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Mass Spectrometry & Spectroscopy

High-Field Asymmetric Waveform Ion Mobility Separation and its Application to Small Molecule Analysis

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High field asymmetric-waveform ion mobility spectrometry (FAIMS), also commonly referred to as differential mobility spectrometry (DMS), is an atmospheric pressure gas phase separation technique which exploits the difference between the mobility of an ion under high and low electric field conditions, as they pass between two electrodes. FAIMS can either be employed as a standalone mobility device or used as an orthogonal pre-separation technique hyphenated with chromatography and/or mass spectrometry. This report will discuss the development of modern FAIMS instrumentation and its application to the analysis of small molecules and peptides.

FAIMS Theory

The first report describing FAIMS (then called field ion spectrometry) in English was published by Buryakov and co-workers in 1993 [1]. The technique was then developed further in the late 1990s by the Guevremont group, who published many papers on the subject [2]. FAIMS has recently been the subject of a textbook published by CRC press in 2008 [3].

FAIMS separation occurs on the basis of the differences between the mobility of ions in alternating high and low electric fields. Ions travelling between two electrodes in a carrier gas stream at atmospheric pressure *(Figure 1)* are subjected to an asymmetric waveform which alternates between high field and low field conditions.

This asymmetric RF waveform is known as the dispersion voltage (DV). *Figure 1* shows the ion path through the device when the lower of the two plates is earthed whilst the DV is applied to the upper plate. In the high field component of the DV, positive ions will be repelled away from the upper plate, and when the waveform switches into the low field component they will be attracted towards it.

The voltage in the high field component is higher than that of the low field component, but the time spent in the low field component is longer (2:1 ratio low field: high field) to compensate. If the high field and low field mobility are the same, the waveform should focus the ions into the centre of the electrodes.

However, in practice this does not usually occur as mobility changes under high field conditions, and ions will accumulate a net drift towards one of the electrodes, which will result in the ions colliding with the electrode and being neutralised. Ions of differing mass, charge and conformation will behave differently in the same waveform.

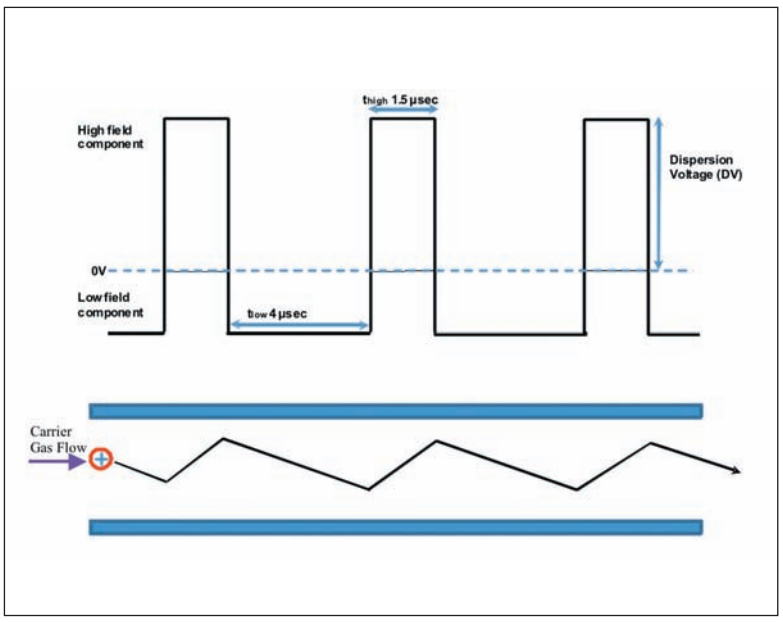

Figure 1.Graphical depiction of the asymmetric waveform applied to a FAIMS device, and the path of ion motion through a planar FAIMS device.

The mobility of an ion in a high-field electric waveform (Kh) can be described using this equation:

$$Kh(E) = K0(1+ \alpha (E))$$

where K0 is the reduced mobility at low electric field, E is the high-field component of the electric waveform and alpha (α) is a coefficient for the dependence of mobility of an ion on the electric field strength. This difference in mobility is determined by the α value, which describes which direction the overall net drift will be at any given dispersion voltage.

In order for the ions to pass through the device a voltage is applied to one of the electrodes to reverse the drift of the ions towards the electrodes [1]. This is termed the compensation voltage (CV) [2]. The CV for ion transmission is compound specific and dependent on the α value of an ion resulting from the previously described mobility differences in high and low fields. By scanning the FAIMS device across a range of CV voltages, a differential mobility spectrum can be obtained, with species separated on the basis of the CV required to enable transmission. *Figure 2* shows an example CV scan obtained using a mixture of vancomycin, reserpine and the test peptide MRFA, each of the three compounds can be partially resolved on the basis of differential mobility. The device can also be used as a filter by using a constant CV value, to transmit only ions which traverse the device at a specific CV value, whilst excluding all other species.

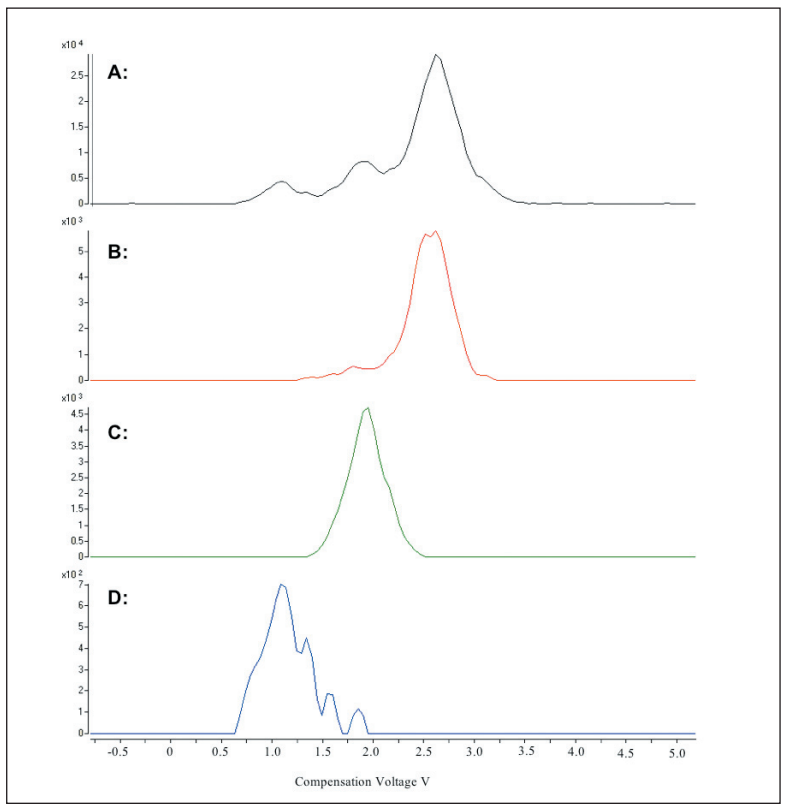

Figure 2. UltraFAIMS CV Scan (-1 to +6V) of test mixture: A = TIC, B = MRFA [M+H]+, C = Reserpine [M+H]+ D = Vancomycin [M+H]+.

FAIMS electrode designs

There are several different electrode designs used in FAIMS and these have been discussed in more detail elsewhere [4], in brief, they can all be broadly categorised into two groups, those that have cylindrical (coaxial) electrodes or those with planar electrode geometry. Planar FAIMS have greater resolving power than cylindrical systems at the cost of reduced transmission. Residence time in a planar FAIMS (<10s to scan full CV range) is much shorter compared to that in a cylindrical system (~1 min to scan full CV range). The shorter scan makes it much easier to interface planar systems into LC-MS experiments while still maintaining adequate chromatographic resolution. Ultra-FAIMS is a recent development in planar FAIMS, this technology uses a microchip with multiple channels for ion transmission. In this miniaturised system the gap between the electrodes is an order of magnitude smaller than that in previous planar configurations enabling higher electric field intensities to be achieved [5]. The ultra-FAIMS chip, showing the scale of the device against a human finger, is shown in *Figure 3*.

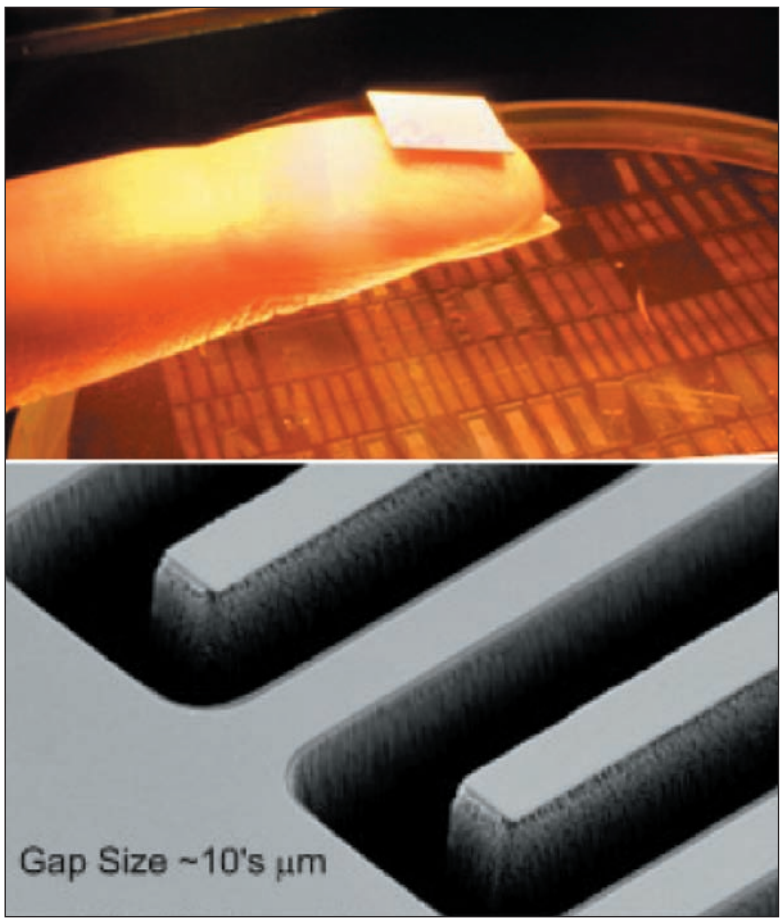

Figure 3. The Owlstone Ultra-FAIMS chip (5).

FAIMS-MS

The first reported use of FAIMS as an orthogonal separation technique for mass spectrometry was in 1998, when a cylindrical FAIMS device was interfaced to a quadrupole mass spectrometer using an electrospray source [2]. Since then there has been a significant body of work using FAIMS devices used both in series and in parallel with mass spectrometry. In this review we will concentrate on the application of FAIMS to the analysis of small molecules including peptide analysis. The use of FAIMS hyphenated to mass spectrometry has been covered in several reviews and more detail about environmental and security applications can be found there [5,6].

Pharmaceuticals

Ion mobility spectrometry (IMS) and FAIMS have been used in a number of studies related to pharmaceutical applications, and a review was published in 2008 which covers FAIMS and IMS applications [6]. FAIMS-MS as a rapid alternative to LC-MS methods shows potential as a fast screening technique for detecting drugs of abuse in complex matrices. Experiments performed on amphetamines in urine using a cylindrical FAIMS device demonstrated quantification down to 0.2ng/mL [7]. In addition, the FAIMS based approach is much faster than established LC based methods, with samples being able to be run and quantified within 20 minutes of arriving at the analytical laboratory.

Thanks to its differential mobility based mode of separation FAIMS has the capability of resolving isomeric species which cannot be separated using MS alone. This has been explored in two separate publications, McOoeye demonstrated that FAIMS-MS can be used to to separate and quantify diastereoisomers and applied the technique to isomers of ephedra in natural health products [8]. A chiral separation has also been demonstrated using FAIMS-MS, divalent metal ions were added to a racemic mixture of terbutaline producing diastereoisomers which were resolvable with a cylindrical FAIMS system [9].

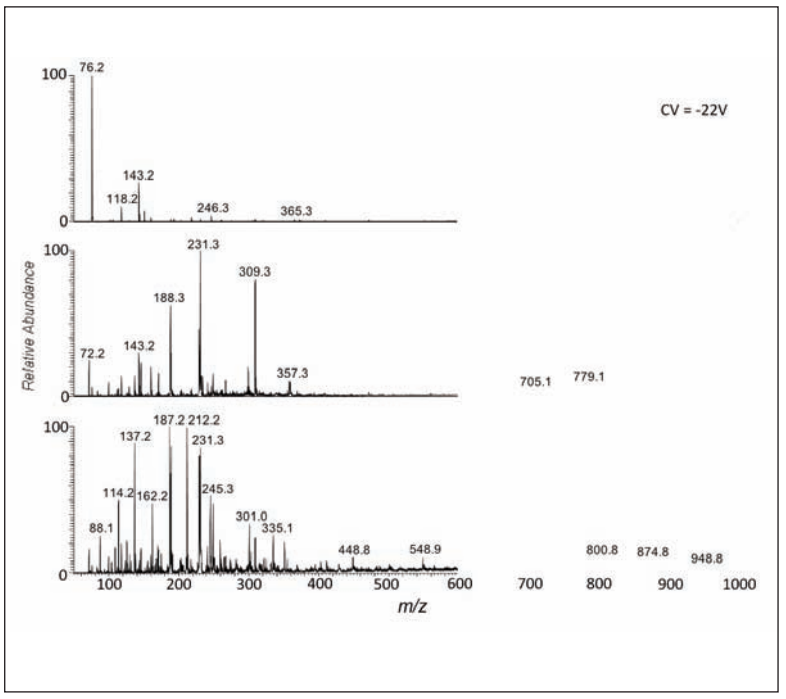

Figure 4. Mass spectra obtained at three different CV values from LC-ESI-FAIMS-MS analysis of a rat urine extract (9).

Metabolite ID/Metabolomics

Metabolomics is an area where FAIMS-MS can be used in several ways to enhance and/or simplify the complex data sets produced from metabolomic experiments. Here at Loughborough we have used a cylindrical Thermo FAIMS system in conjunction with a LTQ linear ion trap to identify biomarkers of ageing in rat urine samples [10].

The FAIMS was set to transmit ions at specific CV values sequentially in the range -12V to -22V, yielding mass spectra of the FAIMS-selected fractions of the complex urine sample across the LC run. *Figure 4* shows the mass spectra obtained at 3 separate CV values following a LC-FAIMS-MS analysis. It can clearly be seen that the spectra obtained at CV values of -12, -18 and -22V are different, enhancing the analytical space compared to MS alone.

The FAIMS-selected fractions were submitted for artificial neural network informatics analysis, which showed that urine from rats of different age groups could be distinguished with >80% confidence based on the data obtained from FAIMS-MS analysis allowing candidate biomarker ions of ageing could be identified.

Peptide analysis – Proteomics

FAIMS has been used in proteomics applications primarily as a pre-separation method used to enhance and simplify complex mass spectra. Another interesting area in which FAIMS has also found an application is in separating different peptide conformers giving structural information not available from standalone MS analysis. The first reported use of ESI-FAIMS-MS for peptide analysis was in 1999, when a study using leucine enkephalin was conducted by Purves et al [11].

This work showed that using FAIMS as a filter and selecting the protonated leucine enkephalin peak at its specific CV value enhanced the S/N ratio considerably by removing interfering background ion species. In addition, it was also possible to use the FAIMS to select a specific charge state of the leucine enkephalin, a capability which could prove very useful when used to preselect ions for collision induced dissociation.

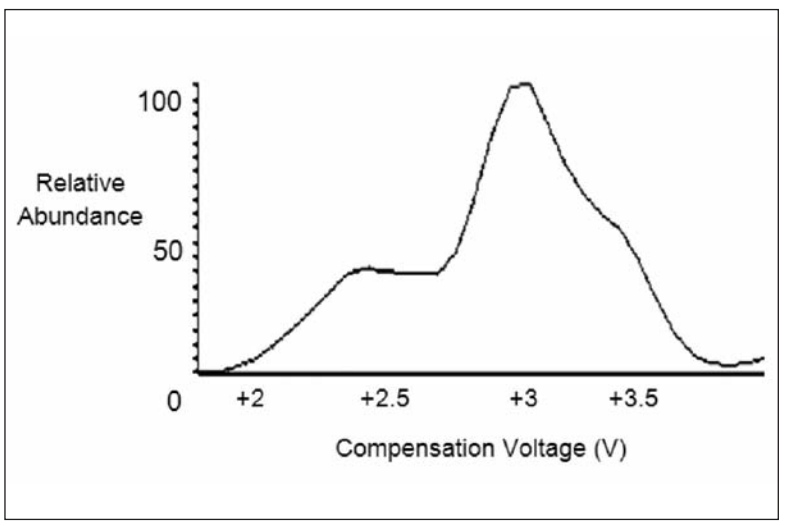

Figure 5. CV scan from the ultraFAIMS-MS ion trap analysis of the bradykinin [M+2H]2+ ion showing the separation of gas-phase conformers of bradykinin (11).

A miniaturised ultra-FAIMS system (Owlstone Ltd) has been hyphenated to time-of-flight (Agilent 6230) and ion trap (Thermo LTQ) mass spectrometers in our lab at Loughborough and applied to the analysis of peptide and protein digests [12]. Using this chip-based planar device we have been able to the acquire data on synthetic peptides and a tryptic digest of α-acid-glycoprotein. A pseudo-peptide mass fingerprint was generated by using the FAIMS to transmit only singly charged species. This was then submitted to a Mascot search which returned α-acid-glycoprotein as the top hit with a statistically significant score of 61. The ultraFAIMS can also be used to preselect doubly charged species prior to tandem mass spectrometry, resulting in an enhancement in S/N ratio of an order of magnitude and allowing ions previously masked by the chemical background to be identified.

Conformor analysis has also been demonstrated using ultra-FAIMS. *Figure 5* shows the CV scan from the doubly charged ion of bradykinin. The extracted ion trace for the [M+2H]+ ion shows distinct partially resolved conformers, demonstrating the potential of ultra-FAIMS for distinguishing between isobaric species on the basis of their gas phase conformation.

Conclusions

Differential mobility separation can be used as an orthogonal technique to mass spectrometry for the analysis of small molecules. Thanks to its fast analysis times and high sensitivity, planar FAIMS can be used as a rugged standalone detector for use in the field and, when hyphenated to mass spectrometry, can be used to enhance selectivity, S/N ratios and limits of detection by reducing interference from background species.

FAIMS-MS analysis can be used to separate and quantify species such as isobaric and isomeric ions that cannot be resolved by MS alone. The development of new FAIMS systems designed specifically to be used in conjunction with mass spectrometry is continuing and new methods, such as ultra-FAIMS, are expected to play an increasingly important role in the future.

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