

The Protein I.D. Autobahn

Automated Nanoelectrospray Mass Spectrometry

Gary Schultz

Two things drive biopharmaceutical researchers: the need for new, more effective pharmaceuticals and the demand to develop those drugs faster. After decades of “shotgun” approaches to testing new compounds, researchers have turned their focus to elucidating protein functions and pathways to understand the underlying causes of health and disease. The goal is to identify promising drug targets and to develop novel therapeutics. Historically mass spectrometry (MS)-based proteomics has been limited by a trade-off between throughput and information content. High sensitivity often comes at the cost of throughput. Ideally, researchers would choose the efficiency of both, but analytical techniques to date have failed to achieve this balance for proteomics. Researchers often list sample introduction as a bottleneck to high-throughput protein analysis. MS has become the technology for protein identification and characterization.

Although MS can analyze any biological sample, that sample must first be converted to gas-phase ions. Electrospray ionization (ESI) is a technique that allows direct analysis of samples in solution by generating highly charged droplets of spray that evaporate to form gas-phase ions.

Nanoelectrospray

In the mid-1990s, nanoelectrospray was introduced as a high-sensitivity protein analysis technology. Operating at low flow rates (10–200 $\mu\text{L}/\text{min}$) yields high sensitivity and consumes small sample amounts, allowing researchers to extend analysis time and mine more mass spectral information from each sample. However, the process is labor-intensive and, consequently, the low throughput of this technique has curbed widespread use.

Skilled users manually perform sample introduction for nanoelectrospray by using



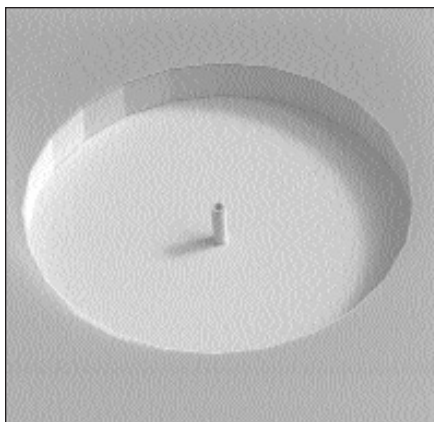
Advion's NanoMate 100 System combines automated sample handling and introduction with the company's proprietary electrospray ionization (ESI) chip to provide a hands-free, robust method for high-throughput sample analysis by nanoelectrospray mass spectrometry.

fragile glass capillaries with tapered tips. Each sample requires a new capillary to be prepared, loaded, optimized, and removed.

Automation

Automated nanoelectrospray integrates sample handling and introduction to give proteomics researchers high throughput, sensitivity, and information content. Combining miniaturization with automation, Advion BioSciences (www.advion.com) developed a system for automated nanoelectrospray called the NanoMate 100. The system uses a chip with an array of 100 nanoelectrospray nozzles etched in silicon. Each nozzle is made to exactly the same specification so that every nanoelectrospray is identical and provides

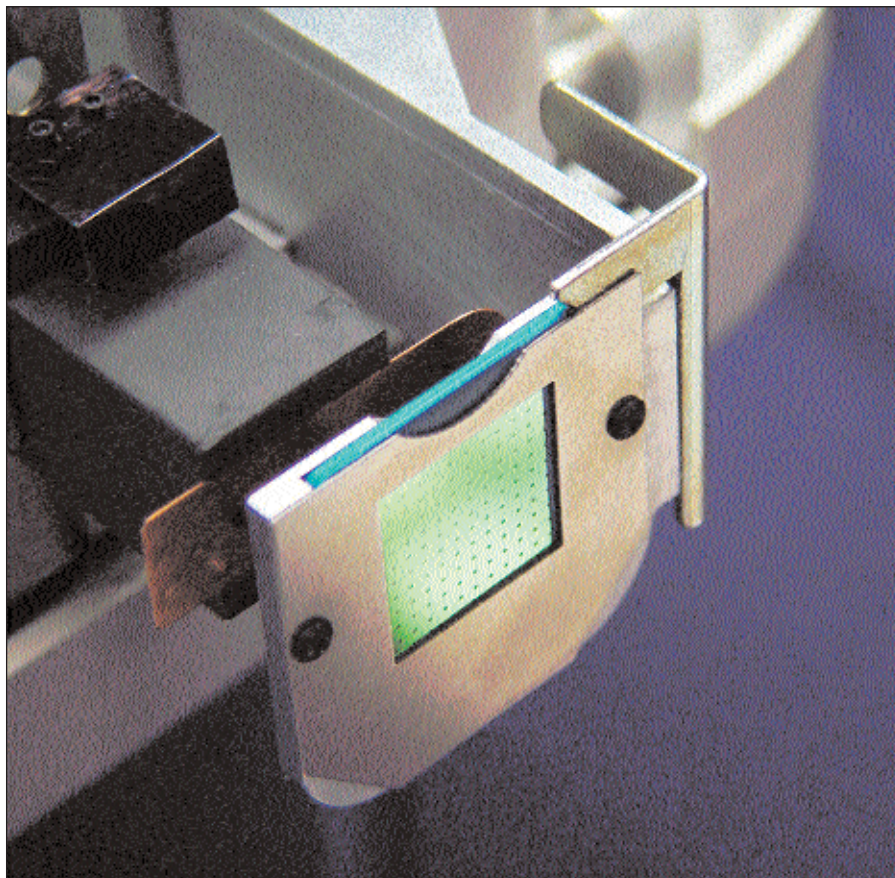
fully reproducible spray. Current methods involving capillaries require that the capillary be positioned precisely, a process that can take up to 20 minutes to achieve an appropriate signal for the mass spectrometer. The mass spectrometer is never fully used because it must sit idle during the positioning of the capillary. The NanoMate can run hundreds of samples per day, as compared with 10–20 samples per day with capillaries. Additionally, with a diameter one-fifth that of a human hair, each nozzle reduces sample and reagent consumption, thus reducing analysis costs and conserving precious samples. Each sample flows through a different nozzle and the ESI chip — used only once — eliminates carryover.



A nanoelectrospray nozzle

The system simplifies nanoelectrospray for the user by accommodating current sample preparation technologies. Samples loaded into a standard 96-well plate are placed adjacent to a rack of 96 pipette tips. A disposable ESI chip is placed in the chip holder and then adjusted for optimal electrospray for all 100 nozzles at once by Advion's software program, ChipSoft. The user then defines analysis parameters with the software and automated, unattended sample loading and nanoelectrospray for up to 96 samples begins. A minute volume of sample (as low as 2 μ L) is aspirated into a pipette tip that then seals against the inlet of a nozzle on the back of the chip. Pneumatic pressure delivers a constant flow of sample for nanoelectrospray analysis. After the first sample is analyzed, the system automatically discards the used pipette tip and moves on to the next sample using a new tip. This process continues until all samples are analyzed.

The system can automatically return any unused sample to the 96-well plate. This is particularly valuable because many proteomics samples are available only in small quantities, and retrieval of unused sample from glass capillaries is difficult and impractical. Advion has designed brackets to mount its system to a variety of electrospray mass spectrometers, including those offered by Thermo Finnigan (www.finnigan.com), Micromass (www.micromass.co.uk), and Applied Biosystems (www.appliedbiosystems.com).



Advion's ESI chip, shown loaded in the NanoMate system, is a microfluidics device capable of ionizing minute volumes of samples and delivering them into a mass spectrometer for analysis. The chip contains an array of nanoelectrospray nozzles, each one-fifth the diameter of a human hair, etched from a standard silicon wafer.

Future developments are likely to focus on integrating products that, like Advion's system, combine automation and miniaturization to increase sample throughput sensitivity and information content from trace biological samples. Decision-making software is also being developed to improve the operation of laboratory mass spectrometers with minimal user interactions. New advances in automated MS will enable the promise of proteomics research to be realized. These advances also provide benefits to drug metabolism and pharmacokinetic analyses, and this combination of applications will pave an express route for getting new drugs to market. **BPI**

Gary Schultz is vice president of research and development at Advion BioSciences, Inc., 15 Catherwood Road, Ithaca, NY 14850, 607.266.0665, fax 607.266.0749, gscultz@advion.com, www.advion.com.