

Effects of chemical dynamics and clustering reactions of chemical modifiers with analyte ions in differential mobility spectrometry (DMS)



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Introduction

Background:

Differential mobility spectrometry (DMS) separates ions according to their specific motion in elevated pressure regimes with alternating high and low electrical field conditions between two plate electrodes. A DC bias voltage, referred to as the compensation voltage (CV), allows to transmit species with a characteristic mobility ratio between high and low field phase, known as the α function (normalized difference between the high and low field mobility). Various physical and chemical mechanisms are potentially responsible for the individual α functions. In particular dynamic cluster formation and destruction with each low/high field cycle leads to distinct differential mobility behavior. Consequently, a chemically modified gas matrix allows to specifically change individual α functions and thus the separation of different ion species [1].

Previous results:

The separation of proton bound water and water/acetone clusters, respectively, in a DMS device was qualitatively reproduced by simulation of the clustering dynamics considering the oscillating electrical field. The simulation results support the notion of clustering as primary separation mechanism for these systems [2]. Supported by literature [3], we propose that this result is also applicable to more complex analytes and chemical systems [1,3].

Present results:

We present experimental results of DMS-measurements with Substance P. Subject of investigation was the effect of gas phase modifiers and gas temperature on its separation behavior, in particular with respect to conformational changes of the molecular structure.

Methods

Instruments:

MS System: 6500 Triple Quad™ (Sciex)
DMS System: SelexION® DMS (Sciex)
Ion Source: Turbo V™ (ESI-mode; Sciex)
Data Processing: Analyst® 1.6.2 (Sciex)

Chemicals:

Gases: nitrogen (5.0)
Modifiers: acetonitrile (ACN; HPLC grade), methanol (MeOH; HPLC grade), 2-propanol (HPLC grade)
Analyte solutions: 2 µg/ml Substance P in ACN/H₂O, 2 µg/ml Substance P in MeOH/H₂O

Experimental parameters:

- Injection of a 10 µl/min liquid flow with a 2.5-ml-syringe and the built-in syringe pump
- Concentration of the modifier in the curtain gas (if used): 3 % ("High"-setting)
- Gas heater temperatures: "low" (150 °C); "medium" (225 °C) or "high" (300 °C)
- Ion-transfer optimized for low fragmentation conditions

Variation of the Modifier

effects on the separation

Figure 1 shows the separation of the doubly and the triply protonated Substance P, with and without a chemical modifier present. Note the complete shift to negative CV values in case of a gas phase enriched with acetonitrile (ACN; 3 %). This strongly suggests two entirely different separation mechanisms for both conditions.

Positive CV values correspond to higher mobility in the low-field than in the high-field phase and negative CV values correspond to lower mobility in the low-field than in the high-field, respectively.

The observed *positive* CV values without modifier present are explained either by hard-sphere collision interactions with the background gas (higher deflection in high-field) [1] or by high-field induced conformational changes of intra molecular bonds [4].

The observed *negative* CV values with ACN indicate dynamic cluster formation with larger clusters in the low-field thus slowing down their mobility in that particular phase.

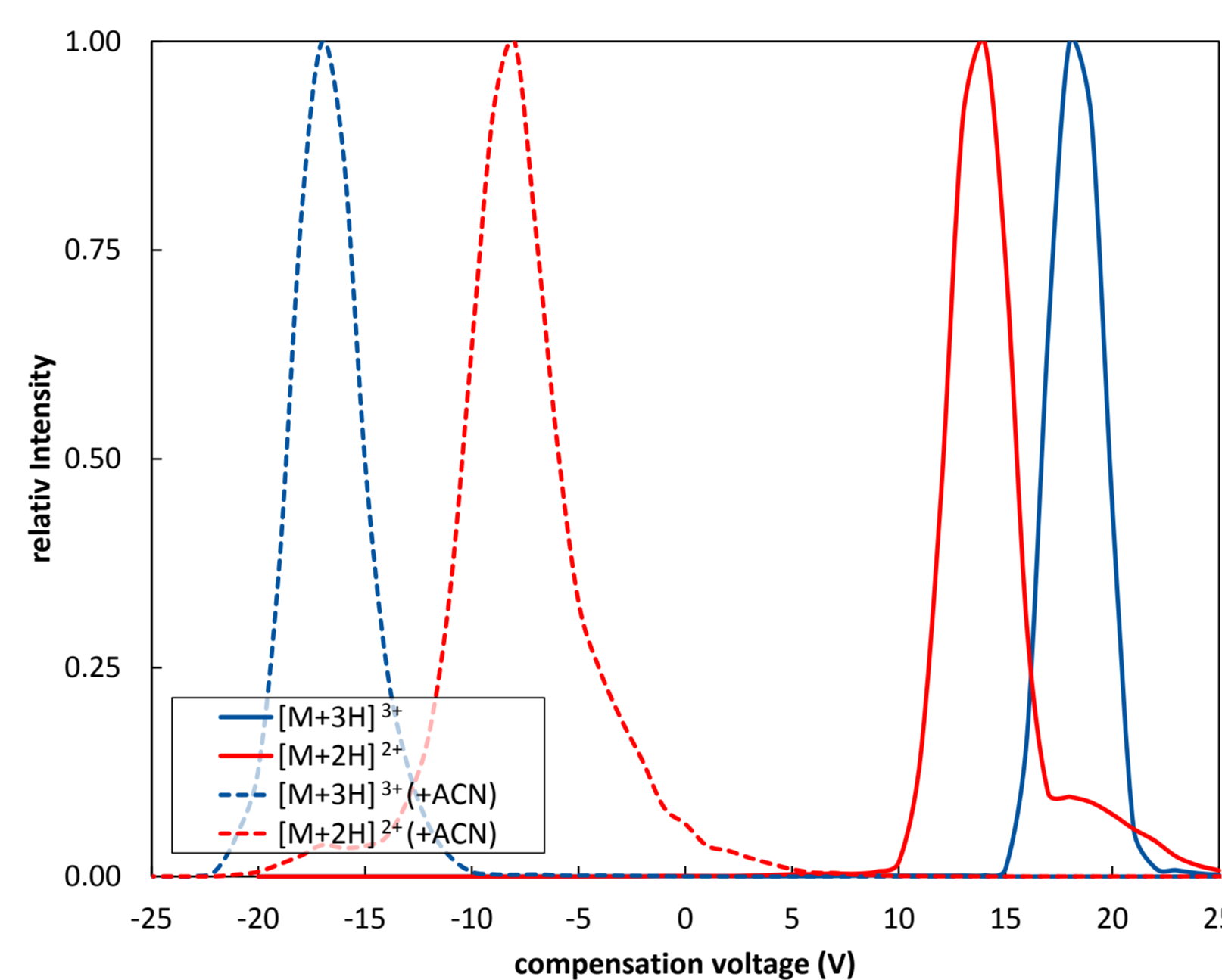


Fig 1: Effect of gas phase acetonitrile on the compensation voltage (CV). The two main ion-signals of Substance P ([M+3H]³⁺ and [M+2H]²⁺) at a separation voltage (SV) of 4000 V (T = 150 °C) are shown.

Figure 2 illustrates the superimposed hard-sphere-like-behavior and the cluster effect in dependence on the separation voltage. Without modifier the CV continuously increases with SV.

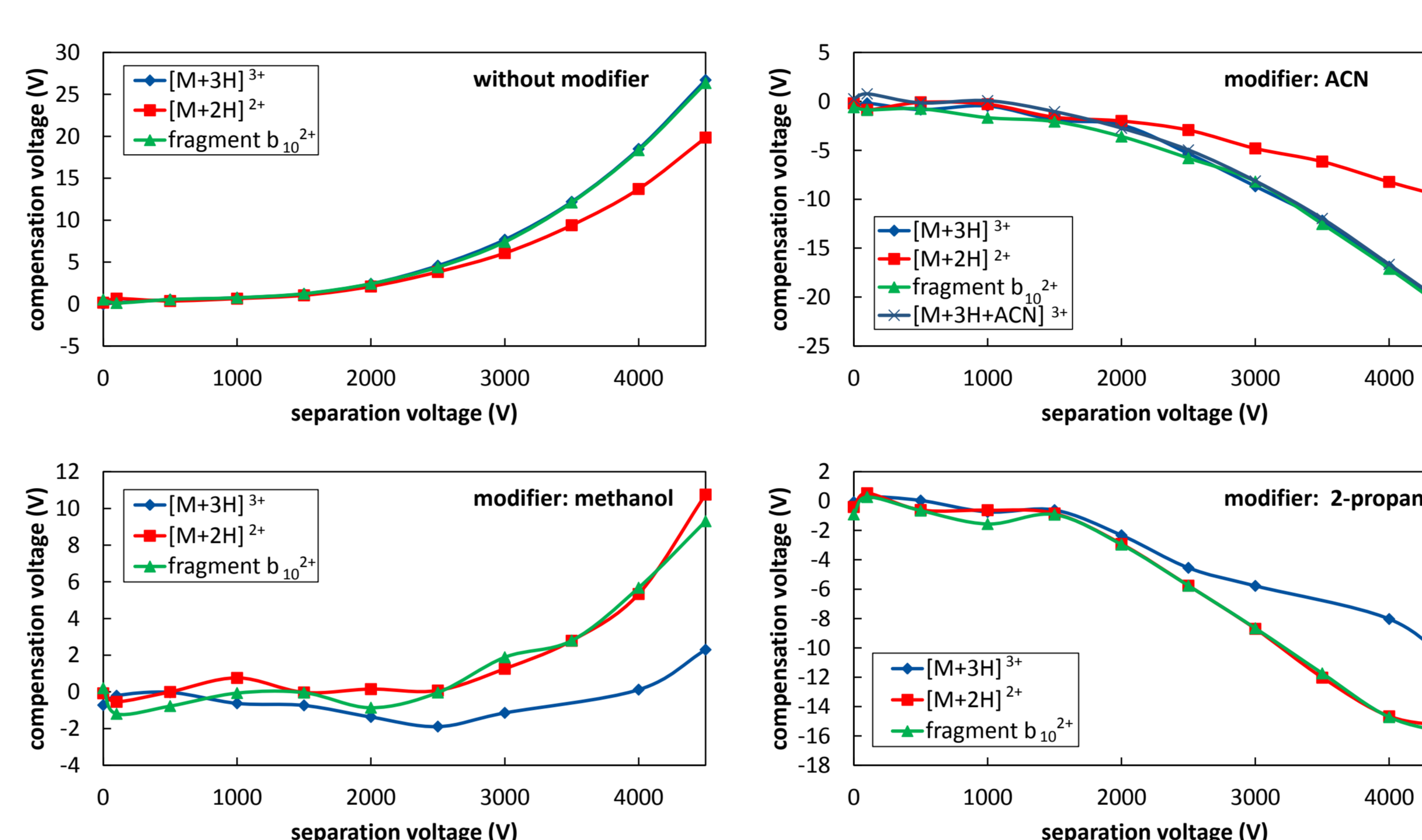


Fig 2: CV dependency on the SV of the two main ion-species of Substance P ([M+3H]³⁺ and [M+2H]²⁺) and the fragment b₁₀²⁺ (T = 150 °C)

effects on the intensity

The type of modifier significantly determines the total intensity as well as the relative ratios between different charge states. As shown in fig. 3, an ACN saturated gas phase causes a decrease in the total Substance P intensity, whereas the relative ratios of the single ion species remain nearly unchanged. In contrast, the modifiers 2-propanol and methanol completely suppress all other species in favor of the doubly protonated ion.

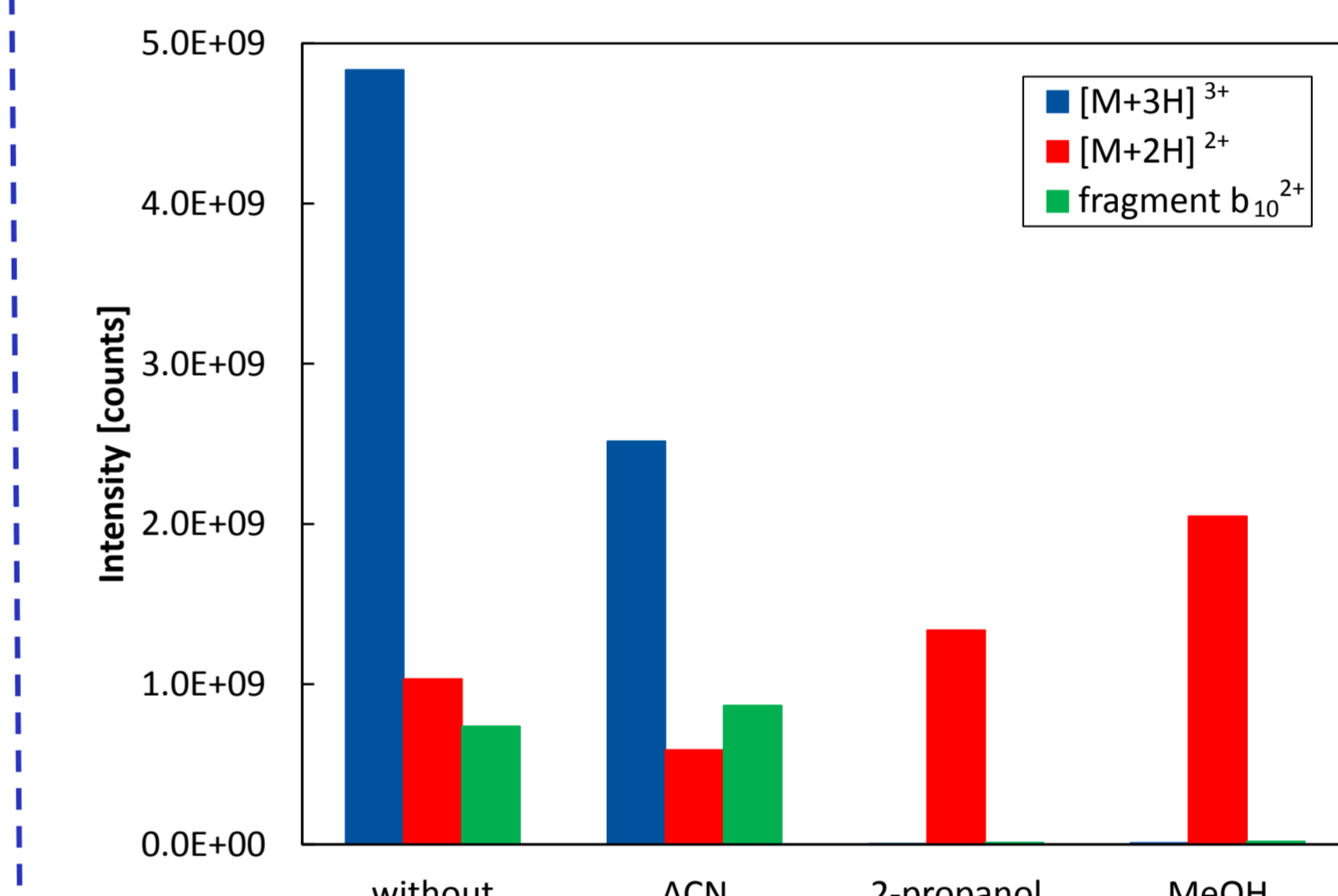


Fig 3: Ion signal distribution of the Substance P species (T = 150 °C) dependent on the modifier.

Conclusions

- Chemical clustering reactions (negative CV) and hard-sphere-like-behavior (positive CV) are of equally importance for the ion separation in DMS. Generally, these two are superimposed.
- The proportion of both effects depends on the analyte structure, the modifier and the reaction conditions.
- Hard-sphere interactions [1] as well as dynamic structural changes [4] provide reasonable explanations for the lower mobility in the high-field (positive CV).
- Reduced cluster stability favors hard-sphere-like-behavior in the DMS separation.
- Strong modifier-analyte-clusters lead to negative CVs, indicating dominance of chemical clustering in the DMS separation.
- The DMS-temperature is crucial to the separation:
 - Increasing temperature favors hard-sphere-like-behavior.
 - The clustering effect decreases with increasing temperature. Above 300 °C the influence of methanol as a modifier nearly vanishes.

Temperature Dependence of the Separation

without modifier

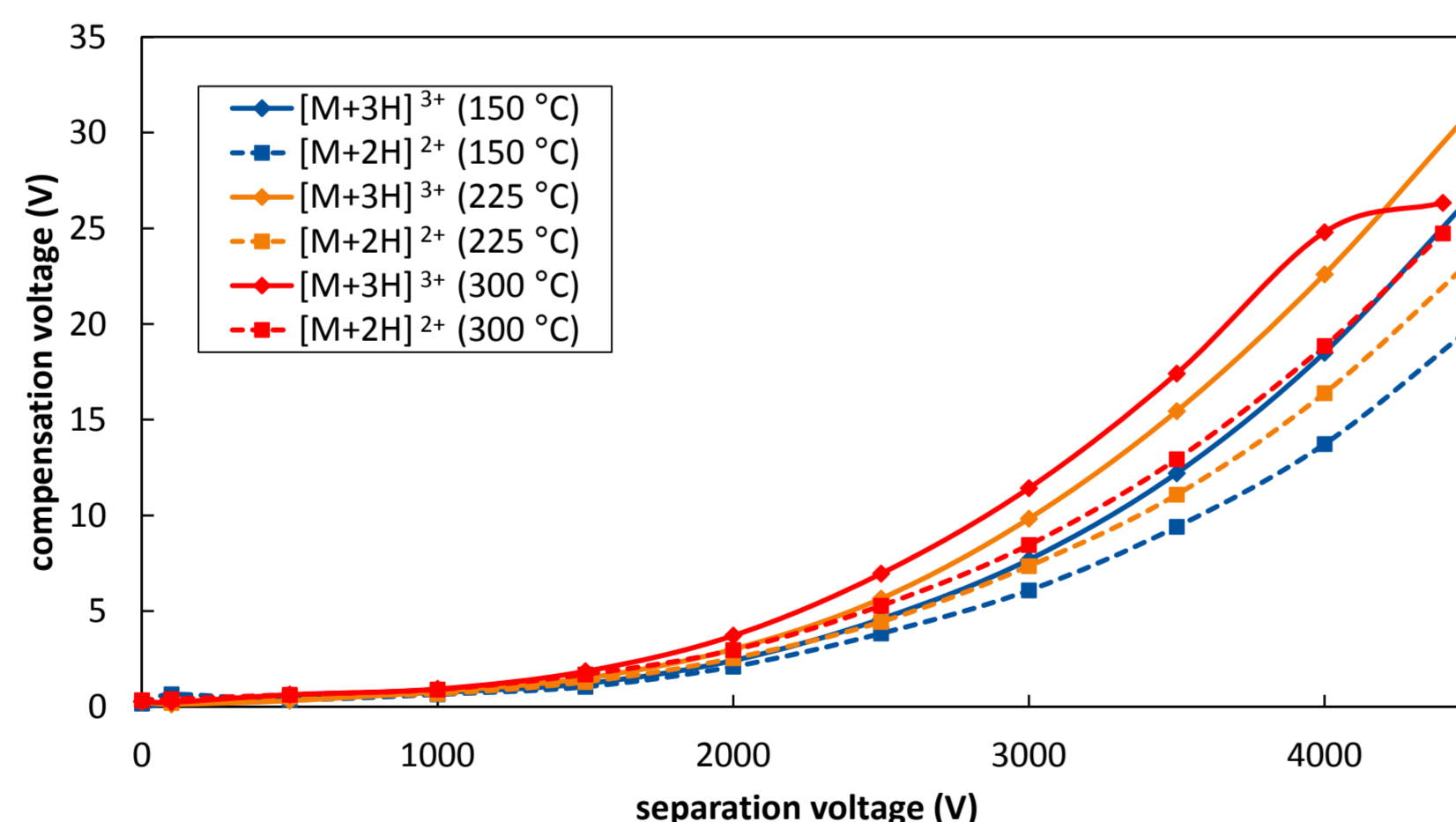


Fig 4: Temperature dependence of the CV of the two main ion-signals of Substance P ([M+3H]³⁺ and [M+2H]²⁺)

In the absence of a modifier, the CVs of both analyte species increase with increasing temperature. This is expected from both effects with hard-sphere-like-behavior [1,4]. High temperatures potentially lead to fragmentation, which could contribute to the observed leveling of [M+3H]³⁺ at 300 °C.

With methanol the CV progression is very similar to the results without modifier. An increase in temperature suppresses the cluster effect.

with methanol

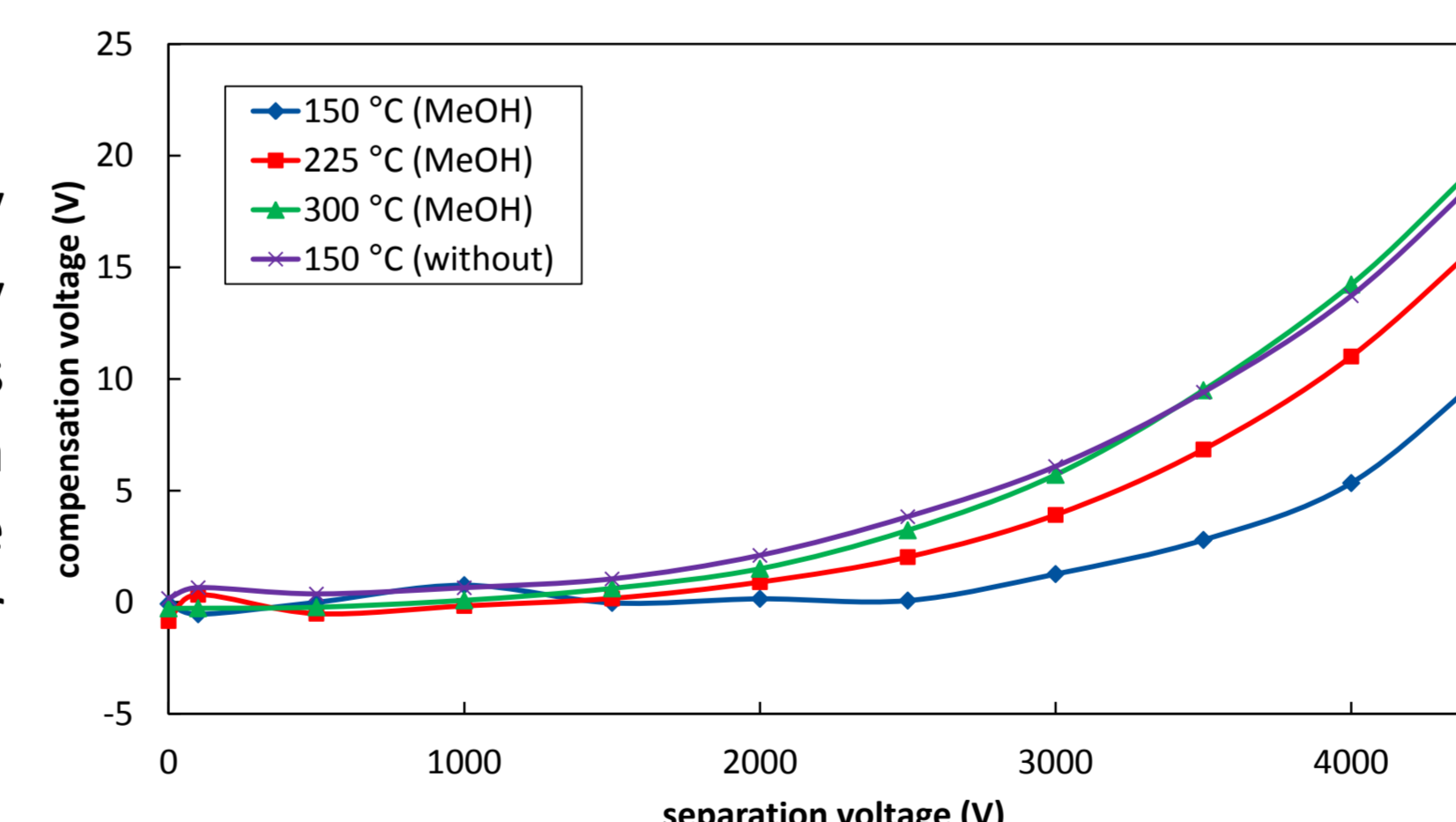


Fig 6: Temperature dependence of the CV of the [M+2H]²⁺-signal

with acetonitrile

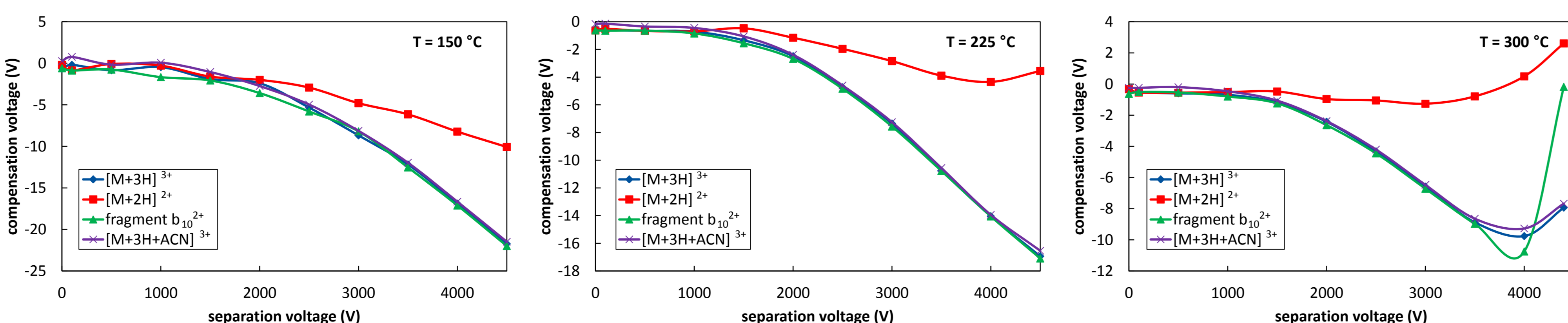


Fig 5: Temperature dependence of the CV of the main ion-signals of Substance P and the first ACN-cluster of the triply protonated analyte ([M+3H+ACN]³⁺)

The cluster size depends on the temperature in the DMS-cell. Both, increasing temperature and increasing SV, lead to decreasing mean cluster sizes.

At low temperature (150 °C) chemical clustering dominates. With increasing temperature (225 °C) the hard-sphere-like-behavior gains importance for the separation at high SV (note the [M+2H]²⁺-signal at high SV). At high temperature (300 °C) this behavior is observed for all species, although chemical clustering is still the main factor.

Outlook

Chemicals:

- Modifier:** New modifiers for deeper mechanistic insights and new applications.
- Analytes:** The use of smaller analytes could reveal the impact of single functional groups.

Combination:

- Simulations:** Comparison of experimental results with ab-initio and molecular dynamic simulations should provide a comprehensive understanding of the basic ion separation effects.
- Supercharging:** Gain deeper insight into the cluster chemistry and the observed supercharging effects.

Cluster strength:

- MS/MS:** The relative cluster strength of analyte-modifier-clusters will be investigated by matching MS/MS experiments.
- Comparative measurements:** For more precise investigation of the cluster effect a softer ion transfer into the mass analyzer is needed.

New Applications areas:

- Chiral Separation with DMS:** Was demonstrated in an IMS [5]. Conceivable for a DMS as well, provided that there is a deeper understanding of the cluster effects.

Literature

- Schneider, B. B.; Nazarov, E. G.; Laundry, F.; Vouros, P.; Covey, T. R.; Differential mobility spectrometry/mass spectrometry history, theory, design optimization, simulations, and applications; Mass Spectrometry Reviews; 35; 687-737 (2015)
- Wissdorf, W.; Schneider, B. B.; Covey, T.; Hager, J.; Benter, T.; Is differential mobility chemically driven? Evidence and simulations for chemical effects as a primary separation factor in DMS; 64th ASMS Conference on Mass Spectrometry and Allied Topics; San Antonio; Texas; USA; (2016).
- Campbell, J. L.; Zhu, M.; Hopkins, W. S.; Ion-Molecule Clustering in Differential Mobility Spectrometry: Lessons Learned from Tetraalkylammonium Cations and their Isomers; J. Am. Mass Spectrom.;25; 1583-1591 (2014)
- Russell, D. H.; Silveira, J. A.; Fort, K. L.; Kim, DoY.; Servage, K. A.; Pierson, N. A.; Clemmer, D. E.; From Solution to the Gas Phase: Stepwise Dehydration and Kinetic Trapping of Substance P Reveals the Origin of Peptide Conformations; J. Am. Chem. Soc.; 135; 19147-19153 (2013)
- Wu, C.; Dwivedi, P.; Matz, L. M.; Clowers, B. H.; Siems, W. F.; Hill, H. H. Jr.; Gas-Phase Chiral Separations by Ion Mobility Spectrometry; Anal. Chem.; 78; 8200-8206 (2006)

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