Differential 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 increased by ~10 times, reaching *R* ~300 for multiply charged peptides**
- **The gain of *R* provided by raising the dispersion voltage and He content in the He/N_2 gas to the limits permitted by electrical breakdown, with operation at up to 75% He**
- ***R* improved further by extending the separation to ~0.7 s**
- **Peak capacity for tryptic digests exceeds 100**
- **Better resolution allows separation of previously unresolved species, e.g., peptide conformers, positional variants of lipids, and small molecule isomers such as leucine/isoleucine**
- **Many lipid classes separated in the FAIMS/MS space, enabling a new approach to metabolomic analyses**

Introduction

- Ion mobility spectrometry (IMS) separates and characterizes ions exploiting their gas-phase transport. Conventional (drift-tube, DT, or traveling wave, TW) IMS is based on absolute ion mobilities (*K*) measured at low field *E*. Differential IMS or Field Asymmetric waveform IMS (FAIMS) utilizes the difference of mobility at high and low *E*, elicited using a periodic asymmetric waveform [1].
- The *K*(*E*) derivative is less correlated to the ion mass than *K*, making MS more orthogonal to FAIMS than to conventional IMS [1, 2]. Hence the peak capacity of FAIMS/MS exceeds that of (conventional IMS)/MS at equal *R* in the IMS dimension. This advantage of FAIMS was blunted by limited *R*, commonly ~10 - 20 vs. 100 - 200 for drift-tube IMS.
- Unlike with conventional IMS, FAIMS resolution is sensitive to gas composition. Adding helium to the N_2 gas normally improves resolution, but the He content was limited to 50% because of electrical breakdown concerns.
- FAIMS resolving power depends on the gap shape, maximizing for planar geometries where the electric field is homogeneous [1]. The value of *R* scales as (separation time $t)^{1/2}$, may be improved by increasing *t* .
- Resolving power scales roughly as the cube of waveform amplitude (dispersion voltage, DV). The DV for planar FAIMS units was previously limited to 4 kV and stabilizing the operation at higher DV was a challenge.

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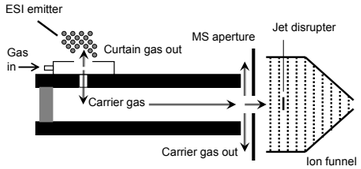
Experimental Methods

Planar FAIMS analyzer

Planar FAIMS design [3], the gap is ~50 mm long and 1.88 mm wide. Ions generated by a single-emitter ESI source, desolvated, and injected in the gap via curtain/orifice interface. At the "standard" gas flow of 2 L/min, the separation takes 0.2 s.



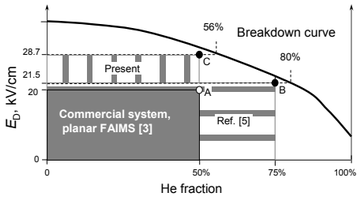
FAIMS unit coupled to Thermo LTQ ion trap MS fitted with ion funnel interface [4, 5].

Tracking the breakdown curve

Helium has about the lowest breakdown voltage of any gas, and the value for He/N_2 mixtures drops with increasing He fraction.

Breakdown thresholds had been estimated for dc voltages, but high-frequency rf allows greater *E*, or, equivalently, higher He content at same *E*. For example, at DV = 4 kV, the maximum He fraction is ~65% for dc, but ~80% for rf at 750 kHz in FAIMS.

Both higher *E* and greater He % tend to improve resolution, but the breakdown constraint requires balancing the two. The optimum depends on the specific ions. To explore the performance at higher DV and lower He %, we developed a generator providing DV = 5.4 kV with stable output.



Controlling FAIMS separation time

FAIMS separation time is inversely proportional to the gas flow. Reducing the flow from 2 to ~0.6 L/min extends the separation to ~0.6 s.

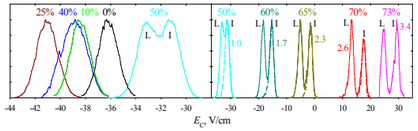
Resolving power up to 200 at 75% He

With DV = 4 kV, operation in He/N_2 is stable up to 75% He. Going from 50% to 75% He improves separation by 2 - 3 times. The data below for t = 0.2 s.

Small molecules

Separation of leucine (L) and isoleucine (I): exemplary resolution test for conventional IMS or FAIMS. L and I cations separated in DT IMS at maximum *R* ~ 150 [6], but not in TW IMS [7].

In FAIMS, separation emerges at ~50% He, resolution increases from 1 at 50% He to >3 at 73% He.

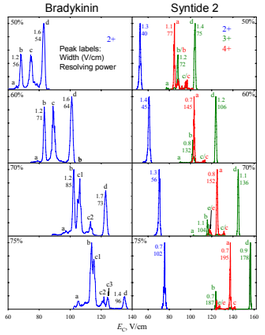


Peptides

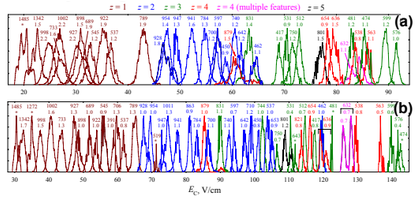
Bradykinin (1060 Da) - common model peptide, makes 2+ ions in ESI. Major unfolding with increasing He content, ~6 conformers resolved.

Syntide 2 (1508 Da) produces 2+, 3+, and 4+ charge states, unfolding insignificant.

R increases at higher He content, up to ~180 at 75% He.



Proteolytic digests



Mass-selected FAIMS spectra of BSA digest. Peak capacity for digests proportional to *R*, increases from ~50 at 50% He **(a)** to >100 at 75% He **(b)** to become competitive with HPLC performance; *m*/*z* marked for each feature with peak width underneath.

Results

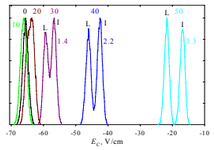
Resolving power >200 at DV = 5.4 kV

With DV = 5.4 kV, operation is stable up to 50% He. Below the performance at {5.4 kV; 50% He} is compared with that at {4 kV; 75% He}.

Leucine/Isoleucine

Separation similar to that at the lower DV, with the He % axis shifted by ~25%.

The resolution at 50% He is same ~3.3 as that at DV = 4 kV and 73% He.



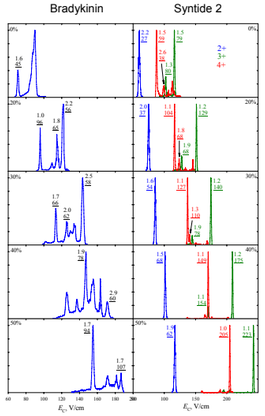
Peptides

By calculations, ions are hotter at DV = 5.4 kV and 50% He than at 4 kV and 75% He.

Indeed, here bradykinin unfolds further than at 75% He.

Syntide shows no systematic unfolding, and *R* reaches ~220.

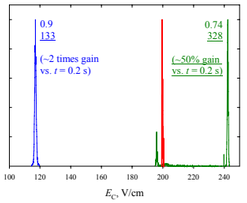
Similar results obtained for Angiotensin I.



Resolving power >300 with extended separation times

Theoretically, increasing *t* from 0.2 to 0.6 s should raise *R* by ~70% [8]. This gain was found in experiment.

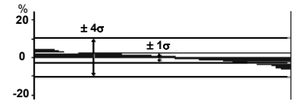
Spectra for Syntide 2 (**2+**, **3+**, **4+**); DV = 5.4 kV, 50% He, Flow 0.8 L/s



Lipid analyses

Lipids were studied by conventional IMS [9]. The trend lines for different classes in the IMS/MS space slightly differ, but are close and the domains largely overlap [10].

Deviation of lipid mobilities from the mean trend, σ = 2.64% [10]

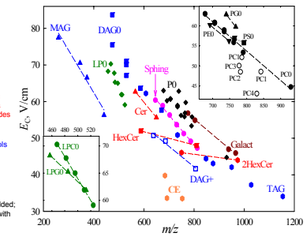


To compare, σ = 7.33% for (1+) peptides

No previous FAIMS analyses of lipids were reported.

Global classification

Lipid classes are separated much better in FAIMS, as expected from its superior orthogonality to MS.



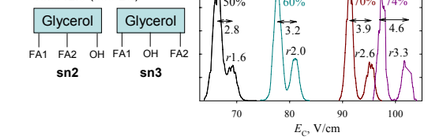
Overall, σ = 11.4% or ~5 times that with conventional IMS

Capability to classify lipids by the location in FAIMS/MS space may be useful for metabolomics and lipidomics.

Targeted separation of isomers and isobars

Many lipid classes, e.g., diacylglycerols (DAG) and triacylglycerols (TAG), comprise numerous isomers that differ in the position of double bond or fatty acid attachment. These are difficult to distinguish by LC or MS/MS.

FAIMS separates regioisomers of DAG 16:0/12:0 (530 Da)



sn2 on the left, sn3 - on the right

Resolution improves with increasing He content; even better separation at DV = 5.4 kV and 50% He.

Conclusions

- **Suppression of electrical breakdown in high-frequency rf fields in FAIMS allows greater DV or He content than those possible in dc fields. In particular, stable operation was achieved using He/N_2 with up to 75% He.**
- **Raising the He content at DV = 4 kV from 50% to 75% increases the FAIMS resolving power (*R*) and peak capacity (pc) by 2 - 3 times, providing *R* up to ~180 for peptide ions and pc > 100 for proteolytic digests. The resolution of amino acid isomers improves over 3-fold.**
- **Instead of increasing the He content, one can increase DV at some He fraction, e.g., to ~5.4 kV at 50% He. The resulting resolution is close to or exceeds that at DV = 4 kV and 75% He, with *R* for peptides reaching ~220.**
- **Extending the FAIMS separation time from 0.2 to 0.6 s increases *R* by another ~2/3, to >300 for some peptides.**
- **For lipids, FAIMS provides much better differentiation than conventional IMS. Many lipid classes occupy distinct domains in the FAIMS/MS space, which may be useful for global metabolomic analyses.**
- **High-resolution FAIMS can separate lipid regioisomers that are hard to distinguish by HPLC or MS/MS.**

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