Affecting FAIMS Separation with Trace Levels of Gas Modifiers

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ABSTRACT

Purpose: Increased separation was demonstrated with the introduction of chemical modifiers (dopants) in the FAIMS Gas.

Methods: Trace amounts of various liquid modifiers were evaporated and mixed with nitrogen and sent to the FAIMS electrodes for analysis.

Results: Adding chemical modifiers to FAIMS gas induces CV shifts that improve separation within cylindrical electrodes.

INTRODUCTION

High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) separates gas phase ions flowing in the gap between two cylindrical electrodes by applying an RF dispersion voltage (DV) to displace the ions within the gap based on their differential mobility.[1] Ions are selectively transmitted through the electrodes and into the inlet of a mass spectrometer by scanning a DC potential called compensation voltage (CV). The resolution of the FAIMS device can be affected by many instrumental parameters, including carrier gas composition, gas flow rate, and electrode temperature. Here, the effect of adding trace amounts of chemical modifiers to the FAIMS carrier gas is described with regards to improving the separation of singly charged ion species.

FAIMS has been shown to improve proteomic analysis by removing the chemical background that obfuscates low abundance precursor ions in biological samples.[2,3,4] This is accomplished by applying a series of CVs, ranging between -30V to -100V, that preferentially transmit multiply charged ions into the mass spectrometer before data dependent analysis. Since it is necessary to maximize the amount of time the mass spectrometer has to interrogate each CV fraction, FAIMS is operated in a low resolution, non-targeted mode, where a small number of CV settings transmit multiple peptidic precursors, while excluding the singly charged ions that are generally transmitted with CVs between 0V and -30V. However, targeted analysis of singly charged ions, such as small drugs and their metabolites, may require increased resolution in order to separate ions that are co-transmitted in regards to CV.

Figure 1. A Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled with a Thermo Scientific™ FAIMS Pro™ interface. The FAIMS Pro mounts on the mass spectrometer with a flange and can be installed with Thermo Scientific™ TNG sources.

Figure 2. Exploded view of the FAIMS electrode set. Electrodes are assembled without tools and do not require a break in the mass spectrometer's vacuum system to install.

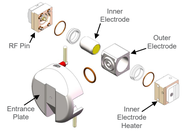
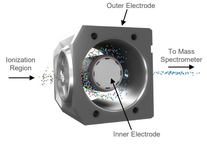


Figure 3. Simulation showing the flow of ions into the FAIMS electrode set. The CV and DV are applied to the Inner Electrode and ions flow around it during separation.



MATERIALS AND METHODS

Analysis was performed on a FAIMS Pro Interface mounted on a modified TSQ Altis Triple Quadrupole mass spectrometer. The gas line of the FAIMS interface was modified to introduce a dopant mixing chamber between the gas valve and the FAIMS electrodes. Liquid solvent was introduced into a flow of nitrogen gas within the mixing chamber using a syringe pump, resulting in molar ratios of modifier to nitrogen between zero and 1% supplied to the electrodes. Methanol, acetonitrile, and isopropanol were each characterized as gas modifiers. CV shifts were determined using Thermo Scientific™ Pierce™ Triple Quadrupole Calibration Solution, Alprazolam (Sigma), and a mixture of Testosterone and Myclobutanil (Sigma).

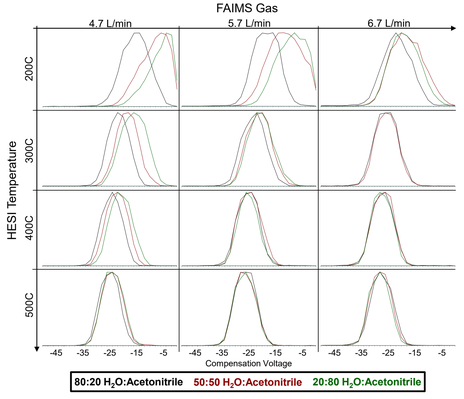
RESULTS

Since FAIMS analysis separates ions by differential mobility within gas phase ion populations, it is important to ensure that the incoming ion stream is sufficiently desolvated before introduction into the electrodes. This is easily accomplished in typical proteomic experiments with low sample flow rates (less than 25 µL/min). However, for analyses with higher liquid flow rates, heating the ionization source region is necessary to remove extra solvent. Additionally, the FAIMS Gas flow rate can be increased in these conditions. This gas flows countercurrent to the incoming ion stream and further aids in desolvation.

For the experiment summarized in **Figure 4**, the mobile phase was delivered at 500 µL/min and the ionization source was placed closer to the FAIMS inlet than usual to demonstrate the effect of poor desolvation in FAIMS analysis. Reserpine was introduced to the FAIMS electrodes by loop injections onto a short C-18 column at various isocratic mobile phase compositions. The optimal CV was determined for each injection by rapidly stepping the CV as the Reserpine was eluted off the column.

In conditions with poor desolvation (low HESI temperature and FAIMS Gas), the optimal CV for reserpine is shifted as the mobile phase composition is changed. This unstable CV position would require the CV to be optimized under the identical conditions as the compound of interest is eluted off the column. However, when the HESI temperature and FAIMS gas are increased, the optimal CV curves exhibit no shift at different mobile phase compositions.

Figure 4. CV scans for loop injections (Reserpine) at 500 µL/min using various HESI temperatures and FAIMS Gas flow rates. Under conditions with poor ion desolvation, top left, CV peak position varies as the organic composition of the mobile phase is varied. When desolvation is improved, bottom right, CV peak position is unaffected by mobile phase composition.



In addition to unintended CV shifts caused by source conditions, CV shifts can be induced in a controlled manner by introducing trace levels of a chemical modifier into the FAIMS gas that flows between the electrodes. Here, a heated mixing chamber, diagrammed in **Figure 5**, was designed so that a liquid modifier flow is sprayed through a nozzle into an incoming nitrogen flow of 5 L/min. The mixing chamber is U-shaped to increase the mixing path and heated to help drive the modifier into the gas phase. The liquid modifier is supplied to the mixing chamber via a syringe pump, with the flow rate calculated by modifier-to-nitrogen molar ratios, shown as a percentage for methanol, acetonitrile, and isopropanol in **Table 1**. Once mixed, the output gas flow from the chamber is directed into the FAIMS electrodes.

Figure 5. Gas Modifier mixing chamber introduces a liquid flow into a nitrogen flow to produce transport gas for FAIMS electrodes.

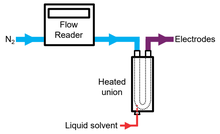
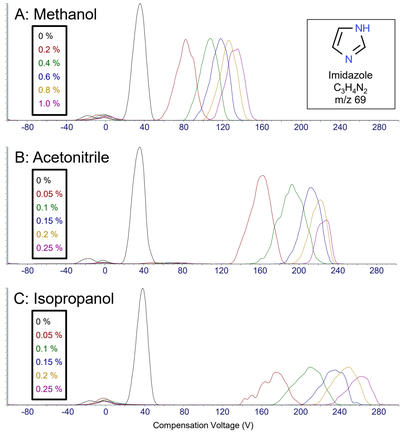


Table 1. Gas Modifier liquid flow rates for mixing with 5 L/min nitrogen for FAIMS Gas flow to electrodes.

Methanol		Acetonitrile		Isopropanol	
%	µL/min	%	µL/min	%	µL/min
0.2	17	0.05	5.5	0.05	8
0.4	34	0.1	11	0.1	16
0.6	50	0.15	16.5	0.15	24
0.8	67	0.2	22	0.2	32
1.0	84	0.25	27.5	0.25	40

Compounds with low molecular weight tend to be sensitive to source conditions such as probe position, ion desolvation, gas flow rate, and FAIMS Gas composition.[5] **Figure 6** shows the effect of adding trace concentrations of methanol, acetonitrile, and isopropanol, to the FAIMS Gas while infusing Imidazole (m/z 69). The optimal CV value required to transmit Imidazole is shifted by a greater magnitude as the molar mass of the chemical modifier is increased.

Figure 6. CV plots for Imidazole using trace amounts of Methanol (A), Acetonitrile (B), and Isopropanol (C). Note that the magnitude of the CV shift increases as the molar mass of the modifier is increased (Methanol < Acetonitrile < Isopropanol).



Although most compounds exhibit a shift in CV position with chemical modifiers, the magnitude of the CV shift is analyte dependent. This is potentially beneficial in instances where compounds do not separate by FAIMS under 100% nitrogen conditions. To illustrate this, a mixture of Testosterone and Myclobutanil was infused into the electrodes (**Figure 7**). These compounds are isobaric and their CV apexes overlap with 100% nitrogen and a DV amplitude of -3000V (top left). The two peak apexes begin to resolve as the DV amplitude is increased (left column), but significant overlap remains at full DV amplitude (-5000V). However, with the addition of 0.2% acetonitrile in the FAIMS Gas, the CV peaks are separated at each DV amplitude.

In addition to improved separation, **Figure 8** summarizes the effect on ion intensity for both Testosterone and Myclobutanil in the presence of 0.2% acetonitrile compared to 100% nitrogen. At low DV amplitudes (below -4000V), ion signal for both compounds is attenuated with 0.2% acetonitrile. However, at higher FAIMS field strengths (above -4000V), significant ion intensity gains are observed for both compounds.

Figure 7. FAIMS separation of Testosterone and Myclobutanil using 100% nitrogen (left column) and 0.2% acetonitrile (right column). Note that optimal CV values for these compounds overlap, regardless of DV applied, but are resolved with the addition of 0.2% acetonitrile.

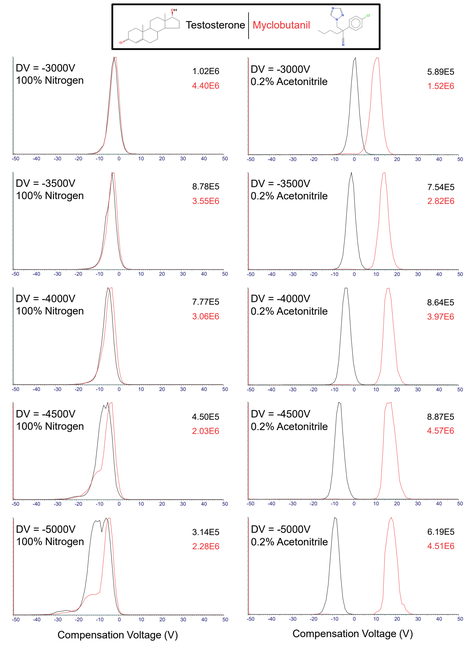
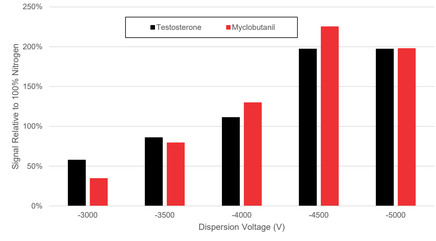


Figure 8. Signal intensities for Testosterone and Myclobutanil when analyzed with 0.2% acetonitrile versus 100% nitrogen at different DV amplitudes. Note that both compounds exhibit increased sensitivity and separation at DV amplitudes greater than -4000V.



CONCLUSIONS

- The desolvation of the ion stream introduced into the FAIMS electrodes is critical to stabilize CV position.
- Trace chemical modifiers can be introduced into the FAIMS gas by mixing finely sprayed liquid solvent into the nitrogen flow.
- The magnitude of the CV shift of an ion increases as the molar mass of the chemical modifier increases (methanol < acetonitrile < isopropanol)
- Ions with overlapping CV peaks in 100% nitrogen can often be separated by CV with the addition of trace amounts of chemical modifier in the FAIMS Gas.
- Ion signal can be maintained (and sometimes increased) with the addition of chemical modifier versus 100% nitrogen.

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TRADEMARKS/LICENSING

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