Nanomanipulation-Coupled Nanospray Mass Spectrometry Applied to the Analysis of Trace Fibers

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Nanomanipulation-Coupled Nanospray Mass Spectrometry Applied to the Analysis of Trace Fibers

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Abstract:

This paper presents novel instrumentation of nanomanipulation coupled to nanospray mass spectrometry, which is used to directly probe trace analytes from individual fibers. New techniques are needed in order to more accurately probe trace analytes from individual fibers with analyte sensitivity and minimally evasive extraction of the sample. We are presenting new instrumentation, that has direct application to fiber analysis and trace species analysis with the capability to improve the current methods of probing. This method requires minimal sample preparation, taking the sample straight from the source to the instrument. We will demonstrate the capability of extracting a single particle of histidine, caffeine, and gunshot residue from individual fibers, and analyzing directly with nanospray MS. Nanospray MS uses small sample volumes and sample sizes, 300nl volume, making it the ideal choice to couple with the nanomanipulator. Future work will show direct cellular applications and trace forensic applications such as microphase-extraction experiments.

Keywords:

Forensic Science, Nanospray, Trace Analysis, Fiber, Micromanipulation, Gunshot residue (GSR)

Direct probing from a sample surface directly coupled to mass spectrometry is a useful tool, helping to eliminate sample preparation and analysis time. Currently there are three techniques at the forefront of direct-coupled surface sampling mass spectrometry (MS); desorption electrospray ionization (DESI)(1), surface sampling probe electrospray ionization(2), and dielectric barrier discharge ionization source (DBDI)(3). DESI sprays charged solvent droplets onto an ambient surface which ionizes neutral analytes, which are then desorbed from the surface and analyzed using MS(1,4). This technique has been used to detect trace amounts of explosives as well as sampling directly from human skin(5,6). Surface sampling probe electrospray MS uses a liquid junction between the electrospray source and the surface to dissolve and then ionize the analyte, which is then electrosprayed into the MS(2,7). This method has been
used to directly sample drugs from thin tissue slices(8). DBDI uses a dielectric barrier discharge to create a stable flow of plasma that desorbs and ionizes the sample off of an ambient surface then analyzes it using MS(3). All of these techniques have great utility, but need a relatively large area, 20-100um, for analysis.

Other methods have been used to analyze samples of GSR to identify the various elements which are desired to identify and analyze. X-ray fluorescence analysis (XRF) spectrometer provides the possibility of recording elemental mappings of samples up to 20 X 20 cm in size. Such distribution patterns are used in GSR investigation, e.g., for shooting distance estimation(9) X-ray mapping technique can offer a new fundamental evaluation parameter in analysis of gunshot residues with scanning electron microscopy/energy-dispersive spectrometry, and new standards could be considered(10). Atomic absorption spectroscopy (AAS) was used to determine the lead (Pb) pattern around bullet holes produced by shots on test targets from the gun. Currently the European forensic laboratories use the method of scanning electron microscopy in combination with energy dispersive X-ray microanalysis (SEM/EDX).

The investigation of gun shot residue particles in forensic laboratories is – among various chemographic coloring methods – usually performed by SEM/EDX. The application of this technique facilitates a concurrent analysis of both elemental composition and morphology of single particles (11).

As the test results can be used as evidence in court, accurate analysis of GSR is of value to forensic scientists. The residues are principally composed of burnt and unburned particles from the propulsive charge, as well as components from the primer, the bullet, the cartridge case and the firearm itself(12). The detection of the inorganic and organic components of the gunpowder is not limited to a representation of a broad list of chemicals that may be found. Besides the two energetic ingredients, nitroglycerin and nitrocellulose, propellant powder composition includes several additives such as gelatinizing agents, flash suppressors, plasticizers, or stabilizers(11). Time is a possible factor if any residue sample is to be gathered from a suspect. Although GSR will be almost non-existent on a person’s hands after a few hours of normal activity, they can be retained for a longer period on hair and face swabs.
and may be retained for weeks on the clothing. Typically, the airborne GSR that can be found in the
surroundings of a shooting gun is of regular or distorted spherical shape. Less frequently they form some
aggregates of spheres of various sizes and objects resembling fragments of sponge or fragments of
spherical shells.(13) If GSRs are expected on clothes, it is no longer necessary to cut out the regions of
interest, as these samples can also be investigated using a specially designed holder for clothes without
damaging the sample(9).

Micromanipulation is a significant tool in the biological and chemical sciences. It is utilized
primarily to manipulate small particles and cellular materials due to its precise movements. It is currently
being used in the biological sciences for single cell transfer(14), to isolate specific bacterial cells from a
group(15), and it has also been employed for sample preparation on MS analysis(16). Mitochondria have
been extracted from cells using micromanipulation which were then analyzed using electrophoresis(17).
Nanomanipulation has higher resolution than micromanipulation (beyond the optical limit), allowing new
advances in the biological sciences to be made through precise movements minimizing cell damage.

There are currently no methods to probe trace analytes directly from a single fiber. The analyses
of fibers collected in the crime scene were previously checked with a low power stereomicroscope at low
magnification. Later, different techniques for further comparison were used to investigate if any of the
fibers were consistent with each other or a known source (18). One of the current methods of probing
trace analytes is the swab method. An object's surface is swabbed using a textile sampling swab that is
then put into solution to extract the analyte of interest(19). This method is not the best to collect trace
analytes, due to analyte losses and dilution of analyte concentration. Multiple steps, comes with a multiple
handling of the analyte, which can lead to sample contamination(20). Improvement in trace analyte
sampling is needed in order to be able to more accurately solve problems and collect trace evidence to
increase analyte concentration if there is not enough analyte on a single fiber utilizing the swab method.

Mass spectrometry (MS) is a useful tool, for trace analysis due to its sensitivity, specificity, and
mass accuracy enabling the analysis of many different types of compounds. Nanospray is an ideal
ionization source to couple to nanomanipulation, because it reduces sample preparation time and requires a small amount of analyte (pmol/ul). Nanospray MS is an ionization technique that only requires a minimum of 300 attograms ($10^{-18}$ g) of analyte with a minimum volume of 300 µL. Also, it is not as affected by salts like electrospray MS is, which reduces sample preparation(21). LC-ESI-MS is used in the trace analysis of explosives(22) and other compounds of interest; nanospray has similar mechanism to the electrospray source except it is miniaturized and requires a lower flow rate nLs/min (23) indicating that nanospray mass spectrometry would be a useful instrument in trace analyte analysis. These techniques can be applied to fiber analysis, expanding the current abilities, so that trace amounts of analyte are now able to be extracted and analyzed.

Materials

The solvents and chemicals utilized were Glacial Acetic Acid, Optima® LC/MS Methanol, L-Histidine, and Caffeine (Thermo Fisher Scientific Inc.; Waltham, MA); no further purification was necessary. Millipore water was obtained using the Milli-Q® Plus (Millipore; Billerica, MA) with better than 18MΩ salt content. We used glass bottom dishes (0) to analyze our samples (Mat Tek Corp.; Ashland, MA), and we probed our analyte from 100% rayon white bemberg lining. For the gunshot residue we used four different unknown fiber samples with 1, 2, and 3 inch shot distances (Alliance Forensics Laboratory, Ft. Worth, TX). A 6 mg sample of gun powder from an unknown source was also provided (Alliance Forensics Laboratory, Ft. Worth, TX). We used the following instrumentation: LCQ DECA XP Plus (Thermo Finnigan; San Hose, CA) with a nanospray ionization source (Proxeon Biosystems; Odense, Denmark) was used to analyze the samples. L200 nanomanipulator (Zyvex; Richardson, TX), the TE2000U Microscope (Nikon; Melville, NJ) and the PE2000b 4-Channel Pressure injector (MicroData Instrument Inc.; S. Plainfield, NJ) were used to retrieve the analyte from the fiber.

Methods
The L-200 nanomanipulator is mounted to an inverted microscope see figure 1. The manipulator employs four positioners; two holders of end effectors either tungsten probes or microgrippers with 10nm resolution and two capillary holders with 1um resolution. A high quality piezo-motor controlled actuator nanomanipulator power supply is used in order to give precise and accurate movement in the fine and course modes of operation. The nanopositioners are controlled by a joystick, a foot pedal and computer program, which allows for precise movement. In the coarse mode of action the nanopositioners have a range of motion of 12mm in the X and Z axes and 28mm in the Y axis with. Two of the nanopositioners have a fine mode of action with a range of 100um in the Z and X axes and 10um in the Y axis. A pressure injector is used to supply up to 60psi of injection pressure and 24Hg of fill pressure to the capillaries allowing us to retrieve the analyte of interest. The nanomanipulator stage has its benefits, because it is able to hold 6-8 nanopositioners, allowing one to conduct multiple probes at a single time which increases the instrument’s capabilities and effectiveness.

The Au/Pd plated nanospray tips are loaded with the solvent of interest, and then the tip is broken using the nanospray source head. A blank is run to determine any solvent contamination, and a background spectrum of the solvent is taken. The tip is then transferred to the nanomanipulator for trace analyte probing from a rayon fiber that was doped with the analyte of interest, and placed in a glass bottom dish. The rayon fiber was tacked down to ensure minimal movement of the fiber and analyte on the fiber. The particle of interest was found on the fiber, and then the nanospray tip was landed near it, less than a micrometer away. The nanospray tip injects the solvent of interest onto the analyte, which dissolves in the solvent. The solvent with the dissolved analyte is then retrieved back into the nanospray tip. The nanospray tip is then transferred directly to the nanospray ionization source and the sample analyzed. Figure 2(a) shows one of the positioners of the nanomanipulator with a nanospray tip retrieving sample. Figure 2(b) shows the tip mounted onto the nanospray assembly.

Caffeine and histidine are used to illustrate this technique. When sampling caffeine, methanol with 1% glacial acetic acid was used as the solvent, and 3ul of the solvent was loaded into the tip. The tip
was landed next to the analyte is shown in figure 3(a). The nanomanipulator used an injection pressure of 20.8psi for duration of 11msec delivered from the pressure injector, and a fill pressure of 65psi with a fill time of 50msec. The sample is then analyzed using nanospray MS. When sampling histidine two capillaries were used the nanospray tip filled with 3ul of 60:39:1 MeOH, H2O, and HA the MS solvent and another capillary filled with water seen in figure 3(b) The capillary tip injected the water onto the histidine which dissolved in the water and then the nanospray tip retrieved the water with the dissolved analyte. This shows the utility and flexibility of the method. The capillary was injected at 1.3psi for 100msec and the nanospray tip filled at 76.0psi for 0.5 sec repeated once. The sample is then transferred to the nanospray MS and analyzed.

The technique was used in a similar fashion to analyze gunshot residue. Prior to analyzing the gunshot residue from the fiber, a standard was prepared by placing a sample of gunpowder (5ug) on a watch-glass and introduced heat to dissolve. The extraction solvent used was prepared by using a combination of 50:50 chloroform: methanol with 1% acetic acid, due to the more nonpolar nature of the extracted organics in the GSR. To collect the analyte from the watch glass, 6ul of the solvent was poured using a micropipette from the rim of the watch glass. Using a new micropipette tip, the micropipette was used to fill the analyte and transfer to a microcuvette for temporary holding. To analyze the sample, a capillary was loaded with 5ul of the analyte and transferred to the nanospray ionization source to identify the compounds from burning gunpowder.

The four different fabric sample swatches were labeled with the proper distance shot and from each of the swatches a single fiber (~1mm) was pulled from the entry point using forceps. Each fiber was observed under the microscope to identify positive identification of foreign particles. Once the particles were located on the fiber, the nanospray tip was landed near the fiber in order to extract the particles which is followed by injecting the analyte with the solvent. The solvent used was prepared using a methanol and chloroform (50/50) and 1% acetic acid, which was placed in the capillary tip. The nanospray tip injects the solvent of interest onto the analyte, which dissolves in the solvent. The solvent
with the dissolved analyte is then filled into the nanospray tip, which is then transferred directly to the nanospray ionization source and the sample analyzed compared to the gunpowder standard.

Results and Discussion

The main purpose of this paper is to introduce this novel instrument of nanomanipulation coupled to nanospray MS, as an effective tool to analyze trace fibers. Both histidine and caffeine trace particles were sampled directly from a single rayon fiber using the nanomanipulator, and then directly analyzed using mass spectrometry. Figure 4 shows the mass spectrum of caffeine (a) and histidine (b) after directly probing trace particles from the rayon fiber. The caffeine particle had an area of 156.65$\text{um}^2$ calculated using the Nikon software to measure the particle. The area of the histidine particle was 47.92$\text{um}^2$ before injection took place. The limit of detection of histidine is 7pg particles when using nanospray, making this an ideal method to analyze and retrieve trace analytes from fibers.

From the swatches containing the GSR, we were able to identify the different organic components detected in the analyte extracted. We correlated eight compounds to the gun powder standard. As each set of data was analyzed, the relative abundances of the organic identifiers diminished based on the placement distances of the swatches. In one sample we even able to identify the inorganic compounds, nickel and barium. The intensity of the inorganic compounds was low; however this was an unexpected result. The metals cations may have formed salts, making them visible in the nanospray MS.

Several organic compounds were found consistent throughout each sample analyzed (Figure 5). The compounds that stand out in each of the samples analyzed are the plasticizers and lead. The plasticizers include dibutyl phthalate, diethyl phthalate, dimethyl phthalate and lead. In the data collected from the gunpowder, contact shot and one inch shot, we observed a consistency of the four previous components plus ethyl centralite. All organic compounds identified showed to have similar relative intensities throughout each of the data collected from the samples analyzed. However, the relative intensity of dibutyl phthalate is the greatest from the data collected from the two and three inch samples.
Our results clearly show that the nanomanipulator coupled to nanospray MS is an effective instrument to probe trace analytes from fibers. It is an improvement in trace analyte probing from a single fiber, allowing for new experimental procedures to be created, and smaller amounts of analyte to be sampled. The nanomanipulator reduces cost of sampling from fibers, because of the low sample preparation and the reduced sample preparation time and run time. Computer control of the positioners can be created to automate the procedure. Being able to extract an analyte from a single fiber allows for better analysis of crime scenes, and the reduced sample size and volume required for nanospray MS gives us the ability to retrieve a higher sample concentration. It is important to have a solvent to solubilize the sample as well as give a steady spray flow. Some nonpolar compounds will not dissolve in any of the nanospray solvents, so it is important to utilize a two capillary system with one capillary containing a solvent to solubilize the alalyte, and other capillary containing the nanospray friendly solvent that retrieves the dissolved analyte. Some diffusion will occur and the small amount of nonpolar solvent with the analyte of interest will mix with the nanospray solvent, and then can be analyzed using the MS. Having the multiple nanopositioners allows us to do this. We are also capable of liquid-liquid phase microextractions to sample trace analytes and gain higher sample concentration from a dilute analyte in a liquid sample and then analyze it. Our next paper will illustrate our capabilities of nanomanipulating cells and direct cell sampling.

Conclusion

Nanomanipulation coupled to nanospray mass spectrometry is a novel technique that we illustrated using caffeine, histidine and gunshot residue. We recovered trace particles of histidine and caffeine from different rayon fibers, as well as gunshot residue from an unidentified fiber using the nanomanipulator. We were then able to analyze the trace analyte by directly taking the analyte from the source to the nanospray mass spectrometer. This clearly showed the functionality and usefulness of the nanomanipulator coupled to mass spectrometry in the trace analysis of fibers, improving current methods.
Dyes and surfactants could be the reason for the unidentified ions in the GSR spectrum. Since online pre-concentration techniques only produces positive results for metal chelates, a better option might be a pre-concentration technique like evaporation, extraction methodologies or changing the sampling procedure to obtain a more concentrated sample(24). However, one advantage of nanospray MS is having the capability to analyze samples with limited volumes of up to 100 nl/min and has limits of detection. Future research will include direct cell probing and other trace analysis including liquid-liquid microextraction.

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References:


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Schematic of the nanomanipulator workstation on an inverted microscope. 2 positioners are connected to a pressure injector which can be controlled through a foot pedal. The orthogonal 2 positioners are end effectors with 10 nm probing resolution.

110x63mm (150 x 150 DPI)
(a) The nanomanipulator positioner with the nanospray tip probing an analyte. (b) The nanospray ionization source showing the nanospray needle that was transferred directly from the nanomanipulator.

124x86mm (150 x 150 DPI)
The plated rayon fiber doped with analyte before extraction. (a) The caffeine particle is on the rayon fiber and the nanospray tip is landed near the particle. (b) The histidine particle is in between the nanospray tip on the right and the capillary tip on the left.

193x89mm (150 x 150 DPI)
Mass spectrum of analyte after its extraction from a single rayon fiber (a) Caffeine, \([\text{C}_8\text{H}_{10}\text{N}_2\text{O}_2]\text{H}^+, 195.08 \text{ m/z.} \) (b) Histidine, \([\text{C}_6\text{H}_9\text{N}_3\text{O}_2]\text{H}^+, 156.08 \text{ m/z.} \)
Gunshot residue compounds identified from in each of the samples analyzed (A) Dimethyl phthalate (B) Lead (C) Diethyl phthalate (D) Dibutyl phthalate.
Figure I

Schematic of the nanomanipulator workstation on an inverted microscope. 2 positioners are connected to a pressure injector which can be controlled through a foot pedal. The orthogonal 2 positioners are end effectors with 10 nm probing resolution.

Figure II

(a) The nanomanipulator positioner with the nanospray tip probing an analyte. (b) The nanospray ionization source showing the nanospray needle that was transferred directly from the nanomanipulator.

Figure III

The plated rayon fiber doped with analyte before extraction. (a) The caffeine particle is on the rayon fiber and the nanospray tip is landed near the particle. (b) The histidine particle is in between the nanospray tip on the right and the capillary tip on the left.

Figure IV

Mass spectrum of analyte after its extraction from a single rayon Fiber (a) Caffeine, $[C_8H_{10}N_2O_2]H^+$, 195.08 $m/z$. (b) Histidine, $[C_6H_9N_3O_2]H^+$, 156.08 $m/z$.

Figure V

Gunshot residue compounds identified from in each of the samples analyzed (A) Dimethyl phthalate (B) Lead (C) Diethyl phthalate (D) Dibutyl phthalate.