Impact of chemical modifiers on the cluster chemistry during electrospray ionization

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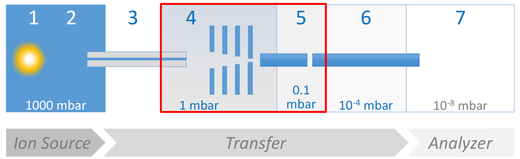
Introduction

State of knowledge:

We have recently begun to fundamentally investigate phenomena regarding the ESI process (i.e., the formation of bare protonated molecules). The multidisciplinary approach builds on literature and our experimental data, which resulted from numerous studies using gas phase API methods. One critical aspect are transformation processes which ions usually undergo on their passage from the elevated pressure inlet region to the mass analyzer. The electrical fields applied in this region easily result in ion activation (e.g. declustering, fragmentation). In contrast, mild transfer conditions, i.e. low electric field gradients, may lead to the occurrence of clusters in mass spectra and may unfavorably affect the ion transmission efficiency and thus sensitivity.



In a thermodynamically controlled elevated pressure region the formation of bare gas phase ions is unlikely, as protonated molecules will readily interact with polar substances present in the background gas for charge stabilization (formation of e.g. protonated analyte-solvent clusters or protonated dimers). Ion activation will ultimately lead to cluster dissociation, transferring the proton to the compound with higher proton affinity.

In ESI, particularly upon formation of multiply charged (i.e. protonated) ions the same rules should apply. In an elevated pressure region protonated groups of e.g. a protein will seek means of charge stabilization via intra- or intermolecular interactions.

"Protonated basic groups which are not stabilized by intramolecular hydrogen bonding will be solvated by one or more solvent molecules. The solvent molecules may have been retained in the transition from droplet to gas phase or acquired later from the solvent vapor present in the atmospheric pressure region of the ES ion source."
– R. E. Cole, Electrospray Ionization Mass Spectrometry, p. 58

Upon cluster dissociation by ion activation, the charge should be transferred to the more affine compound. It may thus be still attached to the analyte or distributed into the gas matrix.

Supercharging:

High charge states are beneficial for electron transfer dissociation MS/MS experiments, which makes targeted shifts of the charge state distribution (supercharging) an important feature for e.g. proteomics applications.

Supercharging can be achieved by addition of reagents to the analyte solution or by saturating the background gas in the ion source. One such agent is acetonitrile, as demonstrated in the commercial Bruker CaptiveSpray nanoBooster ™ device.

This contribution deals with the impact of such reagents or *chemical modifiers* added to the background gas of a nano-electrospray ion source on the detected ion population.

Methods

Experimental Setup

The experimental setup is based on the Bruker CaptiveSpray nanoBooster™, which uses acetonitrile as a chemical modifier for supercharging and improved response.

MS: Bruker micrOTOF, esquire 6000 Ion Trap, (Bruker Daltonics, Bremen, Germany)

Ion Source: custom nano Electrospray Ion (nESI) Source [3], sprayer held at 1.2-2 kV, 15 µm nano spray tip (PicoTip™ ,New Objective). In source CID: Variation of the capillary exit/skimmer DC potential.

Gas Supply: Nitrogen 5.0 (Messer Industriegase GmbH, Germany).
All gas flows are controlled by mass flow controllers (MKS Instruments, Germany), mixing ratios of chemical modifiers are determined by their respective saturation vapor pressure and the gas flow directed through a liquid reservoir, the total gas flow is maintained at 800 ml/min

Chemicals: Acetonitrile, Methanol, Water, Formic Acid and Substance P were purchased from Sigma Aldrich, Germany, and used without further purification

Experimental results

Bruker micrOTOF:

Initial experiments were carried out with a time-of-flight instrument equipped with a capillary-skimmer inlet stage. Substance P was used as a model analyte.

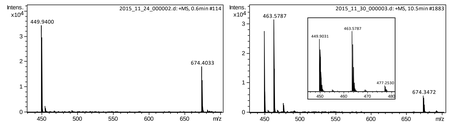


Figure 1a: nanoESI mass spectrum of Substance P diluted in 50% aqueous acetonitrile with 0.1% formic acid (left); background gas in the ion source saturated with acetonitrile leads to M+nACN cluster formation, but only for the triply protonated molecule (right)

Figure 1b: nanoESI mass spectra of Substance P diluted in 50% aqueous acetonitrile with different mixing ratios of methanol added to the background gas in the ion source (left) and intensities of the doubly and triply protonated molecule signal (right); the abundance of the $[M+3H]^{3+}$ signal decreases as methanol is added; at higher mixing ratios protonated methanol-water clusters are detected

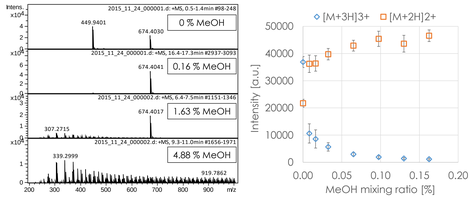


Figure 1c: Mass spectrum showing series' of solvent clusters; 4.9% methanol added to the background gas; at higher MeOH mixing ratios the distribution is shifted to higher numbers of MeOH-molecules

Bruker esquire 6000 Ion Trap

With the ion trap instrument no acetonitrile clusters were observed, however adding acetonitrile to the ion source background gas lead to better Signal-to-Noise (S/N) ratios for the detected ions.

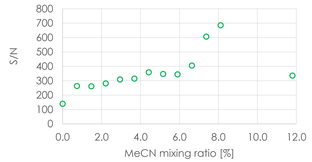


Figure 1d: S/N ratio of the $[M+3H]^{3+}$ ion signal in dependence of the acetonitrile mixing ratio added to the background gas

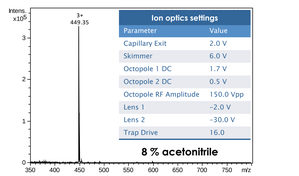


Figure 1e: No MeCN clusters were detected with the ion trap instrument, even at very mild transfer and trapping settings

micrOTOF – in source CID

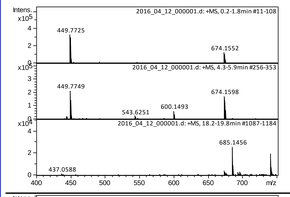


Figure 2a: Mass spectra of Substance P at different in source CID potentials, 15 V (top), 50 V (center) and 300 V (bottom); minute activation leads to fragmentation of the triply protonated molecule (m/z 499.8), strong activation results in complete loss of $[M+3H]^+$ and poor overall ion transmission

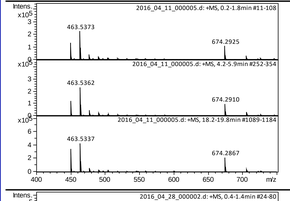


Figure 2b: Mass spectra of Substance P at different CID potentials, 15 V (top), 50 V (enter) and 300 V (bottom), with 5.9 % acetonitrile added to the background gas leads to formation of analyte-acetonitrile clusters. Increasing the CID potential results in higher signal intensities, no fragmentation is observed

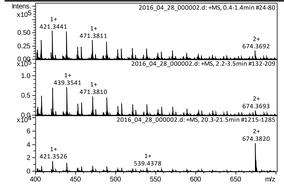


Figure 2c: Mass spectra of Substance P at different CID potentials, 15 V (top), 65 V (center) and 300 V (bottom), with 3.3 % methanol added to the background gas; increasing the CID potential results in decrease of the MeOH-H_2O cluster abundance,

Pressure dependency

The formation of clusters may result from changes of the gas expansion into the first vacuum stage of the micrOTOF MS, which would have an impact of the upstream transmission. Therefore the pressure was varied between 2.8 and 4.1 mbar by adjustment of the pumping capacity of the roughing pump. The distance between the inlet capillary exit (length = 20 cm, inner diameter D_0 = 0.5 mm) and the first skimmer is 3.9 mm. As seen in Figure 3a the position of the Mach disc shifts towards the skimmer as the pressure drops, but does not reach the skimmer tip. If clusters are formed in the adiabatic expansion, they should appear in mass spectra if the skimmer samples upstream of the Mach disk, which is not the case. Additionally, ion acceleration in this region due to electric fields should lead to declustering, which is not achieved for analyte-acetonitrile clusters at high acetonitrile mixing ratios above approximately 4 %, in contrast to protonated solvent clusters. At acetonitrile mixing ratios below 4 % both dissociation of the analyte-acetonitrile clusters and fragmentation of the analyte was observed. Increasing the pressure resulted in higher stability of the ions, probably because of lower energy uptake due to a smaller mean free path.

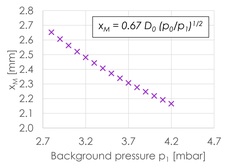


Figure 3a: Calculated distance x_M between the inlet capillary exit and the Mach disc as a function of the background pressure in the first vacuum stage

Nevertheless, increasing the background pressure to the maximum value (a higher pressure led to shut down of the split-flow turbo pump) a shift of the intensities of the clustered and unclustered analyte signal was observed even at highest acetonitrile mixing ratios (cf. Figure 3b).

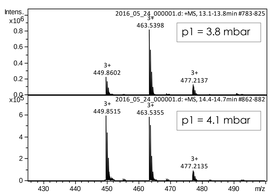


Figure 3b: Mass spectra of Substance P obtained at different background pressures in the inlet stage , background gas saturated with acetonitrile

Conclusions

Acetonitrile addition leads to the formation of analyte-acetonitrile clusters, observed with the micrOTOF instrument. The clusters are only detected for the triply but not the doubly protonated molecule, resulting in a net shift of the charge state distribution towards the higher charge state. The selectivity of the cluster formation may be linked to the additional charge available at the $[M+3H]^{3+}$ ion. The clusters appear to be very stable, leading to absence of cluster dissociation and fragmentation even if very high CID potentials are applied in the ion transfer stage. Surprisingly, also the $[M+2H]^{2+}$ ion does not fragment under CID conditions, even though no clusters are detected. This suggests that a stabilizing interaction between $[M+2H]^{2+}$ ions and acetonitrile does take place as well.

Adding methanol to the background gas leads to depletion of the $[M+3H]^{3+}$ signal, while the $[M+2H]^{2+}$ is maintained and even amplified. At high mixing ratios protonated water-methanol clusters appear in the spectra with high abundance, concealing the analyte signal. CID leads to declustering and the analyte signal appears again. Again, no fragmentation is observed, hinting again at energy disposal into the bath gas

These observations give some insights on the processes taking place. It is conceivable that two protonated groups of Substance P are stabilized by intramolecular hydrogen bonding, whilst the third charge is not and therefore readily interacts with suitable compounds. The interaction with methanol results in proton transfer from Substance P to methanol when dissociation is forced, whereas acetonitrile stabilizes the protonated group but does not remove the proton. The improvement of the S/N ratio at the ion trap instrument supports this assumption because proton transfer to contaminants present in the setup may be prevented when a protecting reagent is present in large excess.

One open question is why the acetonitrile clusters are observed with the TOF but not the ion trap instrument. It may be possible that the clusters are dissociated during the trapping process. However, adjustment of the transfer potentials and reduction of the trap drive to almost the lowest value possible did not lead to detection of the clusters. Another explanation may tied to in the inlet stage of the instruments. Even though the ion trap is also equipped with a capillary-skimmer assembly the geometry and dimensions differ from the TOF instrument.

Outlook

- Validation experiments with another micrOTOF instrument and the original CaptiveSpray nanoBooster™ setup
- Characterization of the expansion into the first vacuum stage and impact on the cluster interactions
- Systematic investigations of the influence of chemical and physical properties on cluster interactions

Literature

[1] R. R. Ogorzalek Loo, R. Lakshmanan, J. Loo, *What Protein Charging (and Supercharging) Reveal about the Mechanism of Electrospray* Ionization, J. Am. Soc. Mass. Spectrom. 25, 1675-1693, **2014**

[2] R. B. Cole, *Electrospray Ionization Mass Spectrometry – Fundamentals, Instrumentation, and Applications*, Wiley-Interscience, John Wiley & Sons, Inc. **1997**

[3] M. Thinius, Nele Hartmann, T. Benter, *nESI and cAPCI applications of a controlled ion activation stage ("ion tunnel")*, Proceedings of the 63th ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, USA, **2015**

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