**Different Ion Mobility Spectrometry (FAIMS) with Resolving Power up to 300 and its Application to Lipid and Peptide Analyses**

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**Overview**

- Resolving power of planar FAIMS systems increases by ~10 times, reaching ~300 for multiply charged peptides.
- The gain of F provided by raising the dispersive voltages and He content in the field-free gas to the limits permitted by electrical breakdown, with operation at up to 75% He.
- He improved further by extending the separation times.
- Peak capacity for tryptic digests exceeds 100.
- For lipids, FAIMS provides much better separation and identification than conventional IMS. Many lipid classes occupy distinct domains in the FAIMS/MS space.
- Capability to classify lipids by the location in FAIMS/MS space may improve further by extending the separation times.
- Indeed, here the peak capacity of FAIMS/MS exceeds that of (conventional IMS)/MS at equal gas composition. Adding helium to the N2 stream improves resolution, but the He content was limited to 50%.

**Introduction**

- Ion mobility spectrometry (IMS) separates and characterizes ions separaring two gas phases through different conventional IMS systems. Resolving power of planar FAIMS systems (FAIMS) allows the difference of mobility high and low, C, elicited using a periodic asymmetric waveform (1).
- The KGD derivative is linearly correlated to the IMS mass that is raised to a mass that is not different than conventional IMS (2). Hence the peak capacity of FAIMS exceeds that of conventional IMS over a wide region of the IMS space. In the work here, the KGD waveform was applied to a planar 20 cm radius cylinder at 1.6 cm long orifice, and 100–200–200–100–200–100–200–100–200–100–200-μm-diameter tube IMS.
- With conventional IMS, FAIMS resolution is sensitive to gas composition. Adding helium to the He improves resolution but the gas normally improves resolution by 20%, whereas because of electrical breakdown concerns. FAIMS research is performed on the gas phase mixture, increasing for planar geometries where the electric field is homogeneous (3). The value of the electric field in FAIMS is ~10 V/μm, may be increased by improving F.
- Resolving power scales roughly as the cube of waveform amplitude (dispersive voltage). DV: the planar FAIMS ions were previously limited to 4 kV and stabilizing the operator at higher DV was challenging.

**Experimental Methods**

**Planar FAIMS analyzer**

- Planar FAIMS design [5], the gas is 1-mm long and 1.8-mm wide, ion beams are separated by a stream of He carrier gas and the gas is used in the conventional IMS (3). Gas flow of 2 L/min, the separation channel is 0.6 mm in diameter.
- The distance for separation improves by 2–3 times. The data below for T = 2.2 s.

**Results**

- **Resolving power up to 200 at 75% He**
  - With DV = 4.5 kV, operation is stable up to 75% He. Going from 0% to 50% He improves separation by 2–3 times. The data below for T = 2.2 s.
  - **Small molecules**
    - Resolution for fast-fragment (1) and isodicenic (2) (example traditional resolution test for conventional IMS of FAIMS). L and C separated in DT IMS at 160%, 140% 110%, 80%, and 50% He.
    - In FAIMS, separation emerges at ~60% He, resolution increases from 1 to 40% He to ~2x at 75% He.

- **Resolving power >200 at DV = 5.4 kV**
  - With DV = 5.4 kV, operation is stable up to 50% He. Below the 50% He, the resolving power of the same IMS instrument increases by ~2.8x, as the He content decreases.

- **Leucine enkephalin**
  - Separation is similar to that of the lower DV, with the He at 50% was ~50% He, and at 73% He.
  - The resulting resolution at 50% He is twice ~2x as the DV = 4 kV and 75% He.

- **Peptides**
  - By calibrating, ions are separated at DV = 5.4 kV and 50% He, then at 74% He, and 75% He.
  - Indeed, here the resolution of peptides further than 75% He.
  - The resulting resolution is close to or exceeds the 0% DV 73% He and 75% He, with 4-fold for peptides reaching ~22.

**Global classification**

- Lipid classes are separated in FAIMS, as expected from its superior orthogonal to MS.
  - Overall, ~11.4%, or ~5 times that with conventional IMS.
  - Capability to classify lipids by the location in FAIMS/MS space may be useful for metabolomics and proteomics.

- **Conclusions**
  - Suppression of electrical breakdown in high-frequency of fields in FAIMS improves separations for He content than those possible in dc fields. In particular, stable operation was achieved using He content as high as 75%.
  - Raising the He content from 4 kV to 50% 75% He increases the FAIMS resolving power (R) and peak capacity (c) by ~2–3 times, providing up to ~180 for peptides and up to ~100 for 50% He for proteolytic digests. The resolution of amino acid isomers improves over 5K.
  - Instead of increasing the He content, one can increase DV of some He fraction, e.g., for 5.4 kV at 50% He. The resulting resolution is close to or exceeds the 0% DV 73% He and 75% He, with 4-fold for peptides reaching ~22.

**Targeted separation of isomers and isobars**

- Many lipid classes show dipole (DAI) and tripoles (TABI) isobars, are sensitive to the dual effects of FAIMS and IMS. The resolving power of FAIMS is ~220.

**Lipid analyses**

- Lipids were studied by conventional IMS (MS). The trends for different ion mobility analyzers with greater capability for He content, but close to and the domain largely overlie [11].

**References**