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High-Resolution Differential Ion Mobility Separations/Orbitrap Mass Spectrometry without Buffer Gas Limitations

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ABSTRACT: Strong orthogonality between differential ion mobility spectrometry (FAIMS) and mass spectrometry (MS) makes their hybrid a powerful approach to separate isomers and isobars. Harnessing that power depends on high resolution in both dimensions. The ultimate mass resolution and accuracy are delivered by Fourier Transform MS increasingly realized in Orbitrap MS, whereas FAIMS resolution is generally maximized by buffers rich in He or H₂ that elevate ion mobility and lead to prominent non-Blanc effects. However, turbomolecular pumps have lower efficiency for light gas molecules and their flow from the FAIMS stage complicates maintaining the ultra-high vacuum needed for Orbitrap operation. Here we address this challenge via two hardware modifications: (i) a differential pumping step between FAIMS and MS stages and (ii) reconfiguration of vacuum lines to isolate pumping of the Orbitrap ultra-high vacuum region. Either greatly ameliorates the pressure increases upon He or H₂ aspiration. This development enables free optimization of FAIMS carrier gas without concerns about MS performance, maximizing the utility and flexibility of FAIMS/MS platforms.
linear IMS. Hence, FAIMS resolution was wanting despite superior orthogonality.

With the development of novel planar-gap analyzers using a homogeneous electric field and extended separation time, buffers rich in He or H₂, and high-definition waveforms, $R_{\text{FAIMS}}$ Pose to $>100$ ($>200$ for multiply-charged ions). While linear IMS has since improved to similar $R$ values, especially for the trapped IMS (TIMS), the orthogonality advantage of FAIMS allows disentangling species merged in linear IMS: isotopomers are the prime example. High-resolution FAIMS effectively separates lipid and peptide isomers and protein conformers, including peptides with variant positions of post-translational modifications (PTMs).

The utility of high-resolution FAIMS was constrained by its combination with MS instruments of modest resolving power ($R_{\text{MS}}$), mainly quadrupole ion traps with $R_{\text{MS}} \sim 10^5$. Frontline analyses of complex biological and environmental samples demand much greater $R_{\text{MS}}$ ($10^7 - 10^9$) and mass measurement accuracy (mma) under 1 ppm provided by Fourier Transform (FT) MS: FT Ion Cyclotron Resonance and Orbitrap™ MS. These metrics permit distinguishing nominal isobars and deducing stoichiometries based on “exact” $m/z$ up to $\sim$1 kDa, which is crucial for studies in proteomics, petroleomics, and other areas.

An increasingly prominent tool in proteomics is electron transfer dissociation (ETD) that severs the peptide backbone bonds without abstracting the weakly bound PTMs. ETD reaction time (here 15 ms) easily fits the long ion storage times mandatory for sought FTMS resolution (~1 s). Hence, ETD is available in many Orbitrap MS platforms but rarely in those without ion trapping such as time-of-flight (ToF) MS. On the other hand, ETD can process continuous ion beams output by FAIMS, but not short packets from dispersive linear IMS techniques of drift-tube and traveling-wave IMS. That makes FAIMS an obvious match to FTMS, even thoughToF's now deliver $R_{\text{MS}} \sim 5 \times 10^4$ (at the floor of FTMS range).

High-resolution FAIMS generally required mixtures of 40 - 85% He or H₂ with N₂ or CO₂. The He/N₂ compositions are most common, but H₂/N₂ maximize $R_{\text{FAIMS}}$ for proteins and large peptides, whereas He/CO₂ reveal the structurally informative isotopic shifts. All turbomolecular (turb) pumps evacuate light molecules with faster thermal speed less efficiently. The vacuum systems of commercial MS instruments are not designed to pump He or H₂, causing a pressure rise throughout the manifold with losses of MS resolution, mma, and sensitivity due to collisional scattering, accelerated wear of turbos, or shutdown by the safe pressure interlock. A destructive internal arc discharge can also happen, as He and H₂ have lower electrical resistances than N₂ or air, and here (to the left of Paschen curve bottom) the elevated pressure reduces them further. This aspect is universal to MS, but is worse for FTMS that requires $\sim 10^{-10}$ Torr pressure versus $\sim 10^{-5}$ Torr for transmission quadrupoles or traps and $\sim 10^{-7}$ Torr for ToFs. That has obstructed adding high-resolution FAIMS to Orbitrap MS.

Lower He or H₂ fractions can often be offset by higher $E_r$. By raising DV from the previous maximum of 5 kV to 6 kV, we separated peptide isomers in N₂ with mean resolution of $\sim$70% that in He/N₂ mixtures. The upgraded FAIMS system was mounted on an Orbitrap XL instrument and is readily attachable to any mass spectrometer without influencing its operation. However, one desires the resolving power of Orbitrap MS with ETD while retaining full FAIMS resolution, lower DV, and flexible buffer choice unrestricted by the MS instrument.

Here we present a high-resolution FAIMS/Orbitrap MS platform that achieves these goals by largely decoupling the FAIMS carrier gas from the Orbitrap stage in two distinct ways. The performance is shown for our established standard of monomethylated complete histone tail variants.

**Experimental methods**

All tested configurations connect an ambient-pressure FAIMS device via an ion funnel interface to an LTQ Orbitrap XL (Thermo Fisher Scientific, San Jose, CA) with the ETD option (Figure 1). The MS instrument (not counting the ETD stage) has five turbo pumps: one on the linear ion trap (LTQ) region and four on ultra-high vacuum (UHV) regions including the Orbitrap cell. All are backed by two mechanical forevacuum (fore) pumps. The pressures are measured by ion gauge (in the LTQ region), Penning gauge (in the Orbitrap region), and Pirani and convection gauges for rough vacuum in the backing line and front MS chamber (Figure 1).

Ions generated by an ESI source using a chemically etched glass emitter of 20 μm internal diameter (i.d.) enter the FAIMS analytical gap via a curtain plate/orifice inlet on the side of one electrode. The He/N₂ or H₂/N₂ mixture formulated by digital flowmeters (MKS Instruments) from UHP grade gases is passed through a universal filter and supplied to that inlet at defined volume flow rate ($Q$). The larger part (~70%) desolvates entering ions, and the rest pulls them through the gap ($g = 1.88$ mm). The residence time ($t$) scales as $1/Q$, here $t \sim 140 - 70$ ms at relevant $Q = 2 - 4$ L/min. The optimum bisinusoidal waveform has 2:1 harmonics ratio, a frequency of 1 MHz, and DV = 4 kV wherein the electrical breakdown caps the He fraction to 65%. The CV is scanned at 0.1 - 0.3 V/min to record FAIMS spectra or fixed for ETD.
and waveform are loaded on the inlet and opposite electrodes respectively, with both biased to stay above the MS inlet voltage at any used CV.\textsuperscript{24}

The FAIMS device sits in a plastic bracket in front of the funnel chamber. The spacing between electrodes and MS inlet capillary should be minimized for best ion transmission, but exceed \( g \) to preclude arcing from the high-voltage rf electrode to the capillary at low dc voltage. With \( g = 1.88 \) mm, a reasonable distance is \( \sim 2.5 \) mm. That area is open, decoupling the FAIMS cell from the MS vacuum suction. As ions need no desolvation after FAIMS, the inlet heating devised for that in ESI/MS systems is reduced to minimize diffusional ion losses, in all systems to \( \sim 100 \) °C from the regular 200 - 300 °C without FAIMS.

We first assessed each arrangement based on high-vacuum pressures measured by cathode ionization gauges. Both are calibrated for \( N_2 \), but the gas identity matters and true pressures \( (P) \) of He or \( H_2 \) are far above\textsuperscript{51} the raw "\( N_2 \) equivalent" values \( P_{N_2} \). The correction factors \( S = P_{N_2}/P \) somewhat depend on the pressure and gauge construction, but the values of 0.18 for He and 0.48 for \( H_2 \) are typical.\textsuperscript{52} The pressure for a mixture with known component fractions \( (f) \) is:\textsuperscript{51}

\[
P = P_{N_2} \sum_{i=1}^{n} f_i S_i \tag{1}
\]

We used eq (1) to project true pressures in the MS manifold. The rough vacuum pressures measured by thermal conductivity gauges depend on the gas nature less, and for He specifically \( S = 1 \) within the error margin.\textsuperscript{52} Therefore, we accept \( P = P_{N_2} \) for those quantities.

The actual analyses involved H3.1 tails (ARTK\textsuperscript{Q}QTARK\textsuperscript{STG} KAPRKQLATK\textsuperscript{23}AARKSAPATGGVKKPHRYRPITVALRE, monoisotopic mass 5338.1 Da) with me on K4, K9, or K23 site.\textsuperscript{33,34} Mixtures of these isomers in 10+ charge state \((m/z = 536.22)\) yield characteristic FAIMS spectra in He/\( N_2 \) buffers, which we picked to benchmark the resolution of PTM localization variants and sensitivity of their identification by FAIMS/ETD.\textsuperscript{24,34} These peptides assembled by Fmoc solid-state synthesis and native chemical ligation were purified by liquid chromatography (LC).\textsuperscript{33} The variant mixtures were dissolved in 99.9:0.1 water/formic acid and infused to the ESI source at 0.5 \( \mu \)L/min.

The Orbitrap MS was operated in the magnitude mode with the transient duration controlled by set \( R_{\text{ion}} \) value.\textsuperscript{53}

### Instrument development and evaluation

#### Original system with single-funnel interface (SFI)

Ion funnels (electrode stacks with internal bore narrowing along the axis) transport and compress large ion plumes with virtually no losses.\textsuperscript{31} The Dehmelt pseudopotential of rf field repels ions from electrodes, while the axial dc field and MS vacuum suction drag them along to the exit.

In the in-line SFI adopted in our FAIMS/MS work to date (Figure S1),\textsuperscript{24,31} the MS inlet is on the funnel axis with the capillary replaced by a wide tube (to ameliorate ion losses to the walls). The 100-mm long funnel comprises 100 metallized 0.5 mm thick PCB electrodes, layered with insulating plates of same thickness.\textsuperscript{24} The 25 mm opening is wide enough to encompass ion beams expanding from MS inlets, while the 2 mm exit fits within the acceptance diameter of a standard quadrupole lens - here Q00 of LTQ. The funnel chamber is pumped through the instrument (Figure 1). Ions enter through a thin metal sheet with a slit of 11 holes spread over a 4-mm segment approximating the shape of beams emerging from the FAIMS gap.\textsuperscript{23} The open area of this non-contiguous aperture equals that of standard LTQ capillary with 0.43 mm i.d. and gas conductance \( Q_{\text{ms}} \) of \( \sim 0.6 \) L/min.\textsuperscript{24} Substituting an aperture for the capillary raises \( Q_{\text{in}} \) to \( \sim 0.8 \) L/min, which matches or slightly exceeds the outflow from FAIMS gap at \( Q = 2 \) L/min. The resulting funnel pressure (for air or \( N_2 \)) is 1.7 Torr, at which the peak rf voltage of 50 V at 1 MHz frequency and dc gradient of \( \sim 15 \) V/cm efficiently convey ions over a broad \( m/z \) range.

As He is added, the \( P_{\text{ms}} \) in funnel, LTQ, and Orbitrap regions rise - to respectively 2.7, 2.1 \( \times 10^{-5} \), and 4.9 \( \times 10^{-10} \) Torr at 100% He (Figure 2a). The increase of 7\( \times \) in Orbitrap versus \( \sim 30\% \) in LTQ regions illustrates the outsized consequence of He for UHV. Given the inefficiency of turbos for He, the pressure must increase in LTQ much more than in the funnel, i.e., by \( \gg 60\% \). That happens once we apply eq (1), with \( P \) rising by 7\( \times \) (to 1.1 \( \times 10^{-4} \) Torr) in LTQ and yet greater \( \sim 40\times \) (to 2.7 \( \times 10^{-9} \) Torr) in the Orbitrap region (Figure 2b). The gas flow to the LTQ region (and hence to the Orbitrap analyzer) may contain less He than the FAIMS buffer because of ambient air sucked into the open MS inlet.
However, both enclosures are continually enriched in He by preferential removal of N$_2$ molecules. Thus, the real pressure in either may be below or (except at 100% He) above the calculations in Figure 2b, but they convey the magnitude of elevation. Nonetheless, we obtained quality FAIMS and FAIMS/ETD data in this regime. The m/z and widths of MS peaks do not materially differ from those with N$_2$ buffer at equal R$_{MS}$ = 30,000 (Figure 3a). In the FAIMS spectrum for K4me/K23me with K9me trace, we assigned features A, B, B1 to K4me; C, C1 to K23me, and D to K9me based on redundant data for individual variants and mixtures under identical conditions with LTQ read-out. All species are perfectly separated at major conformers B, C, D.

Figure 3. Data for K4me/K23me/K9(trace) with SFI: (a) MS spectral window for z = 10; (b) FAIMS spectrum over shown m/z range. The minor feature D is selected for ETD and is critical for practical proteomics. In particular, existing middle-down histone analysis workflows discover most abundant isoforms, but others may have major epigenetic roles.

The data for K9me versus K4me or K23me (Figure 3b) for the first time demonstrate the ability to detect <1% variant concentrations. Such dynamic range is critical for practical proteomics. In particular, existing middle-down histone analysis workflows discover most abundant isoforms, but others may have major epigenetic roles.

The ion counts in A, B, and C suffice for confident ETD identification (Figure 4). The dominant informative fragments remain complementary c$_{54}^{4+}$ and z$_{31}^{5+}$ with s/n = 40 - 70 upon 10 min averaging (at R$_{MS}$ = 15,000 - 30,000). The parallel spectra with N$_2$ buffers evidenced contamination by K23me at B and K4me at C in ~3% amounts, but none is seen here. As present peak heights for major isoforms are similar at R$_{MS}$ = 30,000 and higher at R$_{MS}$ = 15,000, this verifies the superior variant resolution in He/N$_2$ media. We again encounter the z$_{35}^{5+}$ product of K23me, with m/z peaks differing from the adjacent for z$_{31}^{5+}$ of K4me by 0.07 Th at most. That is enough for baseline resolution with present R$_{MS}$ = 30,000 (Figure 4), but indistinguishable using the LTQ. The interferences may cluster even closer, e.g., some isotopic peaks of z$_{43}^{7+}$ and z$_{31}^{5+}$ split by m/z = 0.01 Th. Resolving those requires R$_{MS}$ ~ 100,000, achievable for the present precursor signal with slightly lower s/n ~ 30 (Figure 5).

The absence of fragments for isomeric impurities at peak apexes prevents leveraging them to evaluate sensitivity as with N$_2$ buffer. However, the presently similar signal and s/n for fragments indicate close inter-isoform detection limits of ~500 fmol with a priori assignment. In the accurate mass and time (AMT) tag approach, proteolytic peptides are identified by the LC elution profile in LC/MS (rather than MS/MS) for higher sensitivity. Within that paradigm, one may assign variants using the catalogued E$_c$ values. Then the LoDs are just ~2 fmol, judging by feature D (Figure 3b). The much greater edge of AMT in this context derives from lower informative fragment yields in ETD (~0.5 %) compared to collisional dissociation in bottom-up proteomics.

Some ubiquitin conformers selected by FAIMS exhibit distinct ETD fragment patterns, likely controlled by backbone accessibility in different folds. This can mean varying informative fragments across conformers and/or variants. That is not noted here, possibly as peptides cannot conceal sites internally as well as larger proteins and/or conformational differences are smaller here because of more extensive unfolding upon stronger field heating of ions by presently greater field in He/N$_2$ buffers. In general, one should not assume same informative fragments to be most pronounced for all FAIMS peaks.

While these data prove high-resolution FAIMS/FTMS analyses employing up to (at least) 65% He feasible in the arrangement validated with N$_2$ buffer, the pressure rise is a hardware reliability risk. In some newer Orbitrap models (e.g., Q-Exactive and Orbitrap Fusion), the FTMS region is less isolated from the MS inlet and thus would experience
faster pressure rise upon He addition, perhaps preventing operation at the same fractions. We did not attempt H$_2$/N$_2$ buffers with this system for instrument safety reasons, but the results with the upgraded interface below suggest a failure already at single-digit H$_2$ percentages. To facilitate use of He/N$_2$ buffers and try H$_2$/N$_2$ important for intact protein analyses, we have switched to a tandem-funnel interface.

**Differentially pumped tandem-funnel interface (TFI)**

In TFI, the funnel terminating in the first MS vacuum region (as in SFI) is preceded by a separately pumped funnel facing the MS inlet. The pressure there can be raised up to ~25 Torr (versus 1 - 4 Torr in SFI) while maintaining the requisite MS vacuum. This permits opening the inlet in proportion (usually by ~5x), normally to improve sensitivity by better capturing wide ion plums that arrive from ESI or other atmospheric-pressure ion source. That is moot after FAIMS, where ions hit the inlet over a small area that can be covered by a slit as described. The purpose of the wider inlet here differs - diluting the FAIMS “elucent” by ambient air to cut the He or H$_2$ fractions in gas flow to the instrument. With present $Q_{IN} = 4$ L/min, those should drop by ~5 - 2.5x with $Q = 2$ - 4 L/min (e.g., to ~13 - 26% from 65% and to ~20 - 40% from 100%).

With SFI or TFI, the MS inlet can be perpendicular to the tunnel axis. This geometry (similar to the Z-spray concept) adopted here is anticipated to suppress chemical noise by directing neutrals and poorly desolvated ions away from the funnel exit.

In our implementation (Figures 6, S2), both funnels are built from 0.5 mm-thick PCB electrodes with inside edges metallized to 1.5 mm margins and 0.45-mm thick insulating plates. The front funnel with 20-mm long straight and 105-mm long conical sections has the 52 mm opening and 4.5 mm exit. The heated cone from LTQ is moved to the funnel side, with the original capillary (0.58 mm i.d.) replaced by a wider (1 mm i.d.) 108-mm long steel capillary ending at the 7 x 7 mm window in the straight section. Ions emerging therefrom are deflected 90° by the dc-only repeller electrode. The gas jet shoots across through the holes in opposite wall (made by cutting out 45-mm long plate sectors) toward the intake of external forepump (Edwards 30). The pressure inside is measured by second Pirani gauge.

The back funnel of the “keyhole” geometry lies 11 mm behind the front funnel with a lateral displacement of 3 mm to exclude direct gas jet penetration from the front funnel to the LTQ chamber. Ions traverse a 10-mm long straight section with a 20 mm opening, a 30-mm long conical section with a 4 mm exit, a 10-mm diverging section, a 15 mm-long straight section of 20 mm diameter, and 30-mm long final conical section with a 3.4 mm exit.

The rf waveform in both funnels has 110 V peak voltage at 0.8 MHz frequency. The dc voltages are deployed at two and four points in the front and back funnels respectively, here adjusted to the gradient of 6 V/cm along both. The minimum pressures in the front and back funnels (with N$_2$ or air) are 6 and 0.5 Torr; higher values are reachable by choking the external pump. Here we set a pressure of 13 Torr in the front funnel. Then the pressure in the back is 0.7 Torr - about 40% that in SFI. However, the area of exit aperture is ~3x that in SFI (9 vs 3 mm$^2$), and the pressures in LTQ and Orbitrap regions are equivalent to those with SFI: $1.7 \times 10^{-3}$ and $7 \times 10^{-11}$ Torr.

Adding He lifts all measured pressures more gradually than with SFI, as intended. With $Q = 2$ L/min, the raw $P_{IN}$ in the back funnel or LTQ region inch up by ~5 - 10% at 65 - 100% He. The reading in the Orbitrap region goes up more as with SFI, but still by just ~50 - 100% to 1.1 - 1.4 $\times 10^{-10}$ Torr. These observations support the above picture of He dilution at MS inlet: use of 13 - 20% He with SFI increased $P_{IN}$ by ~5 - 10% in the funnel or LTQ and ~70 - 120% in the Orbitrap region (Figure 2a). The pressures per eq (1) are only a bit higher, 1.9 - 2.1 $\times 10^{-5}$ Torr in LTQ and 1.2 - 1.7 $\times 10^{-10}$ Torr in the Orbitrap. The dramatic difference of these from those for SFI (Figure 2b) reflect both lower raw pressures and lower He fractions in TFI.

This performance enabled us to investigate greater He/N$_2$ flows and H$_2$/N$_2$ buffer. With $Q = 4$ L/min (the realistic limit for present FAIMS device), the $P_{IN}$ in Orbitrap region increased to $1.8 - 2.1 \times 10^{-10}$ Torr at 65 - 100% He. This compares to $1.7 - 2.4 \times 10^{-10}$ Torr with SFI at 26 - 40% He (Figure 2a), confirming our proposed dilution...
mechanism. The corresponding true pressures are 2.2 - 2.9 × 10⁻¹⁰ Torr.

The mass spectra for K4me/K9me/K23me mix obtained at \( Q = 2 \) L/min and 65% He (Figure 7a, b) exhibit the requested \( R_{\text{MS}} \) of 30,000 or 100,000, correct \( m/z \), and signal similar to that with SFI. This shows proper Orbitrap MS operation with TFI, expected from the results at higher pressures with SFI. The FAIMS spectra (Figure 7c) follow that in Figure 3b, save for a much larger peak \( D \) resulting from greater amount of K9me in the sample. Slightly varying peak height ratios at two \( R_{\text{MS}} \) (Figure 7c) are common for acquisitions on different days. The peaks seem

**Figure 8.** Schematic representation of the FAIMS/LTQ XL/Orbitrap XL/ETD platform with single ion funnel interface in front. The vacuum pumping reconfiguration is depicted in red lines.

marginally wider than with SFI (here mean \( w = 1.4 \) V/cm for \( B, C, D \), possibly as stronger suction from TFI inlet diminishes \( t \), but are substantially narrower than with \( N_2 \) buffer at similar \( Q \) and signal.\(^{34}\)

In sum, the increase of true pressure in Orbitrap region is within 2.5\( \times \) at our default \( Q = 2 \) L/min and 4\( \times \) at maximum \( Q = 4 \) L/min with near He, and lower 2\( \times \) at 65% He. This should not cause problems even in the long term. However, \( H_2/N_2 \) buffers remain prohibited: \( P_{\text{Nit}} \) in Orbitrap rocketed by \( \sim 25\times \) (to \( 1.7 \times 10^{-9} \) Torr) already at 20% \( H_2 \). To fix that, we have taken an alternative direction of reconfiguring the vacuum pumping.

**Vacuum pumping reconfiguration**

The residual pressure of any gas achievable by a turbo is the compression ratio \( (c) \) times the pressure in backing line \( (P_{\text{back}}) \). While a low \( c \) for He and (more so) \( H_2 \) is inevitable, \( P_{\text{back}} \) depends on the vacuum engineering. Essentially all gas in the present backing line comes from the LTQ at lower vacuum, resulting in a substantial \( P_{\text{back}} = 1.5 - 2.6 \) Torr with SFI at 0 - 100% He (Figure S3). The \( P_{\text{back}} \) with TFI is lower at 0.7 - 0.9 Torr primarily because of lower pressure in the LTQ, which helps reducing the Orbitrap region pressure.

One can drastically decrease \( P_{\text{back}} \) for the turbos on UHV regions by backing them separately from that on the LTQ. We kept the original backing for the turbo on the LTQ, but split off the joined line from other turbos to an external forepump (Edwards 30), Figure 8. The Pirani gauge stays on that line (the pressure in hose from LTQ pump is unmonitored). While this is feasible with any front MS interface, here we used SFI as only one suitable external pump was available.

In this arrangement, the pressures (with \( N_2 \) buffer) are 1.7 Torr in the funnel, \( 1.6 \times 10^{-5} \) Torr in LTQ, and \( 5 \times 10^{-11} \) Torr in Orbitrap region (Figure 9a). While the first two values equal those prior to reconfiguration, the reduction of the last reflects \( P_{\text{back}} \) plummeting to 0.01 Torr. The \( P_{\text{back}} \) in the Orbitrap region do not move up even at 100% He (with \( Q = 2 \) L/min), although \( P \) presumably does. The pressures in the funnel and LTQ edge up to 2.1 Torr and 1.8 \( \times 10^{-5} \) Torr at 100% He (Figure 9a). Both increase less than prior to reconfiguration because of more efficient pumping of the funnel and backing of LTQ turbo by dedicated forepumps.

Encouraged by this success, we retried \( H_2/N_2 \) buffers (Figure 9b). With \( Q = 2 \) L/min, we now get to 60% \( H_2 \) before \( P_{\text{Nit}} \) in LTQ becomes a concern at \( \sim 6 \times 10^{-5} \) Torr. At that point, the values in the funnel and Orbitrap are fair (2.8 Torr and \( 3 \times 10^{-10} \) Torr, respectively): the \( H_2 \) fraction is now limited by the pressure in LTQ. The true pressures in the LTQ and Orbitrap regions are again higher, though by less than with He because of greater \( S \) for \( H_2: \sim 8 \times 10^{-5} \) and 4\( \times \) \( 10^{-10} \) Torr, respectively. This result is promising, but the ultimate resolution of protein conformers necessitates\(^{35}\) \( H_2 \) fractions up to 84%.

As raising \( Q \) increases the load of light gas and thus the pressures in all stages, we can decrease \( Q \) to reverse the effect. The optimum \( Q \) in FAIMS drops for larger ions that diffuse slower and thus can stay in the gap longer at equal sensitivity. For macromolecules for which \( H_2/N_2 \) buffers are most pertinent, \( Q < 2 \) L/min is appropriate. At \( Q = 1.4 \) L/min, we can go to 100% \( H_2 \) with \( P_{\text{Nit}} \) of \( < 6 \times 10^{-5} \) Torr in LTQ and \( 3 \times 10^{-10} \) Torr in the Orbitrap region (Figure 9c). At any \( H_2 \) fraction with either \( Q \), we still see \( P_{\text{back}} = 0.01 \) Torr. This
again testifies to superb UHV backing, with the pressure limitation moved to LTQ stage.

The instrument normally also receives gas (N$_2$) through the higher-energy collision dissociation (HCD) cell disposed downstream of the Orbitrap region. The MS1 and ETD MS/MS modes employed here do not involve HCD, so the gas supply to HCD can be turned off to reduce high-vacuum pressures (the pressure in funnel is naturally not affected). We see constant $P_{\text{Ne}}$ shift down with any He/N$_2$ or H$_2$/N$_2$ buffer: 7 - 8 x 10^{-6}$ Torr in LTQ and 2 - 3 x 10^{-6}$ Torr in the Orbitrap region (Figure 9 a - c), while $P_{\text{back}}$ falls below the measurable range starting at 0.01 Torr. This makes less of a difference at elevated pressures with high H$_2$ fractions, but can help in borderline situations.

Conclusions and Future Directions

We have integrated high-resolution FAIMS employing any buffer with Orbitrap MS while overcoming steep pressure rise in UHV regions upon substantial inflows of He or H$_2$ not evacuated well by turbo pumps. This was attained by (i) inserting a differentially pumped tandem-funnel interface (TFI) after the FAIMS stage to dilute its eluent by ambient air or (ii) rearranging the vacuum system to provide turbo pumps on UHV regions with dedicated backing apart from that for pumps on LTQ stage. The 1$^{\text{st}}$ modification allows all He/N$_2$ buffers at practical flow rates with only a modest pressure increase in any stage, but not H$_2$/N$_2$ mixtures with significant H$_2$ fraction. The 2$^{\text{nd}}$ modification is more efficient, handling He/N$_2$ buffers with negligible pressure increase and H$_2$/N$_2$ with up to 60\% H$_2$ at the “normal” flow rate or 100\% H$_2$ at reduced rate. In analyses not exploiting the HCD cell, the gas supply to HCD can be switched off as the last step to minimize the pressures.

The performance is demonstrated in analyses of benchmark histone tails. The resolution of variants with methylation on different residues matches that obtained using same He/N$_2$ buffers with low-resolution MS and exceeds that achieved in the FAIMS/Orbitrap MS employing N$_2$ buffer. The sensitivity is similar to that with N$_2$ but with isomeric LoD of several fmol if based on FAIMS separation and hundreds of fmol by ETD fragments. While the full $E_c$ spectra shown here took ~30 min to acquire, sufficient precursor ion signal at known peak $E_c$ in targeted analyses is measurable in a few seconds.

This work addresses the He/N$_2$ and H$_2$/N$_2$ mixtures that are common to FAIMS and most difficult because of the lowest molecular masses of the constituents. Hence, present developments ought to cover other buffers, including He/CO$_2$ preferred for isotopologue separations and He/SF$_6$ that yields extreme non-Blanc effects. One can now optimize FAIMS separations without regard for compatibility with ultra-high-resolution MS performance.

The persisting pressure increase with H$_2$/N$_2$ buffers can be mitigated by combining our two modifications. The outcome can be predicted by inspecting Figure 9 within the TFI ranges (that at $Q =$ 1.4 L/min is narrower because of greater dilution of slower FAIMS eluent). The Figure 9b indicates the possibility of using H$_2$/N$_2$ with up to 100\% H$_2$ at $Q =$ 2 L/min and higher, while staying within the allowed LTQ pressure range. A further reserve is fitting the TFI with a valve leaking N$_2$ into the front funnel while augmenting the pumping (using the spare capacity) to dilute the FAIMS eluent yet more. Those options may be warranted for Orbitrap models with UHV region more open to the MS inlet (e.g., the latest Fusion with resolving power of 500,000). Efforts along those directions are underway.

While this research has focused on a planar-gap FAIMS device, the buffers rich in He or H$_2$ also benefit resolution and/or sensitivity in FAIMS systems with curved or multichannel microscopic gaps. Also, linear (in particular, drift-tube) IMS strongly benefits from He buffer that allows most robust and accurate first-principles ion mobility calculations for structural elucidation purposes and (ii) accelerates separations while improving sensitivity and/or resolution. Present developments facilitate coupling of both linear and nonlinear IMS analyzers to Orbitrap MS.

CONFLICT OF INTEREST DISCLOSURE:

A.A.S. has an interest in Heartland MS that makes the ion funnel and FAIMS systems for mass spectrometers used in this work.

SUPPORTING INFORMATION AVAILABLE:

Photos of single and tandem ion funnel interfaces, the plot of backing line pressure with SFI.

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