Characterization of oil sands naphthenic acids by negative-ion electrospray ionization mass spectrometry: influence of acidic versus basic transfer solvent

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ABSTRACT

Considerable effort and progress has been made over the past decade with respect to development of analytical tools for the determination of naphthenic acids and related components in environmental samples. However, experimental variables that influence the analytical results have not been fully explored. The relative contributions of $O_x$ classes are of particular interest in data obtained using negative-ion electrospray ionization mass spectrometry. Using two types of ultrahigh resolution mass spectrometers (Orbitrap and FT-ICR), the apparent pH of the transfer solvent was observed to have a significant impact upon compound class distributions. A basic transfer solvent favored the detection of $O_x$ species of lower oxygen content, while acidic pH favored the preferential observation of organic compounds with higher oxygen contents. These observed trends were independent of the instrument type. In addition, when using an acidic transfer solvent, the overall observed response was reduced by a factor of $\sim 20$. Thus, the apparent pH of the transfer solvent has critical influence upon detection and upon the profile of different components observed within a complex mixture. In turn, this significantly impacts oil sands environmental monitoring for toxicity, forensic interpretation, and quantitation; when comparing data sets from different laboratories, these findings should therefore be taken into account.

KEYWORDS

Oil sands process-affected water; Fourier transform ion cyclotron resonance; Orbitrap; mass spectrometry; environmental monitoring; naphthenic acids
The Athabasca oil sands deposits, located in the province of Alberta, Canada, are estimated to contain 1.7–2.5 trillion barrels of oil (Burrowes et al., 2009; Clemente et al., 2003). These non-conventional oil reserves require approximately 2–4 barrels of water to produce 1 barrel of crude oil (Barrow et al., 2016), generating a large volume of oil sands process-affected water (OSPW), which is expected to reach $10^9$ m$^3$ by 2025. (Johnson et al., 2011). OSPW must be stored in tailing ponds as per the zero discharge policy that the industry is required to follow (Government of Canada, 2012; Martin, 2015). Storage of OSPW in close proximity to natural water sources (rivers, ponds, marshes) is of concern due to the possibility of tailings pond leakage and possible seepage into groundwater. Analysis of groundwaters and adjacent natural waters is required in order to monitor for possible leakage, seepage or accidental discharge, as it has been reported that OSPW is toxic to aquatic organisms (Allen, 2008; Marentette et al., 2015; Swigert et al., 2015). The caustic hot water extraction process used to extract the bitumen from the oil sands is known to concentrate the acidic species, namely naphthenic acids (NAs), and related components which are collectively known as naphthenic acid fraction compounds (NAFCs) (Allen, 2008; Headley and McMartin, 2004; Shell Canada Ltd., 2016). NAFC samples are complex mixtures that contain traditionally defined NAs, comprising compounds with a single carboxylic acid group, which follow the generic formula of $C_nH_{2n+2}O_2$ where $Z$ is the negative, even integer that represents the “hydrogen deficiency” and $n$ is the number of carbon atoms. Alternatively, it is possible to use double bond equivalents (DBE) as a measure of the number of rings and double bonds involving carbon atoms within the molecule. It is now known that the organic contributions to OSPW comprise a wide distribution of other components, including aromatic structures instead of alicyclic, higher oxygen contents ($O_x$) where molecules may include hydroxyl groups or multiple
carboxylic acid groups, and nitrogen- and/or sulfur-containing components (Barrow et al., 2015; Barrow et al., 2009; Grewer et al., 2010; Headley et al., 2011a; Headley et al., 2009).

Over the past decade considerable attention has been given to the development of ultrahigh resolution mass spectrometry to both characterize and quantify NAFC in oil sands related samples (Barrow et al., 2016; Barrow et al., 2004; Bowman et al., 2014; Brunswick et al., 2015; Chen et al., 2015; Headley et al., 2016; Headley et al., 2015; Headley et al., 2014; Headley et al., 2013; Headley et al., 2011b; Huang et al., 2016; Jie et al., 2015; Nyakas et al., 2013; Pereira and Martin, 2015; Pereira et al., 2013; Wilde et al., 2015). Due to the complexity of NAFC mixtures, the results are dependent upon numerous variables associated with the analytical procedure. In addition, awareness of ion suppression, matrix effects, and the need to use additives to create a reasonably stable ionization state (e.g. adding pH modifiers) or using derivatization to enhance ESI response of certain species should be evaluated as these factors can all play a role in the response factor and relative response factors of species as a consequence of the ESI mechanism. To minimize such phenomena, chromatography or the use of appropriate clean-up methods, such as solid phase extraction, may be used to reduce ion suppression and matrix effects. Other variables such as the extraction pH and choice of solvent can lead to significant variation in the levels and profiles of NAFCs detected (Barrow et al., 2016; Headley et al., 2013; Headley et al., 2007; Huang et al., 2015). Furthermore, the experimental parameters of the instrumentation, such as choice of ionization method (e.g. electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), electron ionization (EI)) and ionization polarity (+/-) have been shown to have a profound effect on what is detected (Barrow et al., 2015; Barrow et al., 2014; Barrow et al., 2010). For example, positive ion polarity provides more
favorable detection of basic (nitrogen containing) components and negative ion polarity acidic components. Additionally, APPI permits the detection of neutral species, such as polycyclic aromatic hydrocarbons (PAHs) and sulfur-polycyclic aromatic hydrocarbons (PASHs) that would otherwise not be ionized using ESI. The majority of the numerous analytical protocols reported utilize negative-ion ESI coupled to mid to ultrahigh resolution mass spectrometers. Sample introductions by flow injection without chromatography and with full chromatographic separations are currently used, both with success (Headley et al., 2016; Headley et al., 2013). The latter bears the advantage of decreased ionization suppression without prior sample cleanup (i.e. solid phase extraction) but also the disadvantage of extended analytical run times. Flow injection analyses typically use a transfer solvent containing 0.1% ammonium hydroxide (NH$_4$OH) (c) as a pH modifier to aid in the ionization process while chromatographic methods use 0.1% formic acid (HCOOH) to adjust the transfer solvent to a pH value below the pKa of NAs (~ 4.5) to allow analyte interaction with the liquid chromatography (LC) column phase in order to achieve sufficient separation.

A variable that has been overlooked with respect to its effects on NAFC analysis is the transfer solvent pH. This study reports the effects of the apparent pH of the transfer solvent upon the response of different organic species (namely the O$_x$ compound classes) present within an Athabasca oil sands derived OSPW extract. Measurement of pH in solvents and aqueous solvent systems is difficult with respect to achieving accurate pH measurements, hence the accepted term for solvent system pH measurements is “apparent pH” and is used throughout this work. To demonstrate that the effects are not instrument-specific, negative-ion ESI experiments were performed using two different mass spectrometers: a Fourier transform ion cyclotron resonance
mass spectrometer (FT-ICR MS) located at the University of Warwick, UK, and an Orbitrap mass spectrometer, located at the National Hydrology Research Centre, Saskatoon, Canada. Furthermore, two commonly used methods of sample introduction were utilized, namely direct infusion with a syringe pump for FT-ICR MS and injection into the eluent of a liquid chromatograph for Orbitrap MS experiments. The results indicate that the transfer solvent apparent pH plays an important role, influencing the relative responses of the compound classes detected and trends observed are independent of the instrument used. Furthermore, transfer solvent apparent pH has a critical impact upon data sets that were acquired using different analytical protocols and used for comparative environmental and monitoring studies along with toxicological investigations (Hughes et al., 2017; Morandi et al., 2015).

2 MATERIALS AND METHODS

2.1 OSPW EXTRACT

A large volume extract of Athabasca oil sands-derived OSPW was obtained following the procedures previously described (Janfada et al., 2006; Rogers et al., 2002). Briefly, OSPW (2000 L) was acidified to pH 2 with HCl (Fisher Scientific Company, Ottawa, Ontario, Canada) and serially extracted (3 times) in 2 L batches with dichloromethane (DCM) (Fisher Scientific Company, Ottawa, Ontario, Canada). The combined DCM fractions was evaporated to dryness and the residue was reconstituted in 0.1 M NaOH (VWR International, Edmonton, Alberta, Canada) followed by ultra-filtration using a Millipore®1000 MW cutoff membrane (Sigma-Aldrich Canada Co., Oakville, Ontario, Canada).
Sample characterization was performed using two instruments, the first of which was an LTQ Orbitrap Elite (Thermo Fisher Scientific, San Jose, CA) operating in full scan and negative-ion mode. Mass resolution was set to 240,000 (at m/z 200) with an m/z scan range of 100-600; the measured resolving power at m/z 200 was 268,000. The ESI source was operated as follows: sheath gas flow rate 25 (arbitrary units), spray voltage 2.90 kV, auxiliary gas flow rate 5 (arbitrary units), S lens RF level 67%, heater temperature 50 °C, and capillary temperature 275 °C. As per Composer data analysis, the mass error was < 2 ppm for all mass assignments. For the high pH experiment, the transfer solvent used was 50:50 acetonitrile:water containing 0.1% NH₄OH, while the low pH transfer solvent experiment used 50:50 acetonitrile:water containing 0.1% HCOOH. A flow rate of 200 μL min⁻¹ was used for both eluent pH conditions (apparent pH 9.1 and 3.2) delivered by an Accela 1250 solvent pump (Thermo Fisher Scientific, San Jose, CA). All apparent pH measurements of the eluent solutions were performed using an Accumet AB15 pH meter (Fisher Scientific Company, Ottawa, Ontario, Canada) calibrated using a three point pH calibration method. Under gentle magnetic stirring, sufficient time (~ 3 min) was permitted for a stable pH reading to be established. The OSPW extract was diluted 100-fold in non-pH adjusted 50:50 acetonitrile:water. 5 μL of the diluted extract was injected into the transfer solvent stream using a Thermo PAL-HTC Accela autosampler (Thermo Fisher Scientific, San Jose, CA). The software used for instrument control/data acquisition and molecular analysis was Xcalibur version 2.1 (Thermo Fisher Scientific, San Jose, CA) and Composer version 1.5.2 (Sierra Analytics, Inc., Modesto, CA) respectively.

2.3 FT-ICR MS
The OSPW extract was diluted 900-fold in 50:50 acetonitrile:water. Individual aliquots of this stock solution were used and NH$_4$OH or HCOOH (Sigma-Aldrich Company Ltd., Gillingham, Dorset, United Kingdom) were added at concentrations ranging from 0.025\% - 1\% to produce solutions over a range of apparent pH values. The apparent pH of each solution was recorded using a Hanna pH 20 meter (Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, United Kingdom), calibrated with external standards (Thermo Scientific Orion, Thermo Fisher Scientific, Hemel Hempstead, Hertfordshire, United Kingdom). The apparent pHs are reported in Table 1. Additionally, three organic compounds of varying oxygen content, each containing at least one carboxylic acid group (see Figure S3), were studied using FT-ICR MS. Biphenyl-4-carboxylic acid, anthraquinone-2-carboxylic, and trimesic acid (Sigma-Aldrich Company Ltd., Gillingham, Dorset, United Kingdom) represented the O$_2$, O$_4$, and O$_6$ compound classes, respectively, and were dissolved as 0.02 mg mL$^{-1}$ in 50:50 MeCN:H$_2$O, with the addition of either 0.1\% NH$_4$OH or 0.1\% HCOOH (see Table S1).

Mass spectra were acquired using a 12 T solariX Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), coupled with an Apollo II ESI source. The instrument was operated in negative-ion mode. Nitrogen was used as the drying gas at a temperature of 220 °C at a flow rate of 4 L min$^{-1}$. The nebulizing gas was nitrogen and was maintained at a pressure of 1.2 bar. Samples were infused using a syringe pump at a rate of 200 μL h$^{-1}$ without the activation of in-source dissociation. 4 MW data sets were acquired using magnitude mode, with a detection range of m/z 128-1500. After acquiring 200 scans, the data were zero-filled once and apodized using a Sine-Bell function prior to applying a fast Fourier transform. For the apodized data, the measured resolving power at m/z 200 was 830,000. Data were internally
calibrated using homologous series and analyzed using DataAnalysis 4.2 (Bruker Daltonik GmbH, Bremen, Germany), prior to the data being imported into Composer 1.5.4 (Sierra Analytics, Modesto, CA, USA) for compositional analysis; Aabel NG2 v.5.2 (Gigawiz Ltd. Co., Tulsa, Oklahoma, USA) was used for data visualization.

Tuning parameters play an important role, particularly with respect to biasing the detection of either high or low mass analytes. In order to eliminate/reduce such effects and to focus upon the effects of adjusting the transfer solvent pH, the initial tuning of the two instruments was not changed during the course of these experiments.

Table 1. Apparent pHs of the nine sample solutions analyzed by direct infusion negative-ion ESI FT-ICR MS and two transfer solvents by flow injection negative-ion ESI Orbitrap MS.

<table>
<thead>
<tr>
<th>Additive</th>
<th>FT-ICR MS</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄OH / %</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Apparent pH</td>
<td>11.2</td>
<td>9.9</td>
</tr>
<tr>
<td>HCOOH / %</td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>Apparent pH</td>
<td>3.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*High purity NH₄OH and HCOOH were added to the samples. The sample with 0% corresponds to the original stock solution without the use of any additives.

3 RESULTS AND DISCUSSION

3.1 MASS SPECTRA AND CLASS DISTRIBUTION
For a given sample, analyses using the same instrument parameters but different transfer solvent apparent pH values, the mass spectra showed differences in both intensity and mass distribution. This trend was observed in the data obtained from both Orbitrap and FT-ICR mass spectrometers. For example, Figure 1 illustrates mass spectra obtained using negative-ion ESI Orbitrap and FT-ICR MS under basic and acidic transfer solvent conditions. For the Orbitrap data using acidic transfer solvent, the $m/z$ distribution was centered in the range of $m/z$ 240-285, compared to $m/z$ 195-240 using basic transfer solvent. Similarly, a significant shift to higher $m/z$ species is observed in the FT-ICR MS spectra when using acidified conditions. These trends and acquired mass spectra were reproducible for both the Orbitrap and the FT-ICR throughout the study (rsd = 0.7% and 1.4% respectively) and at a minimum, included duplicate analysis.
Figure 1. Differences in the mass spectra observed under the different conditions: negative-ion ESI Orbitrap MS for a) basic eluent (0.1% NH₄OH, apparent pH 9.1) and b) acidic eluent (0.1% HCOOH, apparent pH 3.2), and negative-ion ESI FT-ICR MS for c) basic solvent (0.1% NH₄OH, apparent pH 9.4) and d) acidic solvent (0.1% HCOOH, apparent pH 3.2) with an inset showing m/z range 600 - 800.
Data processing provided insight into the compositional differences observed in the mass spectra.

For example, class distribution plots (Figure 2) show that basic transfer solvent conditions favor the lower oxygen-containing species, such as the $O_2$ class, while acidic transfer solvent conditions favor the higher-containing $O_x$ classes, with the $O_4$ class being predominant.

**Figure 2.** Top: class distribution obtained by negative-ion ESI Orbitrap MS using basic (0.1% NH$_4$OH, apparent pH 9.1) and acidic (0.1% HCOOH, apparent pH 3.2) transfer solvents. Bottom: class distribution obtained by negative-ion ESI FT-ICR MS using basic (0.1% NH$_4$OH, apparent pH 9.4) and acidic (0.1% HCOOH, apparent pH 3.2) conditions.

As shown in Figures 1 and 2, similar trends for negative-ion ESI MS results were observed by two independent laboratories, each using a different mass spectrometer and different sample...
Due to differences in instrument tuning (influencing operational $m/z$ ranges) and in resolving power, the number and relative contributions for compound classes observed using the two instruments are not identical. Despite this, both laboratories observed the same trends with respect to the effects of apparent pH of the transfer solvent upon sample profiles. In particular, very similar results were obtained when examining the relative contributions of the $O_2$ and $O_4$ classes, in turn affecting the $O_2/O_4$ ratio that has been proposed as having potential for environmental forensics. Frank et al. reported use of $O_2/O_4$ ratios as a diagnostic tool during forensic studies for sample source determination (Frank et al., 2014). It was found that use of a basic transfer solvent favored the detection of the $O_2$ species within the NAFC mixture, while an acidic transfer solvent tended to favor increased relative response of the higher oxygen containing species, such as $O_3$ to $O_6$ (Figure 2). For oil sands-related samples, negative-ion ESI experiments are traditionally used to study compounds expected to incorporate one or more carboxylic acid groups. The carboxylic acid site must deprotonate for the molecule to form a negatively-charged ion and the addition of acid, lowering the apparent pH, increases the probability of any given carboxylic acid site retaining its proton. At least one, overall negative charge is required, however, for the species to be observed using negative-ion ESI. Additional carboxylic acid groups increase the probability of deprotonation, while other oxygen-containing functional groups potentially help to stabilize the presence of negative charge (Hindle et al., 2013). As one example regarding the number of carboxylic acid groups, the first acid dissociation constant ($pK_a1$) of propanoic acid is 4.87, while its dicarboxylic counterpart, propanedioic acid, has a $pK_a1$ of 2.83. As a result, it would be expected that lower apparent pHs will suppress overall detection of oil sands components especially those of lower oxygen contents when using negative-ion ESI experiments. Figure 3 represents one of the central findings, where an $O_2/O_4$ ratio of 1.67 to 6.89 was observed using
basic conditions, a ratio of only 0.03 was observed when using acidic conditions. It is therefore clear that the apparent pH of the solution significantly influences the O$_2$/O$_4$ ratio and has consequences for environmental forensics. For example, point source determination based on naphthenic acid composition would lead to inaccurate conclusions if the same eluent pH was not used by the same or multiple laboratories for the mass spectrometric analysis. This forensic tool may still be valid if results are compared using the same instrumental conditions and transfer solvent pH, but the validity would no longer hold if data from different laboratories, using different transfer solvent pH, were compared. It is therefore essential that this is taken into account when comparing data between laboratories. In addition, recent toxicity techniques (Hughes et al., 2017; Morandi et al., 2015) rely heavily upon mass spectrometric data, both quantitatively and qualitatively, for the identification of principal toxic components that attribute to end-point responses being measured. Depending upon the apparent pH (basic/acidic) of the eluent being used, the interpretation of the mass spectrometric data would have a significant impact upon the assignment of which components are contributing towards the toxicity of the sample. Quantification of NAs entails the detailed analysis of a complex mixture; biasing the response of low O$_x$ or high O$_x$ components (due to the influence of the eluent apparent pH) would impact the final quantitative result for identical samples. This, in turn, may lead to incorrect or inconsistent conclusions with respect to toxicity assignment, with adverse consequences for remediation strategies.
Figure 3. Normalized bar chart showing the change in $O_2/O_4$ ratio with transfer solvent additive.

FT-ICR MS experiments were performed using nine sample solutions, with HCOOH or NH$_4$OH added to the diluted OSPW extract in quantities ranging from 0.025% to 1%. The $O_2/O_4$ ratio was relatively consistent when NH$_4$OH was added and when no additive was used (Figure S1), but inverted, with the $O_4$ class being more prominent, once HCOOH was added. The results demonstrate both the consistency of the observations and the significant change in ratio when changing sample preparation from use of no additives to using just 0.025% HCOOH (apparent pH of 3.6).

As an illustrative example of how oxygen content and structure play important roles in influencing the observed signal, commercially available compounds were examined (see Table S1 and Figure S3), in addition to the characterization of the OSPW, which represents a complex mixture of potentially unknown components. The three compounds were examples of the $O_2$, $O_4$, and $O_6$
compound classes, where one molecule included a single carboxylic acid group, the second molecule included one carboxylic acid group and two carbonyl groups, and the third molecule incorporated three carboxylic acid groups. The use of these compounds is illustrative but does not represent a comprehensive investigation of the effects of functional groups and other structural features, which is not within the scope of the current study. Oxygen may be incorporated as hydroxyl, aldehyde, ketone, ether, ester, or carboxylic acid groups, for example, and such structures will differ in ionization response (Hindle et al., 2013). Furthermore, for functionalized aromatic compounds, resonance stabilization and position of substitution (ortho, meta, and para) will also play important roles in determining stability of the anions relative to the neutral molecules. Finally, solubility of different structures (Headley et al., 2007) will also influence the probability of observation during characterization using mass spectrometry. The signals of the singly-charged $O_2$, $O_4$, and $O_6$ species were measured after addition of 0.1% HCOOH or 0.1% NH$_4$OH to the solutions. Figures S4, S5, and S6 show that the compounds of higher oxygen content have a proportionally higher response, compared to species of lower oxygen content, following addition of acid (lower apparent pH). The $O_2$:$O_4$ and $O_2$:$O_4$:$O_6$ ratios therefore inverted when switching from the addition of base to the addition of acid and were at their lowest when using acidic conditions. These results are consistent with the observations for the OSPW sample.

$O_2$-containing species have been reported as the primary components of concern within the NAFC fraction with regards to toxicity towards aquatic ecosystems (Hughes et al., 2017; Morandi et al., 2015; Yue et al., 2015). Monitoring water systems in and around mining activities for NAFC is required to regulate and ensure leakage or accidental spillage of OSPW is not occurring. Development of analytical methods for monitoring is still ongoing but methods have been recently
published that show improved sensitivity and robustness. For example, Brunswick et al. report
the use of LC coupled time-of-flight (TOF) MS (LC-TOF-MS) for routine analysis of water
samples (Brunswick et al., 2015). This method sums the areas of peaks that match the $O_x$
components detected and reports the total NA concentration from this summation. The method
uses an acidic eluent for the LC separation which may, according to the findings of this study,
enhance the detection of high $O_x$ species but attenuate the detection of $O_2$ species. Since it is almost
impossible and impractical to calculate the response factor of each component contained with the
$O_x$ families, due to a lack of standards for all compositions and isomers, caution is warranted for
comparison of results with other methodologies that employ different pH eluent conditions.

3.2 DBE OF $O_2$ CLASS OF COMPOUNDS

As the $O_2$ class is implicated as the most toxic class of components in OSPW, further data analysis
was performed to reveal the effects of transfer solvent apparent pH with respect to $O_2$ DBE and
carbon number distributions. Figure 4 illustrates the significant effect of transfer solvent apparent
pH on the DBE distributions of the $O_2$ class of compounds from OSPW extract; an alternative
arrangement of the same data can be found in Figure S2. At apparent pH 3.2, there are differences
between the Orbitrap and FT-ICR MS data sets at first glance, but closer inspection reveals the
contributions for entire homologous series are below 1%. As a result, low intensity summed
contributions (e.g. ~ 0.1% - ~ 0.8%) for a given DBE are being compared and caution is warranted
when attempting to draw conclusions. Under basic conditions, the percentage contributions
become much greater and comparisons of the data become more robust. The percentage
contributions for the FT-ICR MS data are lower due to detection of a greater number of classes
than for the Orbitrap MS data, thus each class must represent a smaller fraction. The predominant
DBE ranges differed between the Orbitrap MS and FT-ICR MS data, where higher DBE components were more pronounced in the FT-ICR MS data. As with O₂/O₄ ratios, DBE plots can be used as forensic tool for NAFC source identification. For comparative studies, it is thus imperative to compare data from similar instruments and to keep the transfer solvent apparent pH consistent.

**Figure 4.** O₂ DBE plots obtained from negative-ion ESI Orbitrap (top) and FT-ICR MS (bottom) data, using acidic and basic conditions.

For further insight, the FT-ICR MS data in this study was examined in terms of double bond equivalents (DBE) and carbon number for the O₂ class, as a function of apparent pH (Figure 5). A steady decrease in the intensity and number of peaks associated with the O₂ class was observed with increasing HCOOH concentration (decreasing pH). Under acidic transfer solvent pH
conditions, carbon numbers 15 and higher with DBE 3.5 and 4.5 have decreased or disappeared altogether, once below the detection threshold, as previously highlighted in Figure 4. In addition, overall intensities are approximately 400 times lower for the O₂ class when using acidic transfer solvent containing 0.1% HCOOH. Similar trends were observed for the overall NAFC response with the acidic transfer solvent displaying a response of 20 times less than that of the basic pH transfer solvent.

Figure 5. DBE vs. carbon number plots using FT-ICR MS data, showing the decrease in intensity and reduction of number of peaks associated with the O₂ compound class.
3.3 VAN KREVELEN PLOTS FOR Oₓ CLASSES

While DBE vs. carbon number plots are useful for examining a single compound class at a time, van Krevelen plots can be used to compare contributions from multiple Oₓ classes at once. Combined van Krevelen plots of H/C against O/C ratios for the Oₓ classes (Figure S7) show that samples analyzed under basic conditions exhibit a larger number of data points at low O/C coordinate values. The higher data point density (number of data points within the same H/C vs. O/C space) in the low O/C and low H/C coordinates observed under basic conditions, as compared to data acquired using acidic conditions or using the Orbitrap, can be attributed to the minimum relative abundance filter setting used during data processing; the total number of compositional assignments, is higher for the FT-ICR MS, which affords higher resolving power.

3.4 CONCLUSION

Transfer solvent apparent pH has a significant impact on the overall class distribution of NAFCs detected by negative-ion ESI MS, regardless of instrument type or method of sample introduction. It is clear that the observed O₂/O₄ ratio is significantly influenced by pH, with consequences for environmental forensics. When attempting to make a comparison of data sets from different laboratories, differences in the apparent pH of the transfer solvent should be accounted for to prevent erroneous interpretation. This observation may also hold true for other multi-component analyses where individual standards are not available. It is therefore essential to choose a method that best suits the intended end use of the data. For example, if O₂ species are of most interest, the best overall response (both qualitative and quantitative) is achieved using a high pH transfer solvent when analysis is performed by negative-ion ESI MS. In terms of relative response, acidic eluent either favors the detection of Oₓ classes of higher oxygen content or conversely suppresses
the lower oxygen contents (e.g. O$_2$); the latter is more likely, as overall response is reduced by a factor of ~ 20 when using acidic eluent during negative-ion ESI MS analysis. The findings from two laboratories, using two varieties of mass spectrometer and different methods of sample introduction, illustrate that very similar trends are observed when monitoring O$_2$/O$_4$ ratios. It is important to consider what is being measured and how, and caution should thus be exercised for oil sands environmental monitoring of the O$_2$ class and when measuring ratios of NAFCs classes for inter-laboratory comparisons.

DECLARATIONS OF INTEREST: None

APPENDIX A: SUPPLEMENTARY DATA

Supplementary data related to this article can be found at ___

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