ION MOBILITY-MASS SPEC COMBO

Adding ion mobility to mass spectrometry brings NEW LEVELS OF SEPARATION and information to analyses

CELIA HENRY ARNAUD, C&EN WASHINGTON

THE GROWING USE of ion mobility spectroscopy in conjunction with mass spectrometry in academic labs and commercial instrumentation is making the powerful MS technique even more capable. In addition to providing an extra dimension of separation, ion mobility provides shape information because that separation is also based on the conformation of a molecule rather than on its mass alone.

Despite the extra information that can be acquired from adding an ion mobility component to a mass spectrometric analysis, the combination was initially slow to catch on. Researchers had to build their own instruments, relegating IMS/MS to the labs of instrument jocks. But now, commercial instruments incorporating variants of IMS peddled by Waters Corp. and Thermo Fisher Scientific are bringing the technology into the mainstream. Waters' instrument, called the Synapt High Definition MS System, uses a form of IMS called traveling-wave IMS. Thermo Fisher offers its system, based on high-field asymmetric waveform IMS (FAIMS), as a front end for its quadrupole and ion trap mass spectrometers.

In conventional IMS, also known as drift-time IMS, ions travel through a gas-filled drift tube that has a low applied voltage. Collisions with the gas molecules slow the ions in a way that depends on their conformation. The time an ion takes to traverse the tube, known as its drift time, can be used to calculate the ion's collision cross section, a fundamental property of the ion related to its size and shape.

Compact ions move through the drift tube faster than stretched out ones. Even for those researchers who don’t care about the shape of the ions, this separation mechanism, when combined with MS or liquid chromatography/MS, allows many more components to be resolved in a single analysis than either of those other techniques alone.

In its recent rise in popularity, the IMS/MS combination first won converts in the
METHOD DEVELOPMENT

Overtones Filter By Shape To Improve Sensitivity, Resolving Power

A new ion mobility technique uses the frequency of the applied field to selectively transmit ions through a drift tube. David E. Clemmer of Indiana University, Bloomington, predicts that the technique will improve the resolving power and sensitivity of IMS. In June, he described the method, called overtone mobility spectrometry, in a talk at the American Society for Mass Spectrometry meeting in Denver.

Clemmer realized that modulating the frequency of the drift field could turn a segmented drift tube into a continuous ion filter. Only those ions whose mobilities were resonant with the experimental conditions would be transmitted through the drift region. Ion mobility could then be interfaced with a continuous ion source, such as electrospray ionization, without having to worry about losing ions between pulses.

Clemmer, however, neglected to remind his students, Fabiane Nachtigall and Ruwan Kurulugama, that the fundamental frequency would be at the reciprocal of the drift time. Instead of looking at the fundamental frequency for their test ion, they scanned the frequency as far as their scanner would go, to 50,000 Hz. The resulting spectrum was filled with peaks they hadn’t seen before. “It didn’t take long for us to realize that these were overtone peaks,” Clemmer said. The overtone peaks were much sharper and farther apart than the peaks at the fundamental frequency.

“Once you see these overtones, you realize you’ve built something analogous to a quadrupole mass filter,” Clemmer said. “You can predict where the mobilities will come out. It’s based on shape-to-charge rather than mass-to-charge.” Thus, they could use the fundamental property of mobility to tune the filter to transmit only the desired species, and they didn’t need to empirically determine the frequency required.

At the overtone frequencies the resolving power increases as the peaks move apart by a multiple of the difference between them at the fundamental frequency. For example, two peaks separated by 800 Hz at the fundamental frequency will be separated by five times 800 Hz, or 4,000 Hz, at the fifth overtone. Clemmer was able to separate the isomeric trisaccharides raffinose and melezitose at the fifth overtone but not at the fundamental frequency.
things like glycomics versus proteomics and lipidomics, they try to purify each of the molecular classes ahead of time and then analyze them by mass spectrometry,” McLean says. “With IMS/MS you can rapidly analyze something as complex as a cancer biopsy and see which masses correspond to peptides, lipids, or carbohydrates. You can really integrate all of those ‘omics’ strategies.” This integration is possible because the different classes of biomolecules lie along their own “trend lines” in a plot of drift time versus mass-to-charge ratio.

SOMETIMES, however, the trend lines become blurred. McLean is developing “shift tags” to tease apart these overlapping trend lines. These tags alter the mobility characteristics of biomolecules. The current shift tags consist of positron emission tomography labels with a chelating ring that binds lanthanide metals with high affinity. In phosphoproteomics experiments the tags can be used to distinguish phosphorylated from nonphosphorylated peptides. Multiple states, such as diseased versus normal tissue, can be analyzed by using a different lanthanide for each one.

Likewise, Richard D. Smith of Pacific Northwest National Laboratory uses standard IMS as a way to increase the coverage and throughput of proteomic and metabolomic measurements. Although IMS reduces the need for a chromatographic separation, Smith prefers to use a fast liquid chromatography separation, as well as IMS. “That combination gives us a two-dimensional overall separation power that would take us a couple of hours to achieve with the best LC separations we can manage,” he says. “This provides an order-of-magnitude increase in throughput, along with comparable or better proteome coverage.”

Bowers calls IMS/MS “perfect” for proteomics. “The millisecond separation timescale works perfectly with any initial separation devices and any microsecond time-of-flight mass analyzer,” he says. “I bet in five to 10 years, every mass spectrometry proteomics facility will have associated with it an IMS.”

Another hurdle for IMS/MS in the proteomics arena is data analysis. “If you’ve got mass-to-charge, you’ve got a lot of data,” Bowers says. “If you’ve got mass-to-charge and shape—wow! Look at the tremendous amount of data you’ve got. Handling the data from a computational point of view is going to be significant.”

So far, researchers are using IMS/MS mainly for biological applications, but McLean foresees other applications such as polymer analysis. For example, McLean and David M. Hercules, an emeritus professor at Vanderbilt, used IMS/MS to separate linear and branched aramids, the type of polymer used in Kevlar. No good techniques exist for being able to tell the branching ratio of these polymers, McLean says. The conventional way to do the analysis, he notes, is with gel permeation chromatography in concentrated sulfuric acid.

The commercial IMS instruments incorporate variations of ion mobility. In the FAIMS technique used by the Thermo Fisher instruments, the waveform alternates between two concentric cylindrical electrodes.
voltages. In one direction the ions experience a drift component much like in standard ion mobility, albeit at much higher field strengths. In another direction the ions experience an electrical displacement that threatens to send them crashing into the electrodes. Applying a direct current voltage, called the compensation voltage (CV), allows only certain ions to traverse the gap between electrodes. This CV is scanned to allow different ions to exit the gap. In this way FAIMS acts as a differential mobility filter just as a quadrupole mass analyzer acts as a mass-to-charge filter.

Waters' Synapt instrument employs a method called traveling-wave (T-wave) IMS. In this version the cell is made of a series of ring electrodes. Rather than a low electrical field being applied uniformly across the cell, a high electrical field is swept sequentially from one electrode to the next in the direction of ion migration. This electrical field separates the ions according to their mobility.

The constantly changing field makes it nearly impossible to determine the path length and collision frequency, two parameters that are needed to determine the collision cross section. Users who want to obtain a collision cross section to calculate the size of the molecule must calibrate data from a traveling-wave device against mobility data obtained with a standard drift cell.

FAIMS is the least understood of the ion mobility methods. "It depends on how the mobility changes with field as opposed to just the mobility," says Richard A. Yost, a chemistry professor at the University of Florida. "It doesn't have as much of a fundamental grounding as basic IMS understanding. The trade-off of the relatively simple fundamental understanding of IMS is that FAIMS offers you more potential to affect the separation by changing parameters."

THE LACK OF a theoretical understanding means that identifying the necessary CV for a given ion remains an empirical exercise. "You infuse a compound, ramp the compensation voltage, and find out at what CV the compound emerges from FAIMS," says James Kapron, a strategic marketing specialist at Thermo Fisher.

Even without such theoretical underpinnings, however, FAIMS provides an analytical boost for a wide range of applications. For example, it is used for sports doping analysis, Kapron says. It is also being used for pharmaceutical analysis in combination with LC/MS to remove interferences.

"They turn out to be significantly orthogonal in how they separate ions," he says.

Specifically, he uses FAIMS as a front-end filter for targeted analyses in which he already knows what he's looking for. He sets the CV to transmit a particular class of ions, reducing chemical noise and increasing the signal-to-noise ratio in the process.

Despite the added information and separation it provides, IMS is not a high-resolution technique. Researchers can improve the resolution of conventional IMS by adjusting three parameters: increasing the drift tube length, lowering the temperature, and raising the drift gas pressure. Unfortunately, attempts to improve Synapt's resolution, which is about one-third that of conventional drift tubes, have failed thus far. "Intrinsic factors that aren't clearly understood seem to limit its resolution," Bowers says.
voltage. In one direction the ions experience an electrical displacement that threatens to send them crashing into the electrodes. Applying a direct current voltage, called the compensation voltage (CV), allows only certain ions to traverse the gap between electrodes. This CV is acoustically allowed different ions to exit the gap. In this way FAIMS acts as a differential mobility filter just as a quadrupole mass analyzer acts as a mass-to-charge filter.

Waters has instrument employs a method called traveling-wave (T-wave) FAIMS. In this version, the cell is made of a series of ring electrodes. Rather than a low electrical field being applied uniformly across the cell, a high electrical field is swept sequentially from one electrode to the next in the direction of ion migration. This electrical field separates the ions according to their mobility. The constantly changing field makes it nearly impossible to determine the path length and collision frequency, two parameters that are needed to determine the collision cross section. Users who want to obtain a collision cross section to calculate the size of the molecule must calibrate data from a traveling-wave device against mobility data obtained with a standard drift cell.

FAIMS is the least understood of the ion mobility methods. "It depends on how the mobility changes with the field as opposed to just the mobility," says Richard A. Yost, a chemistry professor at the University of Florida. "It doesn't have as much of a fundamental grounding as basic IMS understanding. The trade-off of the relatively simple fundamental understanding of IMS is that FAIMS offers more potential to affect the separation by changing parameters."

The lack of a theoretical understanding means that identifying the necessary CV for a given ion remains an empirical exercise. "You can use a compound to map the compensation voltage, and find out what CV the compound emerges from FAIMS," says James Kapron, a strategic marketing specialist at Thermo Fisher. Even without such theoretical understandings however, FAIMS provides an analytical boost for a wide range of applications. For example, it is used for doping analysis, Kaplan says. It is also being used for pharmaceutical analysis in combination with LC/MS to remove interferences.

"They turn out to be significantly orthogonal in how they separate ions," he says. Specifically, he uses FAIMS as a front-end filter for targeted analyses in which he already knows what he's looking for. He sets the CV to transmit a particular class of ions, reducing chemical noise and increasing the signal-to-noise ratio in the process. Despite the added information and separation it provides, IMS is not a high-resolution technique. Researchers can improve the resolution of conventional IMS by adjusting three parameters increasing the drift tube length, lowering the temperature, and raising the drift gas pressure. Unfortunately, attempts to improve the conventional IMS system, which is about one-third of that of conventional drift tubes, have failed thus far. "Intrinsic factors that aren't clearly understood seem to limit its resolution," Yost says.

Some researchers, including Bowers, David E. Glemmer of Indiana University, Bloomington; and David H. Russell of the Florida Atlantic University are developing drift tubes as long as 9 meters to improve the mobility resolution. Such long drift tubes might seem impractical, but "practicality is based on the utility," Russell says. "If we show that ion mobility really can do the things that some of our customers claim they can do, I think you would find themselves converting elevator shafts into areas where you build long mobility devices." Bowers finds that the most practical way to adjust resolution is through a combination of length and pressure. His group has tested a 20-meter tube operated at 10 Torr. Russell is developing cryogenic IMS to improve resolution. As the temperature drops, drift times increase and drift time spreads. "It's all about how it freezes out," he says. Peaks coalesce and separate as the temperature rises. Russell also points out that the technological challenge lies in cooling a 1- to 4-meter metal tube to liquid nitrogen temperatures. "You really want to lower the temperature of the bath gas," Russell says, "but you also have to lower the temperature of the surroundings." If the liquid nitrogen experiments ever work as hoped—his group has worked on them for the better part of a decade—Russell will try to push the temperature even lower to liquid helium temperatures.

McLennan advocates variable temperature IMS/MS, not just as a way to improve resolution but also as a new way of performing "When you do separations as a function of temperature, you can pull out thermodynamic and kinetic data for structural changes," he says.

The resolution improves, the spectra may start to expose hidden fine structure indicating new conformational states, Glemmer says. For example, his team recently saw three structures of the protein ubiquitin, compact, partially folded, and elongated. When they used a 1-meter drift tube, the added resolution revealed multiple states built within the three broader peaks, including at least 10 states within the compact one. "There still be states that aren't resolved in these states," Glemmer says. Although the current system can't measure collision cross sections directly, it nonetheless be used to calculate cross sections and determine molecular conformations. Some researchers worry about the accuracy of the calculations used to draw these connections.

Rustolok believes ideal calibrants should have a cross section that is unchanged by instrument parameters. "This rules out a lot of proteins. Proteins can change their overall size pretty easily just by varying the amount of internal energy they have or the amount of time they spend in the instrument," he says. However, Rustolok notes, unfolded protein structures with high-charge
"With IMS/MS you can rapidly analyze something as complex as a cancer biopsy and see which masses correspond to peptides, lipids, or carbohydrates."

States don’t vary as much. He suggests that carbon clusters might make good standards.

Regardless of the standards used, calibration requires careful notation of the instrumental conditions. “If you are going to publish a calibration dataset, you also have to publish very precise instrument conditions that go along with each of those ions,” Ruotolo says. “If you run the instrument in a different mode or use different amounts of activation energy, you’ll get a completely different cross section.”

For now, the key is careful selection of your research questions, Ruotolo says. Robinson’s group, for instance, uses Synapt to determine conformational changes of as much as 30 or 40% in large protein assemblies. “If you take the error out of the equation by looking at things that can only be one thing or the other, given a set of models or situations, you make your life a lot easier,” Ruotolo says. “Choose your problem such that your approach can actually solve the problem. Don’t get into situations where your approach introduces more problems.”

People must be careful when they report collision cross sections determined with Synapt, Bowers says. “There’s definite curvature in these calibration plots. They’re not linear,” he says. “If you want absolute cross section, you’ve got to be sure that.

SEPARATION POWER

The combination of FAIMS and IMS reveals more protein conformers, as indicated by the white boxes, than either technique alone. The temperature at the FAIMS-IMS interface varies from low (left panel) to high (right panel).

FAIMS compensation field, V/mm

7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0

IMS drift time, milliseconds

50 60 70 80 90 100 50 60 70 80 90 100

Omnical Process Safety
Rxn Calorimeter
www.omnicaletech.com

SuperCRC
An easy-to-use microcalorimeter for measuring reaction conditions, kinetic profiles, and max energy release, used by top chemical and pharma companies worldwide.

low-mobility-resolution instrument such as Synapt against data from conventional instruments with higher resolution can be confusing. If higher resolution instruments reveal fine structures that Synapt can’t resolve, “what do you calibrate to?” Clemmer asks. “If you’re within a few percent, that’s what the instrument can give.”

Waters’ goal in developing the Synapt system was making sure that the inherent low duty cycle of traditional IMS methods did not compromise the performance of the mass spectrometer. “We felt that we needed to offer this exciting capability in such a way that we didn’t detrimentally affect the base sensitivity of the mass spectrometer,” says Alan Millar, senior product manager at Waters. To maintain that sensitivity, the company focused on technology it already knew well, having used traveling-wave ion guides in other mass spectrometers.

ONE CHALLENGE with IMS is increasing the number of ions that are analyzed. Because it is a pulsed method, IMS duty cycle issues can result in many ions being discarded while waiting for previously injected ions to separate. Even those ions that make it into the drift tube can be lost because the ions diffuse radially as they travel through the device. Without a device to refocus the ions, less than 1% of the ions would reach the mass spectrometer. Synapt addresses those duty cycle issues by incorporating additional T-wave devices that trap and transfer the ions with minimal loss.

Many people attribute the current success of IMS to the ion funnels developed by Smith and coworkers that gently refocus those diffusing ions onto the exit and entrance apertures of the drift tube and the mass spectrometer. The funnels ensure that
nearly all the ions are captured. The funnels, however, work best at low pressures, in the 1- to 25-torr range. "It would be nice to work at atmospheric pressure because you get better separations," Smith says. But "the ion funnel doesn’t work at atmospheric pressure. We had to compromise," he adds.

Even in systems where most ions are being analyzed, much time can be wasted waiting for one batch of ions to clear the drift tube before injecting the next batch. Smith’s group is working on this problem, as well. They have developed multiplexing methods in which they inject as many as 50 batches of ions before the first batch has exited the drift tube. "It takes an algorithm and a mathematical approach to deconvolute the overlapping distributions of ions and re-

**Flash to Prep Flexibility**

**Buchi SepacoreControl**

The NEW Buchi SepacoreControl software gives you complete chromatography process control.

The flexibility of the Buchi Sepacore system allows you to customize a complete system for your lab. And now you can control and integrate the system with our new industry-leading SepacoreControl software, meeting all your lab needs.

- From 1 to 4 pumps
- Up to 8 detectors
- Up to 5 columns
- Prepare mg to >100 g
- Use any flash or prep column with pressures to 725 psi
- Complete process control with SepacoreControl Software

To configure your custom system visit [www.mybuchi.com](http://www.mybuchi.com).

**BUCHI**

New Castle, Delaware

1-877-MYBUCHI or visit [www.mybuchi.com](http://www.mybuchi.com)

**SHARPER IMAGE** Images based on mobility and mass (MALDI-IM-MS) are able to distinguish between a peptide (green) and a lipid (blue) of the same nominal mass spotted on liver tissue. The image based on mass alone (MALDI-MS) shows an X shape, but images that combine mass and drift time (ATD) reveal individual lines.

cover all that information," Smith says.

Yost believes that in the long run, the major use of PIAIMS and IMS will be as a separation device in front of a mass spectrometer, alone or in combination with liquid chromatography. "It's going to give you an extra stage of cleanup along the way that costs you nothing in time because it's essentially instantaneous," he says. In addition, the methods add selectivity to an analysis without hurting sensitivity. Finally, Yost says, these devices should be relatively inexpensive. The "killer application" that will make IMS and PIAIMS an analytical must-have is yet to be identified, he says.

Although Bowers gives Waters ample credit for taking the leap to commercialize an IMS/MS system, he continues to hope that some company will commercialize an IMS/MS system with a conventional drift cell to enable higher resolution and the determination of absolute cross sections. With the absolute cross sections, companies could then include modeling capabilities in their software packages.

"We're in the ascendancy of this technique," he says. "We've got to get commercial instruments that are based on drift technology to the community. People aren't going to build them themselves."