DMS-Prefiltered Mass Spectrometry for the Detection of Biomarkers

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ABSTRACT

Technologies based on Differential Mobility Spectrometry (DMS) are ideally matched to rapid, sensitive, and selective detection of chemicals like biomarkers. Biomarkers linked to exposure to radiation, exposure to CWA’s, exposure to toxic materials (TICs and TIMs) and to specific diseases are being examined in a number of laboratories. Screening for these types of exposure can be improved in accuracy and greatly speeded up by using DMS-MS instead of slower techniques like LC-MS and GC-MS. We have performed an extensive series of tests with nanospray-DMS-mass spectroscopy and standalone nanospray-DMS obtaining extensive information on chemistry and detectivity. DMS-MS systems implemented with low-resolution, low-cost, portable mass-spectrometry systems are very promising. Low-resolution mass spectrometry alone would be inadequate for the task, but with DMS pre-filtration to suppress interferences, can be quite effective, even for quantitative measurement.

Bio-fluids and digests are well suited to ionization by electrospray and detection by mass-spectrometry, but signals from critical markers are overwhelmed by chemical noise from unrelated species, making essential quantitative analysis impossible. Sionex and collaborators have presented data using DMS to suppress chemical noise, allowing detection of cancer biomarkers in 10,000-fold excess of normal products. In addition, a linear dynamic range of approximately 2,000 has been demonstrated with accurate quantitation. We will review the range of possible applications and present new data on DMS-MS biomarker detection.

Keywords: DMS, MS, biomarkers, detection,

OVERVIEW

Differential Mobility Spectrometry (DMS) is a rapidly gaining universal acceptance for gas phase ion separation. The interfacing of DMS with Mass Spectrometry (MS) offers advantages over the use of a mass spectrometer alone. Such advantages include improvements in mass spectral signal/noise, an ion separation orthogonal/complementary to mass spectrometry, enhanced ion structural analysis and the potential for analyte quantitation without LC. This manuscript introduces a new ESI-DMS-MS system and utilizes it to aid in the understanding of DMS separation and the advantages of integrating DMS with MS for applications such as biomarker detection and identification.

Differential mobility spectrometry (DMS) has been called by many names. These include ion drift non-linearity spectroscopy, field ion spectrometry (FIS), radio frequency ion mobility spectrometry and high-field asymmetric waveform ion mobility spectrometry (FAIMS). The DMS method of ion separation is related to mobility based ion separation in gas media. A coefficient of ion mobility $K$ determines velocity $\vec{\beta}$ of an ion under the effect of a DC electric field $\vec{E}$ between two electrodes. Coefficient of mobility is a characteristic of individual ion species because it depends on reduced masses, cross section and ion-molecular interaction. Identification and/or separation of ions at ambient pressure condition through measurement of ion velocities is used in conventional time of flight type ion mobility spectrometry (IMS). The principle of ion separation in IMS is well known. A packet or swarm of ions is injected into the drift tube and due to effect of a DC electric field, all ions start moving toward the detector. Due to differences in mobility coefficient, the swarm of ions is separated into packages of ion with the same coefficient of mobility and ion identification is provided by drift time measurement.

In contrast to IMS, differential mobility spectrometry does not exploit the absolute value of mobility but uses dependence of mobility from an electric field, the alpha parameter in $K_\alpha(E) = K_0(0)[1 + \alpha(E)]$. There are a number of chemical-physical mechanisms which could affect electric field dependence, such as the collision energy, ion...
declustering at high electric field\(^1\), dependence of polarization cross section on relative kinetic energy, resonant charge transfer and ion dipole alignment under the effect of a strong electric field\(^2\)

![Ion Mobility as an Analytical Chemical Property](image1)

\[ \nu_i = K(E) = K_0 \frac{V_2 - V_1}{L} \]

\[ K = \frac{3e}{16N} \frac{2\pi}{\mu k T_\text{eff}} \frac{Z}{\Omega(T_\text{eff})} \]

\[ K(E) = K(0)[1 + \alpha(E)] \]

**Figure 1.** The ion mobility coefficient depends on collisional cross-section and on the effective collision energy.

Operationally, the differential mobility spectrometer functions as a tunable ion filter.

![DMS Ion Species Filtration in Continuous Mode](image2)

Ion species are carried through a narrow analytical gap in a gas transport stream where a transverse motion is superimposed by a strong non-symmetric waveform high frequency RF voltage (1-2MHz.) and a static DC compensation voltage. Positive and negative ions move with the transport gas through the analytical gap to the detector and exhaust. Ion trajectories depend on the alpha parameter\(^5\). The rf voltage causes the ions to move up or down in the gap; if the ions move such that they touch the upper or lower plate in the gap, they are neutralized and thus not detected the faraday detectors at the end of the gap. By controlling the compensation voltage Vc, a desired ion species can be kept in the center of the gap so that it is not neutralized and can be detected, either at the positive or negative detector for the...
different ion polarities. If the field is zero between the two Faraday detectors electrodes, filtered ions can be directed to a second analytical device, such as a mass spectrometer, where they can be mass identified.

As a result, a miniature DMS sensor can be used as a continuous, tunable ion filter for mass spectrometry. Advantages of the DMS prefiltering include:

- Positive or negative ions are filtered without the need of retuning of the filter.
- The filter can operate in “transparent” mode by turning off the RF voltage and setting \( V_c = 0 \text{V} \), thereby allowing all ions to pass into the mass spectrometer.
- DMS devices are continuous-mode ion filters unlike the standard IMS which operates as a pulsed filter. As such DMS enhances the total ion transfer efficiency of a selected ion to the MS.
- Ion separation occurs according to the alpha parameter which provides enhanced chemical identification.
- This filter allows RF voltage strengths up to \( E = 30 \text{kV/cm} \) which enables to reach optimal separation for defined ion species by regulation RF voltage intensity.

EXPERIMENTAL SETUP FOR AN ATMOSPHERIC PRESSURE MS INTERFACE BASED ON DMS

Figure 3 shows conceptual schematics of two different types of planar DMS couplings to a mass spectrometer inlet. These are described as inline and right angle configurations, indicating the location of the MS orifice relative to the gas stream direction in the DMS analytical gap. An in-line configuration provides high coupling efficiency between DMS prefilter and MS. The disadvantage of this configuration is that all un-filtered ions (neutralized in DMS) and other impurities are also introduced into the MS spectrometer along with the filtered ions. Due to adiabatic expansion of the gas stream in the interface area behind of orifice region, the clusterization processes between filtered ions and neutral analyte molecules may significantly transform recorded mass-spectrum. Right-angle configuration allows exclusion of neutral molecules from the MS inlet stream but requires some tuning of ion optics for introduction of all ions into the MS. Coefficient of ion transmission may be different for light and heavy ion species due to their different mobilities. Either interface can be used with most types of mass spectrometers. In our work we use the JEOL AccuTOF time of flight mass spectrometer and the single quadrupole Waters Micromass ZQ.

Additionally, we used DMS pre-filters with different ion sources. For samples with sufficient vapor pressure, we used a UV lamp to provide atmospheric pressure photoionization\(^{13}\). In major experiments related to study components related to
Homeland security interests (CWA agent simulants, and explosives) we used a radioactive $^{63}$Ni ion source. For all biomarker and peptide detection experiments, we used a DMS interface with electrospray/nanospray ionization sources.

**ORTHOGONALITY OF DMS AND MS SEPARATIONS**

A goal of mass spectrometry and ion mobility spectrometry is chemical identification. The general principals and operational schematics for both analytical methods are similar. Even the sequence of procedures for these two methods is the same: generate ions from sample molecules by employing any suitable ionization method, separate formed ions, and detect them qualitatively or quantitatively to determine abundance for different ion species. The differences between MS and IMS technology lie in the conditions where ion separation occurs. In mass spectrometer ion separation occurs in vacuum conditions with no interaction between formed ions and neutral gas molecules. Therefore MS provides ions separation by mass-to-charge ration (m/z). In contrast, in IMS technology ions are separated in ambient pressure conditions where the time between collisions is less than $10^{-9}$ s. As a result ions continuously interact with neutral gas molecules and ion separation is based on ion size and conformation rather than simply on mass-to-charge ratio. So, comparison of the conditions of ion separation in IMS and DMS shows these to be complimentary methods. In addition, the enormous amount of interaction between ions and molecules dramatically increases the contribution of diffusion processes in gases. Again, the direct comparison MS and IMS spectra show differences. Mobility based spectra show very broad peaks for ion species in comparison with MS. Formal calculation of resolving power according to the resolving power formula ($R = \text{peak position} / \text{peak width}$) for DMS yields very small values (20-30) in comparison with MS (at least 500). But on the other hand, DMS based technology can provide effective resolution for isomeric and isobaric compounds.

Figure 4 shows that effect of structure of ions in DMS and illustrates one specific advantage of using DMS as a pre-filter to MS isomeric ions. Direct experiments show that DMS can provide separation for not only isomeric ions; it can provide separation of many isobaric components also. Identification of ions with close m/z but with different structures (formulas) can be a problem for mass spectrometry with moderate resolved power.

**Separation of Isomers in DMS**

Figure 4. Superimposed DMS spectra for meta, para, and ortho xylene shows that DMS can resolve structural isomer that cannot be distinguished by mass-spectrometry measurements.

To solve many problems in a single mass spectrometric measurement, accurate exact mass measurements may be needed, which can only be done in expensive high resolution mass spectrometers. Since a DMS-MS system combines two complimentary methods, it can solve such problems. In our first presentation in this meeting we have illustrated the power of such system for example to separate five isobaric ion species which have the close m/z~316 ions by using mass spectrometer with resolution ~ 500 and a miniature DMS prefilter. In this presentation we will present results of our investigation related to using DMS-MS system for selective detection: explosives, CWA simulants and some small molecules which are considered as candidates for biomarkers for radiation exposure (biodosimetry). Analysis of all...
obtained experimental data proves our expectation and provides information that characterizes the power of a DMS-MS system. Pre-filtration of ions enhances the performance of mass spectrometric sniffers due to: improved signal/chemical noise ratio, enhanced limit of detection (LOD) of analyte trace detection, enriched chemical information, reduced chemical noise (interferences) and consequently reduced false alarm rates.

APPLICATION DMS-MS FOR DETECTION TRACES OF EXPLOSIVES

In Figures 5a and 5b, we present results from the DMS-MS interface prototype. DMS operates without change for positive and negative ion species and provide measurement simultaneously for both ion polarities. Figure 5a,b shows that the DMS pre-filter totally suppresses all background negative ions O$_2^-$, and (H$_2$O)$_2$O$_2^-$. Filtration of reactant ions with m/z=32, and 50 occurs when compensation voltage in DMS is turned on Vc = -10V (see bottom DMS spectrum on left frame of figure 5a). When compensation voltage of DMS is turned on Vc= -1.5V which are shown in the left frames only the negative molecular ions (M$^-$) of 2,4-DNT (right frame of figure 5a) is selected. Estimated limit of detection for DNT in this case is less than 1 ppbv.

![Figure 5. DMS spectra (bottom frames) and MS spectra (upper frames) for API formation of a) negative ions and b) positive ions 2,4 DNT.](image)

DMS is able to filter and detect positive and negative ions simultaneously. Surprisingly for us, with increasing analyte concentration of 2,4 DNT, positive polarity DMS channel shows an additional peak (Vc~−1V), Figure 5b. By switching mass-spectrometer polarity on positive mode we have identified this ion species as 2.4-DNT protonated ions (MH$^+$). Intensity of these ions was about five times lower in comparison with negative molecular ions (M$^-$). Peak position in DMS spectrum for these ion differ also for negative molecular ions Vc= -1.5V and for positive ions Vc= -0.95V.

APPLICATION DMS-MS FOR DETECTION OF TRACES OF CWA SIMULANTS

Figure 6 shows an example of ion species separation in DMS and MS spectra for a gas mixture which contains a mixture of nerve and blister chemical warfare agent simulants: Methyl Salicylate (MS, M.W=152) and dimethy methylphosphonate (DMMP M.W=124).

By tuning the compensation voltage of DMS pre-filter system, each component can be selectively recorded with enhanced signal/noise ratio. The first mass spectrum (at the top of the 4 spectra) was obtained without DMS pre-filtering. Therefore, it contains all expected ion species: DMMP protonated monomer ions (MH$^+$, 125 Da), Methyl Salicylate protonated monomer ions (MH$^+$, 153Da), and proton bounded DMMP dimmer ions (M$_2$H$^+$, 250Da). The bottom three mass spectra show that by appropriately tuning DMS compensation voltage, it is possible to selectively record DMMP monomer ions with Vc=−5V, methyl salicylate monomer with Vc=−2V, and DMMP cluster ions with Vc=+2.5V.

This efficient ion filtration can be used in combination with other analytical devices in front of the DMS. For example, it might be different electrospray array systems. All mass-spectra which would be obtained after filtration will not show any background ion species.
DEMONSTRATION OF CHEMICAL NOISE REDUCTION FOR DETECTION OF CAFFEINE IONS.

Decreasing the number of ion species by ion prefiltering before the MS helps to clean up the MS spectrum. A smaller number of ion species is introduced into the MS resulting in fewer fragment ions and a lower probability of interferences with target ions. Figure 7 shows an example. DMS prefiltering reduces chemical noise and consequently it records only selected ions, in this case the monomer ions. Mass spectrum presented on left frame shows that our caffeine sample contains certain amount of impurity (sodium). Therefore without separation, we can see a number of peaks associated with cluster and fragment ions. Complex DMS spectrum (bottom spectrum on right side) shows that in air it is possible to form many complexes. Yet only the peak located in Vc=-12V can be associate with caffeine monomer peak.

Figure 7. Selective detection of caffeine monomer peak in DMS-MS system.
To show the power of filtration we performed the following control experiment (Figure 8). We intentionally introduced into the caffeine sample chemicals which could be considered as a source of chemical noise. These are a polyethylene glycol (PEG) species with different molecular weights. This procedure with PEG is often used for mass calibration. Standalone mass spectrum in this case contains a lot of peaks with high intensity and as a result the caffeine peak (m/z=195Da) intensity close to chemical noise level. When DMS compensation voltage was tuned to filter caffeine monomer ions, the mass spectrum became very clean and contains only one peak related to caffeine monomer. And absolute magnitude of peak intensity is almost the same as on the mass spectrum on left side (~1V).

![Filtration Caffeine Ion from Chemical Noise](image)

(Figure8. Selective detection caffeine monomer peak in complex mixture which containe sufficient amount of chemical noise.

The prefiltering benefits are especially useful for seeking target ions in complex mixtures, for example, detection and measurement of the biomarkers.

**BIOMARKER DETECTION AND SEPARATION WITH nESI-DMS-MS**

Screening for radiation exposure is a critical technology related to Homeland Security. In the event of widespread exposure to radioactive material, a large number of people would have to be screened rapidly. The ideal technology would be non-invasive, and provide selective and specific detection. Metabolomic work is ongoing in collaboration with Sionex that has identified a few candidate biomarkers for radiation exposure. These biomarkers are present in simple biofluids like urine. DMS is being investigated as a much more rapid separation technique than LC as a pre-separation method for mass spectrometric analysis.

As an example of separation and detection of candidate biomarkers, we will show results for azelaic acid, \( \text{C}_9\text{H}_{16}\text{O}_4 \), MW = 188.10485, CAS 123-99-9, obtained using nESI-DMS-MS. Azelaic acid is a medium chain linear dicarboxylic acid containing 7 CH2 groups between the carboxylic acid groups. It is used topically for skin conditions, and is involved in fatty acid metabolism. Azelaic acid is observed in negative ion mode. The ions observed are predominantly the deprotonated anion (AZA – H) at m/z 187, and for higher mass spec inlet cone voltages as a fragment at m/z 125. Figure 9 shows that azelaic acid can be effectively separated from the fragment by DMS-MS.

The azelaic acid sample contained as impurities several related dicarboxylic acids of varying chain length. Analyzing the DMS-MS spectrum for these related species provides a good demonstration of the ability of DMS to separate related compounds, the extended dynamic range of the DMS-MS combination, and the suppression of chemical noise by DMS.
DMS SEPARATION OF RELATED COMPOUNDS AND DYNAMIC RANGE

Because of the high S/N achieved in nESI – DMS – MS, we were able to detect and resolve 5 additional related dicarboxylic acids that were also present in the azelaic acid sample at quite low concentrations. This presented an interesting demonstration of DMS separation of a related series of compounds. This ability of DMS to separate these compounds is illustrated in the following intensity-normalized DMS plot.

DMS REDUCTION OF CHEMICAL NOISE

Biofluids are complex mixtures that may show mass peaks at every unit m/z. Overlapping mass spectra from unwanted compounds and from isotope peaks of unwanted compounds cause errors in quantitative measurements, and result in
false alarms. Because the azelaic acid contains minor impurities, it is possible to use DMS filtration to select one of the minor components and suppress chemical noise at nearby m/z values. This illustrates improvement in quantitative accuracy because the restoration of normal isotopic ratios shows that even the smallest peaks are due only to the target ion. This is demonstrated for n=6 of the previous figure (suberic acid, 4.5% of the azelaic acid concentration) in Figure.

**Chemical Noise Suppression for Suberic Acid Present at 4.5% in Azelaic Acid Sample**

![Graph showing DMS separation](image)

Figure 11. Suberic acid is detected at a 4.5% level in electrospray of our azelaic acid sample.

The presence of several other species contributes chemical noise to the suberic acid signal. This chemical noise is completely removed by DMS filtration. The remaining peaks at m/z 174 and 175 are the expected isotope peaks of suberic acid (predicted intensities 100:9:1.2, observed 100:11:3).

These results on azelaic acid and related species give a dramatic illustration of the separation and noise suppression capabilities of DMS.

**SUMMARY**

In summary, the results obtained in this work shows that DMS prefiltering provides number advantages:

- Enhances chemicals identification information
- Reduces chemical noise by a factor of 10-30. Consequently enhancing the detection limit and quantitation accuracy
- For complex mixtures DMS pre-separation can replace or reduce the requirement and time for separation steps in GC or HPLC.

**REFERENCES**


