cents each) and a piezoelectric transducer (~50 cents each). Moreover, the exposed glass slide is easily cleaned thoroughly, and so the source is reusable. Thanks to the low frequency and power requirements of VSSI, the signal generation equipment can also be easily miniaturized at reasonable cost as demonstrated by many commercialized portable ultrasonic nebulizers. The operation is also straightforward with minimum sample preparation requirements by simply adding a liquid droplet on the edge of

a glass slide or touching the wet surfaces of solid samples with the glass tip. By eliminating the need of a high electric field to generate liquid spray, VSSI provides better integration capability and portability, and enables new applications such as in-situ analysis on living subjects. Finally, it is generally considered that the ionization efficiency is lower for neutral spray than for electrospray due to less excess charges on droplets. In this work, VSSI demonstrates that targets can be easily detected by consuming ~100 femtomoles of sample. The ionization efficiency could be further improved by optimizing solvent components, the interface to mass spectrometer and other operating parameters. Future work of VSSI will include detailed investigation of the droplet generation mechanism, and development of interfaces to liquid chromatography and imaging mass spectrometry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Schematic diagram of VSSI. a) On-substrate spray working mode of VSSI. b) In-situ direct-touch working mode of VSSI. c) Real image of on-substrate direct spray. Frequency: 97.1 kHz; Voltage: 12.7 V_{pp}. d) Real image of direct-touch spray generation on damp cardboard. Frequency: 97.1 kHz; Voltage: 18.0 V_{pp}. The contrast of images was enhanced to facilitate the visualization of fine droplets.

Figure 2.

Size distributions of droplets generated by VSSI under different power input conditions and for different solvent systems. a)-c) Liquid: water. Voltage: 12.7, 19.8, 26.8 V_{pp}, respectively. d) Liquid: H₂O:MeCN (1:1) with 1% acetic acid. Voltage: 12.7 V_{pp}. Number of droplets counted: >1000.

Figure 3.

Mass spectra of a) homocysteine (1 mM); b) Sucrose (1 mM); c) polyaniline peptides; d) ubiquitin (1 mM); e) DNA duplex with Hoechst 33342. Sequence: CAAATTTG Solvent: H₂O:MeCN (1:1) mixture containing 1% HOAc. Capillary Temperature: 250 °C

Figure 4.

Mass spectra of 1 uM acetaminophen at flow rates of a) 5 μL/min b) 20 μL/min. Solvent: H₂O:MeCN (1:1) mixture containing 1% HOAc. Capillary Temperature: 450 °C

Figure 5.

In-droplet denaturation of ubiquitin by MeCN. a) Ion chronogram of two VSSI sources in operation. At 3.04 min, the source with the ubiquitin in water droplet was turned on. After 3.11 min, the second MeCN source was turned on to generate MeCN droplets for denaturation. b) Mass spectrum of ubiquitin prior to turning the MeCN source on. d) Mass spectrum of ubiquitin immediately after turning the MeCN source on. Capillary Temperature: 250 °C

Figure 6.

In-situ analysis of trace chemicals on human fingertips with VSSI. a) Image of a wet finger. b) Direct spray generation from the wet finger with VSSI. c) Mass spectrum obtained from direct spray of a finger exposed to isoleucine powder. d) Mass spectrum obtained from the direct spray from a finger not exposed to isoleucine powder. e) Mass spectrum obtained from on-substrate direct spray of 1 mM isoleucine solution. f) Mass spectrum from direct spray of a finger stained with red food dye, erythrosine. The mass analyzer was operated in negative ion mode for these analyses. Frequency: 97.1 kHz. Amplitude: 12.7 V_{pp}. Capillary Temperature: 250 °C