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1 **Direct Mass Spectrometric Analysis of Naphthenic Acids and Polycyclic Aromatic**
2 **Hydrocarbons in Waters Impacted by Diluted Bitumen and Conventional Crude Oil**

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32 Highlights:

- 33 1. Constructed waters impacted by diluted bitumen (DB) and conventional crude (CC) across
34 salinity and pH range.
35 2. Online membrane sampling coupled to direct mass spectrometry measures trace contaminants
36 in water.
37 3. DB imparts higher concentrations of naphthenic acids with both oils exhibiting marked pH
38 dependence.
39 4. CC imparts higher concentration of naphthalenes with no observable pH or salinity effects.
40 5. Mass spectral data is used to discriminate water impacted by DB and CC oils.
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Abstract

The accidental release of hydrocarbons has well-documented, deleterious impacts on the hydrosphere. There are several classes of toxic petroleum derived compounds, such as naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs), which may present a contamination hazard in the event of an oil spill. We examine the aqueous-phase concentrations of these trace contaminants using a direct sampling, on-line, rapid screening technique known as condensed-phase membrane introduction mass spectrometry (CP-MIMS). Water samples having a range of pH and salinity were equilibrated with either diluted bitumen (DB) or conventional crude (CC) oil. CP-MIMS is employed to directly measure concentrations and isomer class profiles of classical NAs in the aqueous phase using electrospray ionization in negative-ion mode as $[M-H]^-$. Aqueous PAH concentrations are monitored using CP-MIMS coupled to liquid electron ionization (LEI) source in positive-ion mode using selected ion monitoring (SIM) of the molecular ion (M^+). Aqueous NA concentrations are observed to be highly pH-dependent, and are an order of magnitude greater in water samples contaminated with DB compared to CC. Conversely, aqueous concentrations of naphthalene and alkyl-naphthalenes are found to be greater in water samples exposed to CC. Principal component analysis of data generated from CP-MIMS for the NA profile in the aqueous phase enables discrimination of DB and CC as contaminant sources. In addition, we describe the use of online membrane sampling for real-time, direct detection of NAs and PAHs in the same sample. These compound classes are distinguished by both their perm-selectivity behaviour at the membrane and the ionization mode at the instrument. The in-situ analysis of trace organics in water with a simple workflow described here lays the foundation for applications of direct mass spectrometry to rapid screening of large sample sets and monitoring of aqueous-phase contamination and remediation processes in real-time.

1. Introduction

The oil sands deposits in northern Alberta, Canada represent a significant hydrocarbon resource, consisting of a complex mixture of mineral solids, water, and bitumen—a very heavy petroleum product (Mossop, 1980; Strausz and Lown, 2003). Shallow deposits are typically surface mined whereupon bitumen is recovered by using a modified Clark warm water extraction process (Clark and Pasternack, 1932; Masliyah et al., 2011; Masliyah et al., 2013). Bitumen contained in deeper deposits is often extracted *in situ*, for instance, by steam-assisted gravity drainage, whereby pressurized steam is injected into the subterranean deposit to reduce the bitumen's viscosity and allow it to flow back to the surface where it can be separated from water (Czarnecki, 2001; Mullins et al., 2007). In either case, the viscous bitumen is ultimately diluted with lighter hydrocarbons, such as natural gas condensate, to lower its viscosity and enable pipeline transportation to upgraders and refineries (Czarnecki, 2001; Long et al., 2002; Masliyah et al., 2013; Mullins et al., 2007).

Diluted bitumen (DB) contains a complex mixture of hydrocarbons and heteroatom-containing organic molecules of varying size, including several classes of toxic components that may present contamination hazards when introduced into aquatic environments (Dew et al., 2015; Masliyah et al., 2013). These toxic components include naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs). The aquatic behaviour of DB differs from that of better-studied conventional crude (CC) oils, such as those released during the Exxon-Valdez and Deepwater Horizon spills. Therefore, response and remediation techniques for spills of DB can differ substantially from those of CC (Lee et al., 2015). When DB is spilled onto the surface of water, it initially floats because its maximum permissible pipeline transportation density (940 kg/m^3) is less than that of water (Fischer et al., 2008). However, with time, weathering of DB allows a portion of its lighter diluent content to evaporate. Evaporative loss of light oil components increases the density of the bulk oil, potentially allowing a portion to submerge or sink, particularly in turbulent waters with high concentrations of suspended mineral fines which adhere to dispersed oil droplets and form dense oil-mineral aggregates (Hua et al., 2018; King et al., 2014; Stoyanovich et al., 2019). Because DB is much more viscous than CC, it is much less responsive to chemical dispersant application, particularly after weathering has occurred to any appreciable extent (Yang et al., 2018). There is a growing body of literature describing

investigations of appropriate spill response for DB (King et al., 2017), as well as the potential effects of spills off the coast of British Columbia, Canada, where increased tanker traffic is anticipated given the expansion of the Trans Mountain Pipeline (Johannessen et al., 2020). While the physical impacts of bulk spilled oil are an inherent cause for concern, questions have also been raised about the chemical and toxicological impacts that oil spills may have on water quality (Dew et al., 2015). Of particular concern are NAs and PAHs dissolved in the aqueous phase, which are mobile, bioavailable, and known to exhibit toxic effects on organisms (Brown and Ulrich, 2015).

NAs are problematic oil components for both industrial and environmental reasons, including the corrosion of oil pipelines, upgraders and refineries, as well as their acute toxicity towards aquatic life in the mg/kg range, possibly through disruption of the mitochondrial electron transport chain (Brown and Ulrich, 2015; Rundle et al., 2018). NAs are a structurally diverse class of compounds. Historically, NAs were first identified as amphiphilic cycloalkyl carboxylic acids originally isolated from water in the acid-extractable organics fraction of heavy oils. These classical NAs came to be recognized as carboxylic acids bearing any type of hydrocarbyl substituent and characterized by the formula $C_nH_{2n+Z}O_2$, where n is the carbon number (typically 10 to 20) and Z is the hydrogen deficiency due to the presence of rings and/or double bonds (typically $Z = -2$ to -10). The definition of NAs has recently been expanded to include an even broader diversity of compounds containing heteroatoms, aromatic moieties, and other functional groups, often referred to as “naphthenic acid fraction compounds” (Headley et al., 2016). Classically-defined NAs have been shown to be acutely toxic in the low mg/kg range (Hughes et al., 2017), and have been demonstrated to disrupt the endocrine system at sublethal doses (Brown and Ulrich, 2015). NAs present a significant analytical challenge because of their enormous chemical complexity (up to 30,000 individual compounds have been reported), and the lack of an appropriate universal analytical standard for quantitation (Kovalchik et al., 2017). On the other hand, PAHs are a relatively well-defined and discrete analyte class, consisting of molecular structures based on fused aromatic rings. PAHs are well-studied carcinogens that are routinely screened as 16 priority PAHs by gas chromatography-mass spectrometry (Stout et al., 2015). In recent years, interest in various alkyl-substituted PAHs (*e.g.* C1 - C4) has been

growing in terms of their potential toxicity and use as forensic markers (Ahad et al., 2015; Ramirez et al., 2014; Stout and Wang, 2016).

Numerous studies have investigated the bulk release of petroleum into water systems. Recent work by Yang *et al.* investigated the aquatic behaviour of DB in the presence or absence of suspended sediments (Yang et al., 2018). Hounjet *et al.* conducted a systematic study of DB and CC phase distributions in aquatic systems over a range of pH and salinity in which they reported that both DB and CC released more total oil content (alkanes) to the aqueous phase at lower salinity and higher pH (Hounjet et al., 2018). For greater chemical specificity and source identification, techniques such as Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and GC-MS have been used for environmental forensics based on polar (e.g. NAs) and non-polar (e.g. PAHs, alkanes) chemical profiles, respectively (Bayona et al., 2015; Corilo et al., 2013; Headley et al., 2016; Lemkau et al., 2010). After the San Francisco *MV Cosco Busan* oil spill, both techniques were used to determine the aqueous concentrations of polar (Corilo et al., 2013) and non-polar (Lemkau et al., 2010) components indicative of tank leakage. Eide *et al.* used electrospray ionization mass spectrometry (ESI-MS) to differentiate between several bio-oil preparations as well as marine diesel, auto-diesel, and jet fuel (Eide and Neverdal, 2014). These techniques provide detailed information about structurally-unique molecular components of samples due to their high mass and/or chromatographic resolution, providing a comprehensive ‘fingerprint’ with which to differentiate source oils. However, these approaches often require arduous sample clean-up steps to reduce or remove interfering matrix components. Such methods are, therefore, not amenable to *in situ* analysis. In the case of accidental release of petroleum into water, there is a compelling need to quickly and accurately identify the source oil in order to guide response actions and/or inform remediation efforts.

Condensed-phase membrane introduction mass spectrometry (CP-MIMS) is a rapid screening method, which has been employed to directly measure aqueous NAs and PAHs *in situ* (Duncan et al., 2016a; Termopoli et al., 2016; Vandergrift et al., 2019). The technique uses a semipermeable hollow fiber membrane (typically polydimethylsiloxane), which is directly immersed into a liquid or slurry sample. The membrane rejects ionized matrix components and particulate matter while allowing hydrophobic analytes to permeate through and dissolve into a

flowing acceptor phase solvent (Krogh and Gill, 2018). The acceptor phase (typically methanol), containing the membrane-permeable fraction of the sample, is then infused into an appropriate ionization source, often electrospray or liquid electron ionization (Termopoli et al., 2019a; Vandergrift et al., 2020), to provide real-time results, which can be employed to provide both qualitative and quantitative data. This method has been applied to a wide range of analytes and sample matrices, including pharmaceuticals in wastewater (Duncan et al., 2016b), fatty acids in salmon tissue (Borden et al., 2019), NAs in water (Duncan et al., 2016a) and constructed wetlands (Duncan et al., 2020), PAHs in water and soil samples (Termopoli et al., 2016; Vandergrift et al., 2019), and the determination of acid dissociation constants of trace organic contaminants in aqueous solutions (Feehan et al., 2019).

Herein, we determine aqueous-phase concentrations of NA and PAH contaminants in constructed water samples containing DB and CC oil over a range of salinity and pH. We do so by employing CP-MIMS, a rapid sampling and analysis method that uses on-line membrane extraction coupled directly to a mass spectrometer without sample clean-up or chromatographic separation.

2. Materials and Methods

2.1 Preparation of Oil-Contaminated Water Samples

Samples were prepared by Natural Resources Canada (NRCan). A stock solution of synthetic marine water was prepared by dissolving NaCl, Na₂SO₄, KCl, NaHCO₃, MgCl₂, and CaCl₂ in deionized water (18 MΩ·cm, 0.055 μS/cm) to the concentrations reported by Kester (Kester et al., 1967). The resulting synthetic marine water stock solution had a measured electrical conductivity of ~50 mS/cm, comparable to that of natural seawater in the Pacific northwest *ca.* 40 mS/cm (Tyler et al., 2017). Water types of varying salinity were then prepared by diluting aliquots of the marine water stock solution with deionized water by factors of 10, 100, and 1000 to prepare synthetic brackish, fresh, and very fresh water types, respectively. Sub-samples of each were adjusted to pH = 2, 5, 8, 10, and 12 using HCl, NaHCO₃, and NaOH, as needed. Due to precipitation of alkaline earth salts from marine water adjusted to pH 10 and 12, and from brackish water adjusted to pH 12, these samples were instead prepared with only NaCl to give electrical conductivities that were similar to the other marine and brackish water samples (50 and

5 mS/cm, respectively; Table S1). The petroleum products, Cold Lake Blend diluted bitumen (DB) and Mixed Sweet Blend conventional crude (CC), were obtained from pipelines in the Edmonton, Alberta area and stored at 15 °C in well-sealed containers prior to use. Oil-contaminated water samples were prepared by adding 2 g of either DB or CC to 20 mL of each water type in a 40 mL sample vial (2 oil types $\times$ 4 salinities $\times$ 5 pHs = 40 samples; Figure 1). The vials containing the oil and water mixtures were agitated, then left sealed and undisturbed at room temperature for about two weeks before storing at 4 °C until analysis (Table S1).

In addition, several well-buffered water samples were prepared to maintain a constant pH in the oil-contaminated water samples. Buffer solutions were prepared at pH 5.05 (glutamic acid; Aldrich Chemical Co., Oakville, ON), 8.76 and 10.47 (both glutamine, BDH Chemicals, Poole, England). Amino acid buffers were used because of their compatibility with direct CP-MIMS analysis; at pH 3, the buffer components remain ionized in solution, and therefore are impermeable to the polydimethylsiloxane (PDMS) membrane, and so do not interfere with ESI analyses. Buffers were adjusted to the desired pH levels using aqueous 3 M HCl (Fisher Scientific, Ottawa, ON) and NaOH (Fisher Scientific), and maintained a buffer capacity of 10–25 mM. The pH of these buffer solutions was measured before and after addition of oil to samples using an Accumet AR20 pH meter (Fisher Scientific, Ottawa, ON) with an Accumet 13-620-108A glass electrode (Fisher Scientific), calibrated at pH 2.00, 4.00, 7.00, and 10.00 using commercial calibration buffers (Fischer Scientific). Stock oil (2 mL supplied as 50/50 v/v in toluene) was added to 20 mL of the buffered aqueous solution and allowed to equilibrate at room temperature for two weeks.

Sample analysis began with transferring an aliquot of the aqueous phase via pipette into a clean 40 mL TOC vial. To minimize uptake of oil phase along with the aqueous phase, positive pressure was applied to the pipette as it passed through the oil-water interface, and the pipette tip was wiped clean before delivering the aliquot into the receiving vessel. The aliquot was then diluted gravimetrically by factors of 3 to 150 with 18 M Ω ·cm deionized water (Facility Scale Reverse Osmosis/Ion Exchange Water Purification System, Applied Membranes Inc., Vista CA, USA) prior to direct analysis to bring samples into the linear dynamic range (0.010–1.0 mg NAs/kg). For NA analysis, the pH of the sample was adjusted to 3 using 6 M HCl to protonate

carboxylate ions (*i.e.*, ensure that they were converted into neutral carboxylic acids) for membrane permeation, whereas for PAH analysis, the sample was adjusted to pH 10 using 3 M NaOH, to prevent the permeation of carboxylic acids.

2.2 CP-MIMS Analysis of NAs and PAHs

Both NAs and PAHs were measured directly in the diluted aqueous phase by CP-MIMS (Figure 1). CP-MIMS probe construction and design has been described elsewhere (Duncan et al., 2011; Duncan et al., 2020; Duncan et al., 2015; Vandergrift et al., 2017; Vandergrift et al., 2019). Briefly, an immersion probe consisting of a short length (typically 2–8 cm) of a capillary hollow fibre PDMS membrane (Dow Corning, Midland, MI, USA, Silastic Tubing, ID: 0.30 mm, OD: 0.64 mm) was mounted at both ends onto 22-gauge stainless steel hypodermic tubing. The ends of the hypodermic tubing were attached to polyether ether ketone (PEEK) tubing using zero dead volume stainless steel unions (Valco Fittings, Vici, Brockville, ON). An organic acceptor phase, 15/85 heptane/MeOH (v/v), was flowed through the membrane probe and directly into the mass spectrometer at a flow rate of 50 $\mu\text{L}/\text{min}$ using a syringe pump (Chemyx Fusion 100, Stafford, TX).

Figure 1. Schematic diagram of experimental design, CP-MIMS instrumental set-up, and example results. Bottom left: Systematic series of oil-contaminated water samples at four salinities and five initial pHs, for a total of twenty water types for each CC oil and DB. Middle: CP-MIMS instrumental setup—A syringe or HPLC pump delivers an acceptor phase solvent at 50 $\mu\text{L}/\text{min}$ through the membrane lumen, which is immersed in the aqueous sample. Top right: Example CP-MIMS chronogram for NA analysis (y-axis represents the sum of 27 m/z representing 95% of the standard NA mixture). Signal intensity is at baseline in a blank solution, when analyte-free acceptor phase reaches the MS. When the membrane probe is immersed in a sample or standard solution, the signal intensity rises due to the presence of a membrane-

permeable analyte in the acceptor phase. Bottom-right: Example CP-MIMS chronogram for PAH analysis. Stopped-flow methodology is used, so analytes reach the MS as a concentrated slug and sample concentrations are calculated from a calibration curve of the peak height.

2.3 Analysis of NAs

The ESI mass spectra of unresolved NAs from oil-contaminated water samples were measured in negative-ion mode as $[M-H]^-$ on a triple quadrupole mass spectrometer (Perkin-Elmer QSIg 220, Boston, MA, USA). Qualitative data was collected between m/z 100 and 500 with: step size of 0.5 m/z , dwell time of 1 msec at each step, and cone voltage of -20 V. The NA quantitation was carried out by comparing these spectra to those of a commercial mixture of NAs (Merichem Company, Houston, TX). The commercial mixture of NAs is hereafter referred to as “Merichem NAs.” While this mixture has been shown to contain thousands of individual NAs (Grewer et al., 2010), we observe that greater than 95% of the signal intensity is accounted for by 27 nominal mass-to-charge ratios, ranging from m/z 157 to 279 (See Figure S1). Thus, all quantitation was carried out using selected-ion monitoring (SIM) of these 27 m/z with cone voltage of -30 V and a 50 msec dwell time at each. The full list of mass-to-charge ratios used for quantitation and other electrospray parameters appears in Table S2.

To account for any changes in MS sensitivity due to drift and/or ESI-ionization suppression from matrix interference or high analyte loadings, a 5 ppb lauric acid- d_2 (Sigma-Aldrich, Oakville, ON) internal standard was included in the acceptor phase and monitored at m/z 201 (dwell time = 500 msec, cone voltage -22 V). The overall signal intensity was then corrected during quantitation with the formula (Duncan et al., 2015):

$$S'_t = S_t \times \frac{IS_{max}}{IS_t} \quad \text{Equation (1)}$$

where S'_t is the corrected signal intensity (Σ 27 SIMs) at a time t of the chronogram, S_t is the averaged raw signal intensity (Σ 27 SIMs) at a time t , IS_{max} is the maximum signal intensity for the internal standard (m/z 201), and IS_t is the signal intensity of the internal standard at a time t .

The concentration of NAs in an oil-contaminated sample is expressed as an equivalent amount of Merichem NAs giving rise to the same signal intensity in the Σ 27 SIMs. A calibration curve constructed using four Merichem standards with concentrations of 0–1000 $\mu\text{g/kg}$, showed good linearity ($R^2 > 0.99$, see Figure S2) and were used for NA quantitation. The measured signal intensities for each standard and sample were averaged over 3–5 minutes of steady-state signal (Figure 1), then the baseline was subtracted to determine S_t . Sample S_t values were then corrected as above to the corresponding S'_t values.

2.4 Analysis of PAHs

PAHs were analyzed using an Agilent 5975 GC-MS (Agilent Technologies Incorporated, Santa Clara, CA, USA) with a liquid electron ionization (LEI) source. This technique has been described elsewhere (Termopoli et al., 2019b; Vandergrift et al., 2019). Briefly, an 8 cm length of PDMS membrane was mounted on a CP-MIMS direct immersion probe and the acceptor phase was passed through the membrane lumen at 50 $\mu\text{L/min}$ using an HPLC pump (Agilent 1100 Series, Agilent Technologies Incorporated). The outflow from the membrane was passed through a passive flow splitter (*ca.* 250:1), where most of the acceptor phase was directed to waste and a nanoflow of *ca.* 500 nL/min was transferred to the LEI interface. The LEI interface consisted of a silica capillary (ID: 30 μm , OD: 150 μm) inserted 2 mm into the heated MS transfer line of the GC-MS system. The length of the MS transfer line was lined with an additional silica capillary (OD: 800 μm , ID: 400 μm). The combined action of heating from the MS transfer line and nebulization from a coaxial flow of He gas (1.0 mL/min) enacted the vaporization of the acceptor phase and any analytes therein. Liquid flow stability in this system was monitored via the source pressure gauge of the mass spectrometer which remained at $1.2(\pm 0.1) \times 10^{-4}$ Torr throughout all experiments. MS parameters were: Source temperature = 200 $^{\circ}\text{C}$, ionization energy = 70 eV, MS transfer line temperature = 350 $^{\circ}\text{C}$.

To enhance sensitivity, CP-MIMS-LEI measurements were performed in stopped-flow mode (Duncan et al., 2011; Krogh and Gill, 2018; Vandergrift et al., 2020). To do so, a 6-port valve system was included directly upstream from the passive flow splitter. The CP-MIMS probe was plumbed in as an injection loop. To perform a measurement, the valve was set to bypass the CP-MIMS probe (which was filled with acceptor phase), and the probe was directly immersed into a

sample or standard. After 150 s of immersion, the valve was switched, allowing acceptor-phase solvent to flow through the membrane lumen. This stopped-flow approach allows more time for analytes to partition into and across the membrane, accumulating more analyte prior to analysis. This approach generates a concentrated plug of analyte, which manifests as a distinct peak in the MS chronogram (Figure 1). To quantify PAHs in oil-contaminated samples, we calibrated their corresponding peak heights after a 150 s stopped-flow experiment using combined standards of naphthalene, anthracene, and pyrene. The risetime of the PAHs studied here is less than 90 s (Vandergrift et al., 2019), so the stopped flow experiments represent a similar analytical duty cycle to continuous flow mode with a 4-fold increase in sensitivity (Figure S4). PAHs and alkyl-PAHs were detected in SIM mode at unit mass resolution, with a 200 msec dwell time for each m/z . A summary of the m/z ratios used for PAH and alkyl-PAH monitoring is shown in Table S3. Naphthalene, anthracene, and pyrene isomer classes were measured at m/z 128, 178, and 202, respectively. Their alkylated (C_1 – C_4) analogues were measured in positive ion mode at $M+14$ (C_1), $M+28$ (C_2), $M+42$ (C_3), and $M+56$ (C_4).

2.5 Principal Component Analysis (PCA)

PCA was performed on negative-ion ESI fullscan data collected in profile mode using average of 2–3 mins (30–45 scans) of steady-state signal. Raw data was exported from the Perkin-Elmer Simplicity 3Q software (Acquisition version 1.4.1806.29651, Quantitation version 1.4.1806.29651) as a CSV file, which was subsequently imported into Microsoft Excel (Version 16.38; Redmond, WA). In Excel, the signals observed for a blank sample of distilled water adjusted to pH 3, observed within the same experiment, were used for background subtraction. These spectra were then imported into MATLAB (Version 2019b, MathWorks, Natick, MA) and processed using the PLS_Toolbox (Eigenvector Research Incorporated, Manson, WA). Each spectrum was unit vector-normalized to remove the effect of concentration on the qualitative separation. Each m/z signal was then mean-centred to zero the average signal intensity across the sample series. After pre-processing, PCA was performed to visualize the qualitative difference between the spectra obtained from DB- and CC-contaminated samples.

3. Results and Discussion

3.1 Naphthenic Acids (NAs)

NA fractions isolated from crude oils are enormously complex mixtures containing thousands of distinct molecular structures of hydrocarbyl carboxylic acids, which can be independently observed at high chromatographic and mass resolutions (Bowman et al., 2019; Headley et al., 2016; Hewitt et al., 2020; Ross et al., 2012). We have previously shown (Duncan et al., 2016a) that the PDMS membrane-permeable fraction of NAs consists predominantly of the O₂ class (i.e., C_nH_{2n+z}O₂) once the pH of the sample being analyzed is adjusted to a value less than the pK_a (i.e pH < 4). In negative-ion ESI, these are observed as isomer classes at [M-H]⁻. Figure S1 shows the mass spectra of NAs obtained from three different sources by direct CP-MIMS analysis of their acidified aqueous solutions. Each of the fullscan mass spectra derived from CC, DB, and Merichem NAs (a commercial NA standard) have an envelope of *m/z* peaks characteristic of NAs, with base peaks at *m/z* 199, 251, and 237 respectively. Each peak in a spectrum represents an individual isomer class (e.g., C₁₅H₂₆O₂ for *m/z* 237). While there are some notable differences in the mass spectra, which may enable source discrimination by multivariate statistical approaches, such as PCA (Bro and Smilde, 2014; Woudneh et al., 2013), quantitation remains a challenge due to the lack of a universal standard and the complex nature of the NA analyte class (Headley et al., 2016; Kovalchik et al., 2017). In this work, the normalized signal intensities of 27 *m/z*, chosen to account for more than 95% of the total signal intensity observed for the Merichem standard, were used for quantitation. This use of summed signal intensities to determine total NA concentrations has previously been employed for quantitation of other NA mixtures (Duncan et al., 2020; Letourneau, 2016). While we recognize the shortcomings of using an imperfect calibrant, this challenge is not unique to the work presented here (Kovalchik et al., 2017), and provides a consistent relative approach for total NA concentrations. This method showed excellent linearity for Merichem standards in the µg/kg range with R² ≥ 0.99 (Figure S2; estimated LOD 0.001 mg/kg), while samples contaminated by either CC or DB generated significant signal intensity among these 27 mass-to-charge ratios. Quintuplet analysis of a DB-contaminated sample yielded an RSD of 8%, similar to that reported for previous analysis of NA by CP-MIMS (Duncan et al., 2020).

Figure 2 summarizes the aqueous concentrations of NAs in water samples of varying salinity and pH that have been saturated with DB or CC. In general, we observe that NA concentrations of

aqueous phases derived from DB are approximately 10 times greater than those from CC. This observation is consistent with DB having a higher total acid number (0.89 mg KOH/g) than CC (< 0.10 mg KOH/g) (Hounjet et al., 2018).

A clear trend appears across the pH range for both DB and CC samples, with higher total concentrations of NAs appearing at higher initial pH of the water sample, as seen in Figure 2 and Table S4. The equilibrium concentration of any single NA structure in the aqueous phase is determined by the following intrinsic and extrinsic factors: 1) its oil-water partitioning coefficient (K_{ow}), 2) its acid dissociation constant (K_a), 3) its initial concentration in the source oil, and 4) the pH of the solution at ambient temperature. The dependence of aqueous NA concentrations on these factors has been previously modelled (Bertheussen et al., 2018a; Bertheussen et al., 2018b; Havre et al., 2003). Modelled plots of aqueous NA concentrations versus water pH exhibit sigmoidal geometries with inflection points at pK'_a :

$$pK'_a = pK_a + pP_{wo} \quad \text{Equation (2)}$$

Where pK'_a is the pH at which half the NA of interest is in the aqueous phase, pK_a is the negative logarithm of the NA's acid dissociation constant, and pP_{wo} is the negative logarithm of P_{wo} , a constant representing the NAs partitioning between the crude oil and water phases, which accounts for NA dimers within the oil phase (Bertheussen et al., 2018b). As the solution pH increases, NAs are deprotonated, leaving behind charged conjugate bases that are more soluble in the aqueous phase. While this pH effect is readily observed for both CC- and DB-contaminated samples (Figure 2), it is more pronounced in the latter due to the higher concentration of NAs in the DB source oil. We have previously reported pK_a values for NA isomer classes to be in the range of 5.1–5.7 (Feehan et al., 2019). Such values are anticipated to result in a levelling of aqueous NA concentrations at $pH > 8$, depending on the value of pP_{wo} , which varies across isomer classes (Bertheussen et al., 2018b). The fact that we do not observe such levelling has prompted further investigation. We found that the pH of the oil-contaminated water samples was substantially lower than the pH of the water prior to the addition of oil. For example, a water sample prepared with an initial pH of 8 was observed to have a pH 4.5 after prolonged contact with the oil (Table S1), presumably due, in part, to its NA content.

Figure 2. Total aqueous NA concentrations as a function of the initial (nominal) pH and salinity in DB- and CC-contaminated water samples, determined using CP-MIMS-ESI. With the exception of marine pH 10 & 12 and brackish pH 12 samples (which were prepared with NaCl solution), samples with solid colours were prepared with synthetic seawater at various dilutions (1, 10, 100, or 1000x) and/or with pH adjustment. Buffered samples (striped bars) were prepared with amino acid buffers in deionized water. Replicate analysis ($n = 5$) of the DB-affected pH 12 freshwater sample yielded a relative standard deviation of 8% (added above for clarity).

Details of the initial and final pH of solutions appear in Table S1. To better understand the relationship between pH and total aqueous NA concentrations, a series of well-buffered aqueous solutions was prepared. We found that buffered DB-contaminated samples had higher aqueous NA concentrations than unbuffered samples at the corresponding initial pH. Furthermore, increases in pH above 10 had relatively modest impacts on the total NA concentrations (striped bars, Figure 2). A plot of the relative abundance of NA isomer classes in the aqueous phase of buffered solutions (Figure S3) are consistent with the work of others, which showed pK'_a at pH *ca.* 8–9 for most NA species (Bertheussen et al., 2018b; Havre et al., 2003). Conversely, in CC-contaminated samples, the presence of a buffer had a less pronounced effect on aqueous NA concentrations over the pH range examined (striped bars, Figure 2).

In general, the water-solubility of trace organic compounds decreases with increasing salinity (Schwarzenbach et al., 2016). However, this trend is not apparent for NAs in waters of varying salinity, as seen in Figure 2. In this case, aqueous NA concentrations appear to increase slightly

at elevated salinities for both CC and DB. The degree to which this trend occurs above and beyond our precision limits ($\pm 10\%$) may be explained by either (1) greater chemical activity of NAs at higher salinity and (2) greater partitioning of NAs into the aqueous phase due to buffering capacity of waters with higher salinity. Higher chemical activity of NAs in saline solution would result in increased driving force for membrane transport (LaPack et al., 1990) and a positive bias in the final NA concentration. However, a control experiment where the CP-MIMS steady-state signal of a NA standard solution was fortified with 3% w/w NaCl (48 mS/cm) displayed no marked change in the signal intensity over a 20-minute sampling window (Figure S5). This suggests that changes to NA concentration is not an artifact of the measurement strategy. Instead, we propose that the increased buffering capacity of the marine and brackish waters provided a higher equilibrium pH, allowing more NAs to partition into the aqueous phase. Given the high pK'_a reported for high MW NAs (Bertheussen et al., 2018b), which dominate the spectra of DB-contaminated samples (m/z 240–300; Figure S1), relatively small changes in pH between 11–13 can have significant impact on the resulting aqueous-phase concentration of high MW NAs.

Figure 3. (-)-ESI Mass spectra of the aqueous phase of DB- and CC-contaminated water samples in various buffered solutions. In general, overall signal intensity increased with increasing pH, accompanied by a concomitant increase in the average m/z of detected species. DF=Dilution factor of aqueous sample prior to analysis, NL: Normalization limit for MS signal intensity.

3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

In addition to analysis of NAs in oil-affected waters, we also screened for three PAH isomer classes and their corresponding C₁–C₄ alkylated analogs using CP-MIMS. The parent PAH isomer classes were C₁₀H₈ (Naphthalene; *m/z* 128) C₁₄H₁₀ (anthracene, phenanthrene; *m/z* 178), and C₁₆H₁₀ (pyrene, fluoranthene; *m/z* 202). Measurements were made using a LEI source and a single quadrupole mass spectrometer (PAHs measured as M⁺⁺ ions) using a stopped-flow methodology to enhance sensitivity (Figure S4). This method exhibited parts-per-billion (µg/kg)-level sensitivity, with limits of detection (LoDs) of *ca.* 1–2 µg/kg for the three PAH classes measured with an analytical duty cycle of *ca.* 5 mins/sample. Triplicate analysis of a 10 µg/kg standard of each naphthalene, anthracene, and pyrene gave signal relative standard deviation (RSD) of 9.5 %, 14 %, and 23 %, respectively. Aqueous concentrations of PAHs were in the 5–100 µg/kg range, with both CC- and DB-contaminated samples exhibiting similar and low aqueous concentrations of anthracene-class (C₁₄H₁₀) and pyrene-class (C₁₆H₁₀) PAHs (5–20 µg/kg). However, significantly higher concentrations of naphthalene (Figure 4A) were observed in waters contaminated with CC (*ca.* 80 µg/kg) compared to those with DB (*ca.* 25 µg/kg). This observation is consistent with the order of magnitude higher concentration of naphthalene in the parent CC oil over the parent DB oil at 432 mg/kg and 39.7 mg/kg, respectively (personal communication). No discernable trends were observed in aqueous PAH concentrations across the pH or salinity range tested. The lack of any observed effect of sample pH on the aqueous PAH concentration is expected, as these compounds lack functional groups that would impart pH-dependent water-solubility. A reduction in the water solubility of PAHs in saline aqueous solutions is described by the Setchenow salting constants (Schwarzenbach et al., 2016). In marine water samples (50 mS/cm) salt concentrations of *ca.* 0.5 M the PAH solubility is expected to be reduced by *ca.* 30 ± 2 %, however in brackish (5 mS/cm) and freshwater (0.5 mS/cm) samples this effect quickly becomes negligible at 3‰ and 0.3‰ respectively (Table S5, Table S6). We do not believe increased membrane transport (as discussed for NAs) during analysis compensates for the reduced PAH concentration because the salinity of samples were levelled by 10–20 fold dilution and pH adjustment to 10.6 (0.4 mM NaOH) to prevent co-permeation of NAs prior to analysis. Instead, we propose that any reduction in PAH concentration as salinity increases is insignificant relative to the precision reported here (Table S5). The measured relative standard deviation assessed from triplicate analysis of each diluted

CC- or DB-contaminated sample was less than 20% for naphthalene, but higher for anthracene and pyrene due to their lower concentrations present in these samples.

Figure 4. Summary of CP-MIMS results for PAH and alkyl-PAH concentrations for the series of DB-and CC-contaminated water samples. A) Global average of naphthalene, anthracene class, and pyrene class concentrations for all salinities ($n = 24$ total, 3 for each salinity / pH combination) at pH = 2 and 8, error bars represent the standard deviation. B) Alkyl-naphthalene profile averaged across all samples at pH = 2, alkyl-naphthalene concentrations were calculated from the calibration curve for the parent PAH. C) Alkyl-anthracenes for all samples at pH = 2, concentrations were calculated from the calibration curve for the parent PAH. D) Alkyl-pyrene class profile for all samples at pH = 2, concentrations were calculated from the calibration curve for the parent PAH.

Aqueous concentrations of C₁–C₄ alkyl-PAHs were also measured in oil-contaminated water samples by monitoring signal intensities at m/z M+14, M+28, M+42, and M+56, respectively, and quantifying the signals corresponding to the parent PAH (Figure 4 B to D). Alkyl-PAH isomers are not resolved, and their concentrations are expressed as an equivalent concentration of the parent PAH that would give rise to the same signal count. The aim of analyzing these alkyl-PAH species is to provide additional, orthogonal data with which to distinguish oil types rather

than to provide absolute quantitation. For alkyl-naphthalenes (Figure 4B), we observe a distinct drop in relative concentrations for the CC-contaminated samples, from 80 $\mu\text{g/kg}$ for naphthalene (C_0) to 10 $\mu\text{g/kg}$ for C_4 -naphthalenes. On the other hand, DB-contaminated samples exhibit a more constant profile between the parent- and alkyl-naphthalenes, each exhibiting concentrations of *ca.* 20–25 $\mu\text{g/kg}$. For the anthracene and pyrene classes, the concentrations are lower (0–20 $\mu\text{g/kg}$) and any distinction between CC- and DB-contaminated samples is obscured (Figure 4C–D).

Given the negligible differences in PAH concentrations observed across the pH and salinity range, average concentrations across all replicates and salinities are shown in Figure 4 panel A). The aqueous concentrations of parent and methyl-naphthalenes appear to be roughly 3 times higher in waters contaminated by CC than in those contaminated by DB and one-way ANOVA showed significant difference in the mean naphthalene concentration between the two groups ($p \ll 0.005$), whereas differences in the concentrations of higher alkyl-naphthalenes are less significant. While it is clear that waters contaminated with CC have greater relative concentrations of naphthalene-class PAHs than those contaminated with DB, the same cannot be said for the anthracene and pyrene classes.

The PAH screening results presented here using LEI coupled with a single quadrupole mass spectrometer are limited by modest sensitivity and selectivity. While these limitations can be addressed using tandem mass spectrometry (*vide infra*), we have shown that use of a PDMS membrane is an effective on-line sampling strategy that effectively selects for neutral, hydrophobic analytes in complex samples (Vandergrift et al., 2019). Given the inherent advantages of using single quadrupole instruments for on-site applications (cost, ease-of-use, lack of collision gas, lower power requirements), we feel that the data presented here provides a useful screening application that has the potential to be mobilized to the field.

3.3 Contamination Source Identification

An advantage of direct mass spectrometry over other rapid screening techniques, such as Fourier-transform infrared spectroscopy (Meshref et al., 2020), is the wealth of molecular-level data (m/z) that mass spectrometry provides. Principal component analysis (PCA) is a data-

reduction technique, which can be used to visualize differences in these large, multivariate datasets (Bro and Smilde, 2014). It has been employed to characterize and discriminate aqueous samples impacted by trace organic contaminants. For example, oil samples have been differentiated based on GC-MS measurements of PAHs and alkyl-PAHs (Ahad et al., 2015; Brown and Brown, 2012; Thavamani et al., 2012). Corilo *et al.* correctly identified the source tank on the *MV Cosco Busan* in the San Francisco Bay oil spill using differences in the polar component profiles (Corilo et al., 2013). Ross *et al.* have visualized differences in NA homologue profiles from oil sands process-affected waters, groundwaters, and pore waters (Ross et al., 2012), and we recently reported on changes in the CP-MIMS-derived mass profile of NAs as they were attenuated in constructed wetland treatment systems (Duncan et al., 2020). While CC and DB may be differentiated by the total concentrations of NAs or specific PAHs that they release to the aqueous phase under a set of reproducible conditions, in the event of an oil spill affecting an aquatic environment, such differentiation would be confounded by dilution. To demonstrate how water samples contaminated by different oil sources may be identified by profiling their water-soluble NAs with CP-MIMS, we performed PCA on the concentration-normalized negative-ion ESI full scan mass spectral data for the series of oil-contaminated water samples in buffered solutions.

The mass spectra presented above (Figure 3) were used to generate the score and loading plots shown in Figure 5. As can be seen in these spectra, as the pH of the water sample increases, the relative abundance of higher molecular weight ions increases. Also, the mass profile for the DB- and CC-contaminated samples at high pH center on different ions (m/z 251 and 199, respectively). In the PCA scores plot (Figure 5A), the first principal component (PC 1) discriminates the samples equilibrated under acidic and basic conditions, with low pH samples having positive scores on PC 1. The m/z values that drive this pH separation are shown in the loadings plot (Figure 5B), with lower m/z ions exhibiting higher positive loadings on PC 1. The two oil-types are discriminated on PC 2, with DB (circles) and CC (squares) having negative and positive scores on PC 2, respectively. As the mass profile in the DB-contaminated samples is centered at higher mass than that of the CC-contaminated samples, the m/z with high negative loadings on PC 2 (e.g., m/z 251/[C₁₆H₂₇O₂]⁻ and 237/[C₁₅H₂₆O₂]⁻) and with high positive loadings on PC 2 (e.g., m/z 199/[C₁₂H₂₃O₂]⁻) allow the different sources of NAs to be

discriminated. Unlike the CC-contaminated samples, where the mass spectra change very little as the pH increases in buffered water samples from 8.76 to 12.0 (Figure 3), the DB-contaminated samples contain larger NAs with higher pP_{wo} values (Bertheussen et al., 2018b), and so the spectrum shifts significantly towards higher m/z between pH 8.76 and pH 10.47 as these heavier NA species move from the oil to the aqueous phase. This feature is again reflected in the scores plot, as the DB pH 8.76 sample has a less negative score on PC 2 than the DB samples at higher pH, while the CC samples at pH 8.76, 10.47, and 12.0 all cluster closely together. PCA was also applied to the entire range of sample pH (2–12) and salinity (0.05–50 mS/cm) series of unbuffered samples (Figure S6A). However, the pH drop occurring upon oil addition (Table S1) to these samples shifts their mass spectra towards lower mass and confounds separation between the Merichem standard, DB-, and CC-contaminated samples. An additional PCA model was generated using the high-pH (12) subset of these samples, after which a clear separation between Merichem, DB-, and CC-contaminated samples was observed (Figure S6B).

Figure 5. PCA Scores (left) and loadings (right) plots for buffered CC- (squares) and DB- (circles) contaminated samples. Sample pH is indicated by fill colour, with red, orange, light green, dark green, and blue representing pH 2, 5, 8, 10, and 12, respectively. Intermediate pH (5, 8, and 10) samples were buffered with 10–25 mM glutamine (pH 8 and 10) or glutamic acid (pH 5). Samples equilibrated at high (8, 10, 12) and low (2, 5) pH separate along PC 1, with low m/z ions (e.g., m/z 157, 107) dominating low-pH samples and heavier ions (m/z 237, 251, 199) prevalent in high-pH samples. DB- and CC-contaminated samples separate along PC2, again with heavier ions (m/z 223–251) prevalent in the DB-contaminated samples and lighter ions (m/z 171–199) prevailing in CC-contaminated samples.

Given that diagnostic ratios of PAHs are readily used to differentiate sources without multivariate statistics (e.g. based on pyrene:naphthalene ratios), we elected not to conduct PCA on the

PAH/alkyl-PAH dataset (Tobiszewski and Namiesnik, 2012). We are currently developing more sensitive and selective variants of CP-MIMS for PAH analysis, as well as expanding the range of PAHs analyzed (Table S7), such that the PAH and NA datasets can be fused for improved chemometric discrimination of CC and DB contamination sources. In light of the speed and ease of the direct sampling CP-MIMS measurement technique (which enables high throughput analysis), we are encouraged by the ability to use full scan mass spectra to discriminate water samples impacted by spills of different commercial oils.

3.4 Process Monitoring and Future Work

By obviating sample clean-up, direct membrane sampling lends itself to high-throughput screening, which can provide a powerful complement to existing environmental analysis and forensics techniques, especially when large sample numbers are involved. With CP-MIMS, aqueous concentrations of NAs can be analyzed in as little as 10 min, and PAHs in as little as 5 min. As demonstrated here, this approach provides both qualitative identification and quantitative determination of isomer classes. The selectivity may be further enhanced with modifications of the acceptor phase, ion source, and mass spectrometer. For instance, the selectivity for carboxylate ions can be improved by including Ba^{2+} in the acceptor phase through the formation of $[\text{RCOO}-\text{Ba}]^+$ complexes, which can be monitored by tandem MS in positive-ion mode ESI (Duncan et al., 2016b). Preliminary work using a liquid electron/chemical ionization (LEI/CI) source has measured PAHs as $[\text{M}+\text{H}]^+$ ions; PAHs are protonated by CH_3OH_2^+ ions generated from auto-protonation of methanol in the LEI/CI source. This approach enhances sensitivity towards the target PAHs roughly ten-fold by enabling MS/MS monitoring (Table S7) of PAHs in the $[\text{M}+\text{H}]^+ \rightarrow [\text{M}]^+$ transition (decreased noise), and also enhances selectivity by excluding detection of co-permeating isobars with proton affinity less than that of methanol. Preliminary work screening the oil-contaminated samples described in this manuscript show a similar quantitative pattern where CC-contaminated samples exhibiting significantly higher naphthalene concentrations (Figure S7).

In addition to the rapid screening capabilities of direct, on-line mass spectrometric techniques such as CP-MIMS, these approaches also provide real-time information which can be used to assess dynamic changes in solution-phase concentrations. Figure 6 shows an example, raw

chronogram arising from simultaneous analysis NAs and PAHs in a single sample. To accomplish real-time data acquisition, the otherwise-wasted flow of analyte from a LEI/CI experiment (used for determining PAH concentrations) is directed to a second mass spectrometer equipped with an ESI source. In this dual-MS configuration, protonated anthracene (m/z 179) is detected immediately upon immersing the membrane probe into a combined standard solution at neutral pH ($t = 8$ min). The observed drift in the anthracene signal has not been corrected with the use of an internal standard. Under these conditions, the predominantly anionic naphthenates do not cross the PDMS membrane. Upon acidifying the sample to a pH of 3 ($t = 17$ min), the naphthenates become protonated, the NAs cross the membrane, and are subsequently detected in negative-ion mode as their corresponding $[M-H]^-$ ions simultaneously with the PAHs, by the ESI-MS and LEI-MS, respectively. By changing the conditions of the sample, such as pH, we can tune the analyte classes that can permeate the membrane. At $t = 26$ min, activated carbon is added to the sample with the membrane probe still immersed therein. The rapid drop in signal intensities observed for both PAHs and NAs, occurring after the addition of activated carbon, is attributed to the decrease in their aqueous-phase concentrations as they adsorb onto the surface of the sorbent. The rate and degree of signal decay can be used to directly monitor both kinetic and thermodynamic parameters of adsorption/desorption processes, thus providing a powerful experimental technique to rapidly assess and optimize the use of sorbents as spill-remediation materials. To our knowledge, this is the first demonstration of simultaneous monitoring of PAHs and NAs at trace levels using an online membrane sampling approach.

Figure 6. Real-time, simultaneous process monitoring of aqueous concentrations of NAs and PAHs. The CP-MIMS acceptor phase was passed through the PDMS capillary membrane at a flowrate of 50 $\mu\text{L}/\text{min}$, then passed through a 250 ESI:1 LEI/CI passive splitter. Both mass spectrometers responded to changes in the aqueous-phase concentrations of their analytes. At $t = 8$ min, 0.5 mg/kg Merichem (NAs) and 200 ng/kg anthracene are spiked into an aqueous sample at pH = 8. The anthracene signal (LEI/CI) rises immediately, however the NA signal does not rise until $t = 17$ min when the solution pH is adjusted to 3 to protonate naphthenates and allow NAs to pass through the membrane. At $t = 26$ min, 12.5 mg/g of activated carbon is added to the solution, and the signal intensities for both analyte classes decrease as they adsorb onto the carbon.

4. Conclusions

The aqueous concentrations of NAs and PAHs in DB and CC contaminated water samples across an initial pH ranging from 2 to 12 and salinities encompassing those found in marine through very freshwater types. The analysis was carried out directly in the sample using CP-MIMS and a simple analytical workflow, without the need for sample clean-up or chromatographic separation of analytes. The total aqueous concentrations of NAs spanned two orders of magnitude, from less than 0.025 mg/kg to nearly 25 mg/kg. Concentrations were significantly higher ($\sim 10\times$) in water samples exposed to DB *versus* CC oil. Concentrations of aqueous NAs exhibit a marked pH dependence, with considerably higher concentrations observed in basic solutions, consistent with the greater water solubility of their carboxylate forms. Several PAH classes (and their alkylated

analogues) were observed at concentrations below 100 µg/kg in all samples, with significantly higher aqueous concentrations (~3–4x) of naphthalene and (C₁/C₂) alkyl-naphthalenes observed in CC-contaminated water samples. PCA of fullscan, unit vector-normalized mass spectra of NAs enabled discrimination of waters impacted by CC, DB, and a commercial Merichem NA mixture, especially in alkaline waters where NAs are observed at higher concentrations.

This work demonstrates the utility of membrane introduction mass spectrometry for two important contaminant classes using the pH dependent perm-selectivity to control membrane transport and different ionization strategies amenable to NAs and PAHs, respectively. These techniques are readily adaptable to *in-situ* screening of trace organics in a large number of samples and provides a foundation for their use in process monitoring and eventual mobilization to in-field analysis. One of the long-term goals of this work is to develop portable mass spectrometry-based analytical instrumentation that can be mobilized to the field to characterize natural waters or treatment systems. Such screening tools can be used in an adaptive sampling strategy to complement conventional techniques on a more limited subset of samples, if and where necessary. By minimizing sample clean-up and enabling on-line, direct analysis using selected-ion monitoring of both NAs and PAHs with single-quadrupole instrumentation, the techniques described here could be used to provide useful qualitative and quantitative information and support on-site decisions in the future.

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