Interactive Comment On "Clustering, Methodology, and Mechanistic Insights Into Acetate Chemical Ionization Using High-Resolution Time-of-Flight Mass Spectrometry"

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We thank the two reviewers for useful and insightful comments. The resulting discussion has led to a more rigorous manuscript with increased clarity. Below, we respond to each reviewer comment, and note the resulting changes in our revised manuscript (submitted separately).

Reviewer #1 General Comments

1. Misinterpretation that ionization mechanisms are being studied rather than instrument control of observed ions:
    a. Sensitivity and changing signals

Relevant Reviewer #1 Comments:

"This work is studying the impacts of altering the m/z at which a specific ion product is detected by varying the electric field and thus the extent of declustering or fragmentation, not changing the ionization pathway of the fundamental chemical ionization method."

"When the authors describe changes in sensitivity due to changes in voltages, what is really being discussed is changes in signals detected in a particular time bin."

Author Response: We would argue that sensitivity as traditionally defined is the response of an instrument to an analyte. Collisional dissociation control is almost always occurring to some extent in the ion transfer optics of mass spectrometers. This makes the observed sensitivity a function of ion source chemistry and instrument design/operation. To clarify the reviewer's concern, we have added our definition of sensitivity, which we do not believe is a departure from traditionally defined analytical sensitivity of an instrument.

Added Text (Line 396, page 1):

"Here, we define the sensitivity as the analytical sensitivity of the instrument; the sensitivity is the observed response to a known concentration, which is a function of both ion chemistry and instrument operational parameters."

    b. Where is the process occurring? Need for clarity.

Relevant Reviewer #1 Comments:

"In these experiments the cluster distribution in the IMR is now being fundamentally altered and the ionization reaction pathways are being changed. At a constant voltage setting, if the RH is increased and signal at the formate ion mass increases then in fact the sensitivity to formic acid, as classically defined, did increase as a result of a change in the cluster distribution. Since both processes are studied in this manuscript the language needs to be tightened up to reflect the manner in which sensitivity is commonly used."

"At a very minimum the authors must make it absolutely clear to the reader what is being discussed, actual changes in the sensitivity driven by changes in the cluster distribution in the IMR, or a change in 'sensitivity' as a result of redistribution of signal onto various masses."

Authors Response: We have added some detail to a key statement to add clarity. Also, see Reviewer #1 (General Comment #5).

Original text Line 393, page 11:
"All of these clusters are suppressed by operating the instrument in a declustered mode. We reiterate that while the detection of these clusters can be eliminated, the cluster chemistry is still occurring in the IMR. Operation in clustered mode produces linear calibration curves for all species presented."

Updated text:
"The observation of all of these clusters is suppressed by operating the instrument in a declustered mode. We reiterate that while the detection of these clusters can be eliminated in the ion transfer optics, the cluster chemistry is still occurring in the IMR. Operation in clustered mode produces linear calibration curves for all species presented."

2. <u>Comparison of instrumentation: operational differences exist</u>

   Relevant Reviewer #1 Comments:
   "The result is that the ion-neutral cluster distribution in each instrument is different thus the reactions occurring in the are likely also quite different"

   "Therefore, the detected acetate to acetate-acetic acid cluster ratios observed are not necessarily reflective of the distribution in the reaction region which is where the absolute sensitivity is determined"

   "The fact that the water cluster distributions in that 2008 work and this study may have been very different due to the operating conditions means these results may not be inconsistent rather incomparable."

   Authors Response: This highlights a key difficulty in comparing CIMS instruments. Nearly all mass spectrometer ion transfer optics will bias the observed ion distribution to some extent. For example, the fact that the Veres 2008 quadrupole mass spectrometer uses a collisional dissociation region means that they are also not observing the ‘true’ ion distribution that exists in the CI reaction region. Thus, there are essentially two approaches to attempting to compare acetate CIMS instruments. (1) Operate the mass spectrometer in a maximally clustered mode and report the cluster ratio between the first [acetate + acetic acid] cluster and acetate. (2) Operate the mass spectrometer under normal operational conditions and report the cluster ratio between the first [acetate + acetic acid] cluster and acetate. The first option is susceptible to instrument-to-instrument biases in how gently ions and ion-neutral clusters can be transferred from the ion source region to the detector. The second option tells us little about the conditions in the IMR, but instead tells us about the extent to which the observation of clusters has been eliminated (i.e. the extent of declustering). We agree that the acetate + acetic acid cluster to acetate ratio may not be as important for targeted measurements where a limited number of calibrated acids are measured at lower m/z ratios where clustering will not be as greatly observed.

Ultimately, the fact that two acetate CIMS observe completely different relative humidity dependencies is inconsistent, but we agree with the reviewer that the underlying cluster distribution in the IMR may be completely different in these two instruments; this should be acknowledged. This is discussed on page 14 with the paragraph beginning on line 500. The original text relevant to this comment begins on page 14 line 504 in the same paragraph:

“This is inconsistent with previous quadrupole acetate CIMS experiments that indicate no humidity dependence for formic acid (Veres et al., 2008). However, the ions most susceptible to humidity effects are the ion-neutral clusters; these species are rarely detected because of the operation of the API on the HR-TOF-CIMS in a declustered mode and the use of a CDC on quadrupole instruments.”

In order to clarify this statement, we added the following sentence after this selection of text to line 508:

“As such, the cluster distribution in the Veres *et al.* (2008) study may be completely different than the cluster distribution observed here making comparison between these instruments and relative humidity effects nebulous.”

3. Where do the reactions occur? Claim that ion chemistry occurs in the IMR only.

Relevant Reviewer #1 Comments:

“After reading the manuscript I have failed to see a strong case for such claims”

“My suggestions would be to remove this from the abstract as it is not once of the focal points of the work, and only comment on how some of the results suggest that this is true. Additional work must be done to show that this is the case.”

Authors Response: Figure 4 provides experimental evidence that chemistry and clustering reactions are limited to the IMR or the transfer from the IMR to the SSQ. The voltage scan between the SSQ front and the SSQ entrance plate (top plot in the left column) suppresses the detection of the [acetate + acetic acid]$^-$ cluster. The curve cannot be extended to sufficiently large voltage differences to reduce the acetate cluster ratio to the same extent that is observed with other component relations (e.g. lens skimmer – SSQ back, simmer – lens skimmer ,BSQ Front – lens skimmer). These other component relations can be extended far beyond the dV_{50} because of allowable voltage set points.

As this is not the focal point of the paper and only one line or reasoning leads to this conclusion, we have taken the reviewers suggestion and removed this point from the abstract.

4. Quad vs TOF Comparisons

Relevant Reviewer #1 Comments:

"“Ideally, quadrupole CI is deployed with the intent of measuring specific species with readily available authentic calibration standards and well-characterized interferences.” This can be and should be also said about TOF CIMS.”

“Identify and observe the temporal behavior of compounds that have not previously known to exist or calibrated in a non-targeted approach”. This is done by performing mass scans, the only limitation being the lower duty cycle. You must be more careful in this section discussing the limitations and benefits of the two techniques against each other. This paragraph is in need of some cleaning up so as to not mislead the reader as to the relative advantages.”

Authors Response: We agree with these comments, and have updated the text with the aim of making a more accurate comparison between TOF and quadrupole systems. We also highlight the necessity of authentic calibrations with TOF-CIMS.

Updated text beginning at line 49 page 2:

These features are, essentially, added benefits of TOF-CIMS over quadrupole CIMS instruments; however, we note that there is no substitute for authentic standard calibrations despite the alluring benefits of using TOF mass spectrometers. Quadrupole systems contrast this non-targeted approach, but calibrations remain as important for TOFs as quadrupole systems. Ideally, quadrupole CI is deployed with the intent of measuring specific species with readily available authentic calibration standards and well-characterized interferences. Calibrations are typically conducted for a limited number of compounds, but interferences are difficult to address until they are identified through instrument intercomparisons and careful study. Mass scans are often conducted using quadrupole based CIMS to examine the temporal behavior of uncalibrated species, but these results may be more difficult to understand compared to the mass spectrum acquired by TOF-CIMS because of the lack of elemental information present in high-resolution data.

5. <u>More clarity of what we are studying: ionization mechanisms not directly studied</u>

   Relevant Reviewer #1 Comments:

   "On page 5, line 163, the authors indicate that this work provides "a detailed investigation of the acetate ionization mechanisms". However, as my comments above indicate, it is my belief that the authors have studied mechanisms that are not the same as the acetate ionization mechanisms, rather the effects of inducing fragmentation or declustering through ion optic voltage scanning, RH experiments being the exception." (Now at line 168, page 5)

   Authors Response: The core question is: which reagent ion(s) (or ion-neutral clusters) actually deprotonate carboxylic acids and to what extent? We agree with the reviewer that this question is not directly answered by our manuscript. We have, however, identified that clustering reactions occur and provided constraints on the extent to which they occur. We find that the sensitivity of acetate CIMS is dependent on the cluster distribution as shown by the relative humidity experiments. We agree with the reviewer that this may not characterize the mechanisms in the traditional sense of flowing afterglow or selected ion flow tube techniques, but this work does describe relevant mechanisms that are occurring in these acetate CIMS systems. We have added a few words to highlight the use of collisional dissociation beginning on line 170 page 5:

   Original text:

   "This characterization of the API allows for a detailed investigation of the acetate ionization mechanisms and the impact of controlling for these mechanisms on sensitivity, detection limits, selectivity, and mass spectral ambiguity with the general aim of non-targeted analysis."

   Updated text:

   "This characterization of the API allows for a detailed investigation of the acetate ionization mechanisms and the impact of controlling for these mechanisms with collisional dissociation in the ion transfer optics on sensitivity, detection limits, selectivity, and mass spectral ambiguity with the general aim of non-targeted analysis."

6. <u>Normalization</u>

   Relevant Reviewer #1 Comments:

   "In the data analysis section, the authors describe a normalization procedure, it could be useful to have a discussion either here or later in the manuscript on what the result of this study suggest should be done. Can signals be traditionally normalized? Or is there some other method that can be used? Or are there going to be things that we simply have to calibrate for?"

Authors Response: This is an important point. We agree this is something for which we will have to calibrate. Some of these concerns with regard to RH dependent calibration and normalization are added to the discussion on page 15 in the paragraph starting on line 522 where the complexities of water interferences are discussed. We added the following text:

Updated text line 522 page 15:

“The role of water on acetate CIMS chemistry remains difficult to reconcile. Propionic acid sensitivities are the lowest under dry and very wet conditions (Figure 6), but other trends exist for other deprotonated-declustered acids (SI5.1). The formation of the water-, acetate-, and self-clusters show identical RH dependence for all the calibration compounds: the addition of water shifts the cluster distribution as water is incorporated into acetate clusters. Additionally, normalization methods described herein do not eliminate the relative humidity dependence. Normalization of acetate CIMS data remains a challenge because the information about the cluster distribution is lost when collisional dissociation is sufficiently high that we observe only declustered, deprotonated ions. Relative humidity dependent calibrations may be the most direct method for rigorously addressing the water interference.”

7. Clarity concern
   Relevant Reviewer #1 Comments:

   “The description and logic of the statements made in section 3.3, page 10, lines 352 – 259 is hard to follow. It is important that this is clear, because the conclusions being drawn here, as mention in comments above, are rather important.

   Authors Response: We thank the reviewer for this note and modified the text as follows:

   Original Text (original lines 341-347, page 10):
   “The exit of the SSQ to the lens skimmer (component relation 3) provides a promising region for cluster control compared to the choice of components used in previous studies (Lopez-Hilfiker et al., 2016), which are unable to directly resolve the dV_{50} of the [iodide + water] cluster and limited in their ability to directly resolve the dV_{50} for the [iodide + formic acid] cluster. Similar to that study, scanning the BSQ front and skimmer relation (component relation 5) for the relevant acetate ions and ion-neutral clusters results in the identical problem described by Lopez-Hilfiker *et al.* (2016) when attempting to resolve the entire sigmoidal curve: we are unable to generate sufficiently low electric fields needed to transmit weakly bound clusters.”

Updated Text (lines 351, page 10):

"The exit of the SSQ to the lens skimmer (component relation 3) provides a promising region for cluster control compared to the choice of components used in previous studies (component relation 5) (Lopez-Hilfiker et al., 2016). The dV_{50} of the [iodide + water] cluster cannot be resolved because sufficiently weak electric fields cannot be generated. Similarly, the dV_{50} for the [iodide + formic acid] cluster can only partially be resolved. Scanning the BSQ front and skimmer relation (component relation 5) for the relevant acetate ions and ion-neutral clusters results in the identical problem described by Lopez-Hilfiker *et al.* (2016) when attempting to resolve the entire sigmoidal curve: we are unable to generate sufficiently low electric fields needed to transmit weakly bound clusters."

8. Veres 2008 work "overlook" vs "not investigated": language issue

Relevant Reviewer #1 Comments:

"On page 14, the authors point out "The original development of this method by Veres et al. (2008) overlooks the importance of clustering. . .""

"Overlooking the importance of something and identifying but not going into the details are two very different actions."

"On page 14, line 495, in talking about the TOF results and discussion here the authors indicate the necessity of effective collisional dissociation. This is consistent with previous work, which the authors themselves have already acknowledged "Although Veres et al. (2008) note that a collisional dissociation chamber is important to "dissociate weakly bound cluster ions such as CH3C(O)O-(H2O)n". Another indication the 2008 work does not overlook the importance of clustering on the sensitivity of the ion chemistry."

Authors Response: We have removed the word "overlooks" from line 495 page 14 and reworded the sentence to read:

"The original development of this method by Veres *et al.* (2008) does not investigate the importance of clustering in the ion source due to the use of a quadrupole mass spectrometer, limited mass scan range, and a collisional dissociation chamber (CDC)."

9. Alternative hypothesis to change in sensitivity

Relevant Reviewer #1 Comments:

"On page 15, line 506, have the authors considered any other explanations for the water dependencies such as the hydration of acids to diols, or another water catalyzed reaction occurring in the IMR? Something that comes to mind is the Maroń et al. 2011 Chem. Phys. Lett. work on pyruvic acid hydration to a geminal-diol. Again remember when the RH is changed the reaction in the IMR change fundamentally, and then the effects being studied are the fragmentation and breakup of clusters in the transfer optics."

Authors Response: To our knowledge there is no evidence for water-mediated interconversion of carboxylic acids in the gas phase. Maroń *et al.* (2011) examine this chemistry using pyruvic acid in the condensed phase, which is expected to be very different from the gas phase chemistry due to solvent effects. Water has been observed to change the sensitivity of iodide adduct CIMS (Lee *et al.* 2014) because of the formation of the [iodide + water]$^-$ cluster, which is proposed to undergo ligand switching reactions with analyte molecules. This reflects a true shift in sensitivity, and we find parallel chemistry in the acetate CIMS. Figure 5 in this manuscript shows that during the operation of the API under clustered conditions, the acetate signal actually increases when changing the relative humidity from 0% to 10%, and then stabilizes as the relative humidity is further increased to 80%. Thus, the concentration of bare acetate does not appear to change as a function of relative humidity beyond 10%. The clusters do change across the entire relative humidity range and the sensitivity of the various analytes tracks the change in the cluster distribution. This suggests that these clusters are key to the ionization process.

10. Iodide adduct CIMS

Relevant Reviewer #1 Comments:

On page 17, the author makes the statement that the lessons here could apply to the iodide adduct CIMS. However, if one operates in declustered mode as presented in the acetate work nothing would be detected as iodide does not charge transfer or deprotonate (it could react to form IO as in the case of PAN iodide CIMS).

Authors Response: The recent description of iodide adduct CIMS applied to HR-TOF-MS by Lee *et al.* (2014) would argue otherwise. Specifically, Figure 2 a-b shows that numerous ions exist that do not contain iodide. This is separated from the iodide containing clusters by the "iodide valley", which appears when the high-resolution data are plotted in mass defect space. Iodide is unlikely to charge transfer or deprotonate most molecules. Nonetheless, these ions are a prominent feature of the iodide spectra. Collisional dissociation within the API does not completely explain this phenomenon because declustering of an [Iodide + analyte] cluster should dominantly break apart into the original constituents (I^- and the neutral analyte) because of the high gas-phase acidity and high electronegativity of iodide. The question that remains is: are all of these non-iodide-containing ions the result of clustering followed by collisional dissociation or individual non-clustered ions formed by an alternate mechanism? Increasing collisional dissociation increases the observation of deprotonated, iodide-free ions: Lee *et al.* show that with increasing voltage difference in the API, more acetate ions are observed. These signals may arise from peroxy acids.

11. Calibration allows for ignoring clustering.

Relevant Reviewer #1 Comments:

"I think the authors should take a short amount of time to discuss calibrations however. All of these features of the declustering can essentially be 'ignored' if one is dedicated to calibrating the instrument."

Authors Response: We respectfully disagree with the reviewer's suggestion that calibration enables a user to ignore clustering/declustering effects. The processes described in this manuscript have two potential effects that are not captured by typical calibration techniques: (1) Interference by clusters of smaller mass molecules with water or acetate, and (2) interferences by fragmentation products of larger mass molecules. Thus, we agree that these processes can be ignored in simple, single analyte systems – but in the complexity of ambient or smog chamber systems, interferences may occur that cannot be accounted for by calibration. The observation of strong signals from the $[\text{analyte + water}]^-$ clusters make a strong case that calibration, while critical, is not a panacea for CIMS problems. If measurements focus only on small acids (<100 m/z), numerous interferences can arise from water clusters in this range. The formate ion, for example, has the molecular formula CHO_2^- and propanoate has the molecular formula $C_3H_5O_2^-$. Adding water to these ions produces the observed molecular formula $CH_3O_3^-$ and $C_3H_7O_3^-$, corresponding to $[\text{formate + water}]^-$ and $[\text{propanoate + water}]^-$, respectively. A HR-TOF-CIMS would identify these molecular formulas, which may aid in realizing that an ionization chemistry interference exists as $CH_3O_3^-$ is a surprising molecular formula for a deprotonated ion and would warrant further investigation. Quadrupole systems would be unlikely to catch this interference; arguably, this is less of a problem for quadrupole systems that have built in a collisional dissociation region, but TOF-CIMS systems similar to the one described herein (i.e. with a Tofwerk API) could be tuned to transmit clusters with high efficiency. Further, fragmentation may also result in interfering ions that are not captured completely by typical calibration. Thus, understanding these processes cannot be ignored and is key to performing a more robust measurement.

12. Difference needs to be stressed regarding difference between getting the most out of a data set and making good, quantitative measurements

Relevant Reviewer #1 Comments:

"The elucidation of these details is specifically geared at getting the most and best understood amount of data from a uncalibrated bulk data set. There is a big differentiation between those two concepts, and one that I feel needs to be stressed considering the recent rapid growth in the use of newer TOF CIMS instruments."

Authors Response: The changes made to Reviewer #1 (general comment #4) should address this concern as we make the need for calibration more explicit and point out that many of the benefits of TOFs are added benefits that do not replace the need for calibrations.

Reviewer #1 Specific Comments

1. Page 2, line 43, pluralize the word collect

   Done

2. Page 2, line 48, need to add the word "been" into the phrase 'been known'

   Done

3. Page 2, line 57, instead of absence do the authors mean 'presence'

   Authors Response: We do not mean "presence" instead of "absence". The point here is that the interpretation of CIMS (TOF-CIMS) data is difficult even if clustering, relative humidity, and the exact molecular formula are known. This statement is justified by what follows in lines 57-68, page 2.

4. Page 6, as a result of some of the concerns I included above, knowing the operating pressures of the IMR and other vacuum regions would be beneficial.

   Authors Response: Figure 1 in the main paper contains all operational pressures of the IMR, SSQ, BSQ, and PB region. Additionally, the following sentence has been added at line 198 on page 6:

   "IMR and SSQ pressures are held constant at 100 and 2 mbar, respectively"

5. Page 7, line 228, make "curves" singular

   Done

6. Page 11, line 394, are the authors referring to an acetate-water or RH-water cluster here?

   Authors Response: We are referring to the [acetate + R-H]$^-$ cluster (this is the [acetate + analyte]$^-$ cluster) . Line 82, page 3 defines R-H as the analyte. We had the concern that denoting analytes as carboxylic acids only (e.g. RCOOH) is not sufficiently generalized because other non-carboxylic acids are detectible (e.g. nitrated phenols). As such, R-H was chosen to highlight the fact that the analyte has a proton that may undergo proton transfer reactions to form a deprotonated anion. We note that this may cause confusion with relative humidity, which we abbreviate as "RH" on line 202, page 6.

7. Page 13, line 463, the word 'cluster' needs to be plural.

   Done

8. Page 16, line 559, I believe this should read "dissociation is not a straightforward approach due to"

   Authors Response: The original sentence on lines 558-560, page 16 reads:

   "However, controlling cluster interference in observed mass spectra by collisional dissociation is a straightforward approach to the complexity of acetate CIMS. Other formulations proposed in the literature may oversimplify this problem."

We intended to suggest and encourage acetate TOF-CIMS users to take advantage of the fact that we characterized the voltage difference (dV) dependence of adjacent ion optics for declustering. This is, in fact, an easy way to deal with the complexities of the observed ionization chemistry. The details of declustering are not straightforward, but if sufficient collisional energy is imparted on the ions, then most the complexities fall away. Moreover, this procedure can be easily added to pre-existing voltage configurations. One can simply float the API of an already tuned TOF-CIMS to produce the necessary declustering conditions and observe only declustered ions.

9. <u>On page 17, line 603 the word underestimation is used, however, depending on where you are in the dV space, you could be under or overestimating these bulk parameters.</u>
   Authors Response: This is a good observation that highlights the complexities of calculating the "true" bulk parameters.

   The original sentence reads:
   "Ignoring clustering will result in an underestimation of the average H:C ratio and overestimation of the average O:C ratio, average oxidation state, and average number of carbons."

   This sentence has been updated to read (Lines 624-626, page 17):
   "Ignoring clustering will result in either an over or underestimation of the average H:C ratio, O:C ratio, average oxidation state, and average number of carbons depending on the extent of clustering."

10. <u>Figure 5, 7, and 9 are really difficult to see the relative changes because of the log scale. Perhaps there might be a better way to show this data.</u>

    Authors Response: linear scaling of the y-axis makes it impossible to see the important changes because the changes of greatest importance change over many orders of magnitude. We attempted to plot these data linearly during the preparation of the manuscript, but important information is lost. For example, figure 5 shows that the [acetate + water]$^-$ cluster changes from ~100 cps to almost 10^5 cps, while other ions change very little. A factor of 2 change is fairly unimportant when other ions are changing in abundance by orders of magnitude. We could break these plots up into multiple, individual plots, but the number of figures is already quite high.

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