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Potential of capillary electrophoresis mass spectrometry for the characterization and monitoring of amine-derivatized naphthenic acids from oil sands process-affected water

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ABSTRACT

Capillary electrophoresis coupled to mass spectrometry (CE–MS) was used for the analysis of naphthenic acid fraction compounds (NAFCs) of oil sands process-affected water (OSPW). A standard mixture of amine-derivatized naphthenic acids is injected directly onto the CE column and analyzed by CE–MS in less than 15 min. Time of flight MS analysis (TOFMS), optimized for high molecular weight ions, showed NAFCs between 250 and 800 *m/z*. With a quadrupole mass analyzer, only low-molecular weight NAFCs (between 100 and 450 *m/z*) are visible under our experimental conditions. Derivatization of NAFCs consisted of two-step amidation reactions mediated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), or mediated by a mixture of EDC and *N*-hydroxysuccinimide, in dimethyl sulfoxide, dichloromethane or ethyl acetate. The optimum background electrolyte composition was determined to be 30% (*V/V*) methanol in water and 2% (*V/V*) formic acid. NAFCs extracted from OSPW in the Athabasca oil sands region were used to demonstrate the feasibility of CE–MS for the analysis of NAFCs in environmental samples, showing that the labeled naphthenic acids are in the mass range of 350 to 1500 *m/z*.

Introduction

More than 170 billion barrels of crude oil are considered to be economically viable for recovery in the Athabasca oil sands in Northern Alberta, Canada (Headley et al., 2013a, b). For every 1 barrel of synthetic crude extracted from the oil sands, 3.3 barrels of process water ("tailings") are produced (Headley et al., 2013a, b). Process water from oil sands mining is stored in large tailing ponds, whose total volume has already surpassed 720 billion L (McKenzie et al., 2014). Fresh oil sands process-affected water (OSPW) has been demonstrated to be acutely toxic toward a number of animal species, due in part to the presence of a class of organic contaminants called naphthenic acids (NAs) at concentrations as high as 100 mg/L in tailing ponds (McKenzie et al., 2014; Allen, 2008).

The term "naphthenic acids" suggests the acids of naphthenes (*i.e.*, cycloalkanes) (IUPAC, 1997; Nic et al., 2006; Senning, 2007). In fact, the class of compounds referred to as

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NAs consists of a mixture of aliphatic and alicyclic hydrocarbon carboxylic acids, usually containing 10 to 30 carbons and N, O, and S as ring heteroatoms and functional moieties. A small subset of possible NA structures with C_{8-26} is shown in Fig. 1.

NAs mixture compositions vary according to the geographical location of the crude deposit. Additionally, NAs found in OSPW may have altered molecular structures and toxicity from those found naturally in bitumen (Rowland et al., 2011). Identification of the source of a particular NAs sample is therefore an important challenge for environmental forensics. Currently, there is no standard monitoring system for NA levels in water. This is due in part to the fact that current chromatographic separation methods cannot resolve individual NAs species, including isomers, from the complex mixtures that exist in the environment (Proposal to Add Naphthenic Acids to the NPRI for the 2012 Reporting Year Recommendations of the NPRI Multi-Stakeholder Work Group, 2012).

Currently there are several methods that are used for the characterization of NAs. High-resolution mass spectrometry has shown remarkable success in identifying possible chemical structures (Headley et al., 2016). More recently, liquid chromatography–mass spectrometry (LC–MS) has also been used to further characterize the different species, including those containing oxygen, sulfur, and nitrogen species (Martin et al., 2010). Capillary electrophoresis coupled with mass spectrometry has shown important complementarity with LC–MS in proteomics and metabolomics research (Sun et al., 2016).

Here we propose to use a new method for the characterization of NAs based on capillary electrophoresis and mass spectrometry. Capillary electrophoresis (CE) is an analytical technique that separates analytes based on their differential rates of migration in an electric field, which is determined by the charge-to-size ratio of the analyte ions in solution. Because this mechanism of separation differs from that of chromatographic separations, in which separation is determined based on differential partitioning between a mobile and stationary phase, CE represents a complementary approach with several

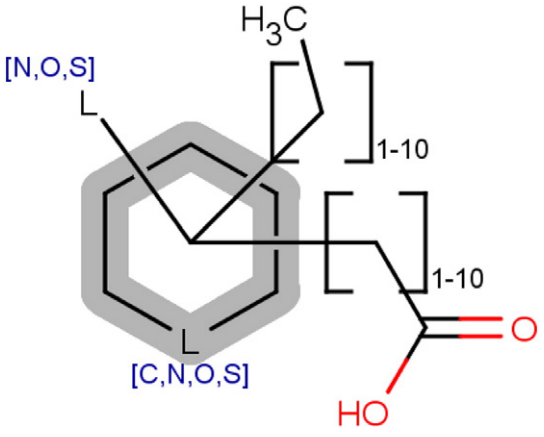

Fig. 1 – A small subset of possible naphthenic acid structures based on C_{9-28}, a single 6-membered ring, 3 heteroatom types at 2 locations (L in the diagram) and a single carboxylic acid group. This small subset contains approximately 259,000 members.

important advantages compared to chromatography: The most salient advantages of CE include (a) the possibility of high separation efficiency in order to resolve complex isomeric mixtures; (b) versatility in its ability to resolve a wide range of analytes (including neutral species) through the addition of additives to the background electrolyte; (c) low sample consumption (nanoliters) and low solvent consumption, which make it suitable for high-volume, routine analyses and environmental applications.

The power of CE can be further increased by coupling with mass spectrometry (MS), which provides an added dimension of resolution and information on the mass-to-charge ratio (*m*/*z*) of the separated species. While CE can be coupled to MS using a range of ionization modes (Maxwell and Chen, 2008), electrospray ionization (ESI) is by far the most common ionization and coupling method.

NAs exist as negatively charged ions in alkaline medium; however, the electrophoretic separation and negative-mode ESI–MS analysis of these molecules in basic aqueous medium has been challenging with the instrument we have. In the present study, three different types of chemical derivatization that convert the NAs into naphthenic amines were investigated to impart greater selectivity of acidic compounds from the sample matrix. The naphthenic amines, which existed as cations in acidic solution, were characterized by CE separation and ESI–MS analysis under positive ion mode. To our knowledge, this work is the first known application of capillary electrophoresis mass spectrometry to the analysis of NAs.

1. EDC/NHS-mediated chemical derivatization of NAs

Chemical derivatization is a common technique for increasing the selectivity, separation and sensitivity of detection of target analytes. The coupling of a carboxylic acid to a primary amine can be facilitated by the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). Both EDC and NHS are widely used in bioconjugate chemistry and peptide chemistry. The general outline of such derivatizations is shown in Fig. 2a.

EDC and NHS activate carboxylic acids *via* different mechanisms. In the case of EDC, the carboxylic oxygen atoms donate electrons to the carbodiimide carbon on EDC, forming a C – O bond to make a transient *O*-acylisourea (Fig. 2b Step 1) (Mikołajczyk and Kiełbasiński, 1981; Williams and Ibrahim, 1981). Subsequent aminolysis of the *O*-acylisourea affords the amine-derivatized acid (Fig. 2b Step 2). In aqueous media, EDC degrades to the urea by-product. This degradation of EDC occurs rapidly in acidic, aqueous solutions (Gilles et al., 1990; Nakajima and Ikada, 1995).

By contrast, the mechanism of NHS activation involves a substitution to form a *N*-succinimidyl ester, with subsequent aminolysis of the ester to afford the amine-derivatized product. The aminolysis of the *N*-succinimidyl ester is first order with respect to [OH^-] (Cline and Hanna, 1988).

In concert, EDC and NHS, buffered at approximately pH 7, enhance each other's ability to mediate the amidation of carboxylic acids (Grabarek and Gergely, 1990; Staros et al., 1986).

Previous strategies for characterizing NAs have leveraged the *N*-acylurea by-product of EDC derivatizations, sometimes referred to as "*N*-acylisourea", suggestive of an imidic acid tautomer (Hermanson, 2008), that forms when there is a large excess of EDC in aqueous protein chemistry, given by IR data of C═O and –NH– stretches (Nakajima and Ikada, 1995), an excess of tertiary amine reacting aromatic carbodiimides with monocarboxylic acids (Kurzer and Douraghi-Zadeh, 1967), in the absence of faster reactions in pH 7 medium, by pH monitoring (Hoare and Koshland, 1967), or in high dielectric organic solvents (Rich and Singh, 1979). The formation of *N*-acylurea is relatively slow and is thought to proceed *via* intramolecular acyl transfer from the ureic O to ureic N (Fig. 3), or in other words an isomeric *O*-acylisourea *N*-acylurea rearrangement, and has been substantiated with Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy (NMR) data (Sam et al., 2010; Volonterio and Zanda, 2005; Wang et al., 2011).

Once the *N*-acylurea derivative is formed, it is recalcitrant to aminolysis and has shown to be generally stable at temperatures up to 140°C (Wei et al., 2006). Woudneh et al. (2013) reported that *N*-acylurea EDC derivatives of NAs give rise to a single fragment (*m*/*z* 129) in LC–(+ESI)–MS/MS that can be monitored for quantification. In contrast, the present study focuses on the full scan mass spectrometric data, produced with both CE–ESI–TOFMS (optimized for high molecular weight) and CE–ESI–QMS, of NAs derivatized to form amines *via* EDC or EDC/NHS-mediated amidation.

2. Experimental

2.1. Materials and chemicals

NAs standard mixture, EDC, NHS, *N*,*N*-dimethylethylenediamine (*N*,*N*-DMEDA), *N*,*N*-diethylethylenediamine (*N*,*N*-DEEDA) and NAs standard mixture were all purchased from Sigma-Aldrich, St. Louis, MO. Both EDC and NHS were stored at –20°C in tightly sealed containers. Dichloromethane (DCM), ethyl acetate (EtOAc) and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific. OSPW extracts were provided by Environment Canada. The electrophoresis background electrolyte (BGE) consisted of methanol (LC–MS reagent grade, J.T. Baker, Avantor, USA), formic acid (Anachemia, Canada), and deionized water.

2.2. Derivatization procedure

This study implemented three types of derivatizations for the analysis of NA compounds using capillary electrophoresis–electrospray ionization–(low resolution) mass spectrometry (CE–ESI–MS): Isolated NAs were derivatized with a primary–tertiary diamine using (1) EDC and NHS in DMSO, (2) EDC in DMSO, or (3) EDC in DCM. EDC/Sulfo-NHS-mediated amidations in DMSO were also explored, but the analytical advantages compared to more economic alternatives were not significant enough to warrant its use at this time. Since the efficacy of

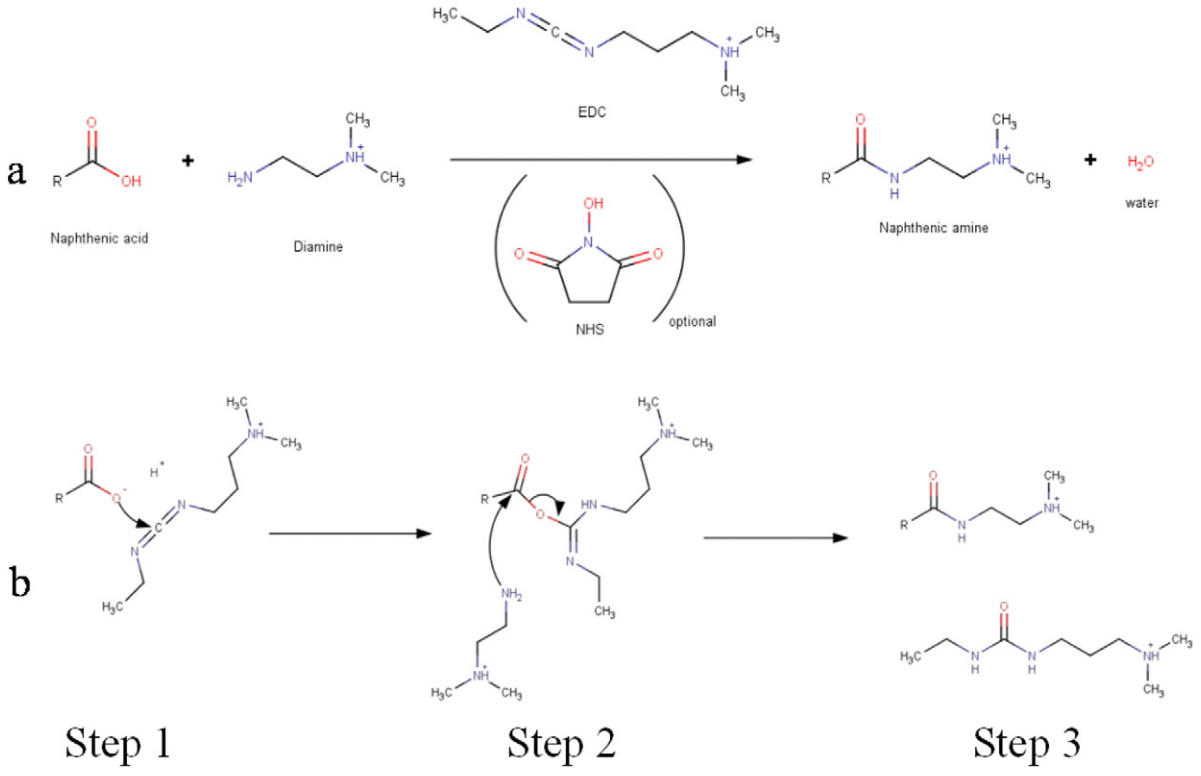

Fig. 2 – (a) General reaction equation for the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (and optional *N*-hydroxysuccinimide) mediated amidation of carboxylic acids (*e.g.*, naphthenic acids) with a primary–tertiary diamine to form (naphthenic) amines; (b) additional information on the reaction mechanism. (Step 1) The electrophilic carbodiimide carbon in EDC accepts electrons from R-carboxylic acid to (Step 2) produce the O-acylisourea derivative of EDC. Subsequent aminolysis of the O-acylisourea releases (Step 3) the EDC urea by-product (far right, lower structure) to produce the diamine-derivatized carboxylic acid (far right, upper structure).

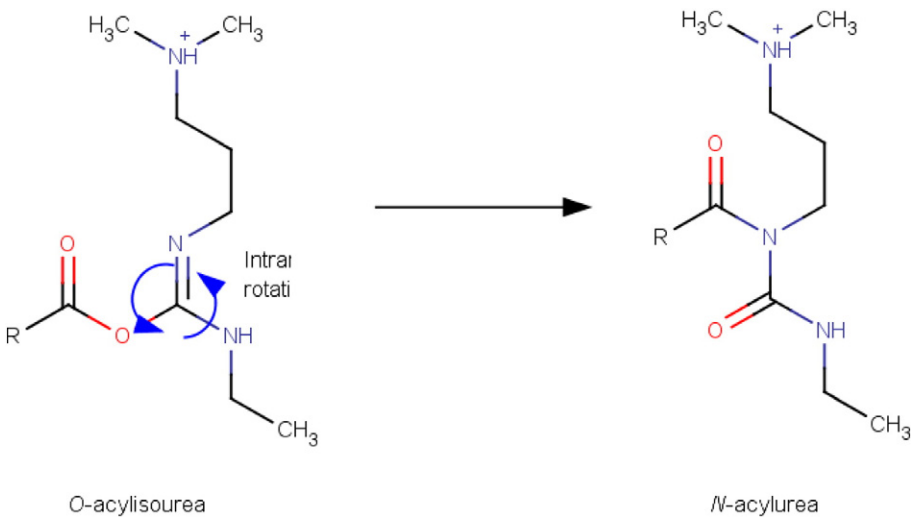

Fig. 3 – Isomerization of O-acylisourea EDC derivatives of naphthenic acids to N-acylurea derivatives based on naphthenic-acyl transfer.

derivatization and associated side reactions appear to be highly dependent on the lability of protons in the reaction solution, aprotic solvents were used in this work to suppress side reactions.

Prior to derivatization, NAs were extracted from sample solutions by liquid–liquid extraction with EtOAc. For the NAs standard solution, 1 mL of 100 mg/L solution of Aldrich Standard NAs in 0.1 mol/L $NaOH_{(aq)}$ was acidified by an equal volume of 1.0 mol/L HCl. NAs were extracted from the acidified solution using three extractions of 0.5 mL EtOAc. For the environmental tailing water samples, 50 μL of large-volume OSPW-extracted naphthenic acid fraction compounds (NAFCs) in 0.1 mol/L $NaOH_{(aq)}$ was acidified with 50 μL 1.0 mol/L HCl and extracted into 50 μL EtOAc three times. Following the extraction, the combined EtOAc layers were evaporated to dryness by vacuum centrifugation (Vacufuge, Eppendorf, Wesseling-Berzdorf, Germany).

The extracted NAs were derivatized by adding 5 μL of 10 mmol/L of a reagent solution containing EDC and 10 mmol/L NHS (or only 10 mmol/L EDC) in DMSO orDCM, followed by 5 μL of a second reagent solution containing 0.2 mol/L *N,N*-DMEDA in DMSO or DCM. After 20 min, the reaction mixture was diluted with 40 μL BGE and injected directly into the CE–ESI–MS system.

2.3. CE–ESI–MS system

CE–ESI–MS of amine-derivatized NAs was carried out on P/ACE MDQ Glycoprotein System (Beckman-Coulter, Inc.) coupled to Micromass QTOF (Waters-Micromass) with mass resolution of approximately 500 (*i.e.*, "low resolution"). The CE and MS instruments were coupled using a previously described low-dilution "junction at the tip" electrospray interface (Jayo et al., 2014; Lindenburg et al., 2013; Maxwell et al., 2010, 2011; Soliman et al., 2012; Zhong et al., 2011a, 2011b). Data acquisition and visualization, as well as ESI and mass spectrometer parameters were controlled *via* MassLynx Software V4.0 (Waters-Micromass). The electrospray voltage was set at +4500 V and the MS inlet temperature was 150°C. The modifier solution was delivered through a separate capillary with inner diameter of 75 μm located at the CE instrument inlet. The modifier solution was identical in composition to BGE and was kept at 0.2 psi (approximately 100 nL/min) to stabilize the electrospray. The mass spectrometer was operated in positive, full scan mode scanning between 50 and 2000 *m/z* at a spectrum acquisition rate of 1 Hz.

2.4. CE

All CE separations were carried out using a 75 cm long fused-silica capillary (50 μm inner diameter, 365 μm outer diameter, Polymicro Technologies, Phoenix, AZ, USA). Prior to use, the interior walls of the capillary were treated with trimethoxysilylpropyl modified polyethyleneimine (Gelest, Morrisville, PA, USA) to form a positively-charged covalently bound surface layer.

CE separations were controlled using 32 Karat software, version 7.0 (Beckman-Coulter, Inc., Brea, CA, USA). Samples were injected by applying a positive pressure (0.5 psi for 5 sec) to the sample inlet vial, corresponding to an estimated sample injection volume of 4.4 nL. Separations were carried out in reverse-polarity mode at −30 kV. Under these conditions, the bulk flow (electroosmotic flow) in the covalently treated capillary was toward the capillary outlet, whereas the electrophoretic migration of the positively-charged derivatized NAs opposed the direction of the bulk flow.

The composition of BGE was optimized using a Simplex protocol based on the empirical evaluation of 5 factors. Optimization experiments were carried out by varying the proportions of methanol and formic acid while maintaining a constant capillary temperature (15°C). The optimum BGE composition was determined to be 30% methanol, 2% formic

acid in water; this BGE composition was used for generating the CE–ESI–MS data displayed in the figures below.

2.5. Other software

MSConvert (ProteoWizard) was used to convert mass spectrometric data files to open source formats (Chambers et al., 2012). TOPP view software (OpenMS) was used for fast visualization and generation of detailed CE–ESI–MS electropherograms (Kohlbacher et al., 2007; Sturm et al., 2008). Data analysis for simplex optimization, finding unique *m/z* peaks, polynomial regression and plot diagram creation were carried out in R statistical programming language (R Core Team, 2014) using in-house scripts (MacLennan, 2016).

3. Results and discussion

3.1. EDC derivatives of NAs prior to aminolysis

Prior to the amidation step of the derivatization, CE–ESI–MS data was obtained for the EDC *O*-acylisoureas of NAs. This data, obtained by scanning the quadrupole mass analyzer, showed three swaths of peaks (Fig. 4). The groups of peaks had *m/z* differences of approximately 71 and 45 amu.

If we allow that rearrangement to *N*-acylurea has occurred, the most probable fragmentation series is neutral loss of ethylisocyanate (71 amu) to form naphthenic tertiary amine derivatives and subsequent neutral loss of dimethylamine (45 amu) to form the 2-naphthenic-5,6-dihydro-1,3-oxazinium derivatives. In fact, the formation of *N*-acylurea and loss of isocyanate during electrospray mirrors the reverse of the two-step synthesis of EDC reported by Sheehan et al. (1961). When the mixture was left overnight in separation BGE (formic acid, methanol, water), CE–ESI–MS analysis showed no discernable peaks, suggesting that the EDC had largely degraded in acidic, aqueous BGE, as expected.

Interestingly, the fragment *m/z* 129 previously reported by Woudneh et al. (2013) as diagnostic of EDC-derivatized NAs in LC–(+ESI)–MS/MS was also clearly present in CE–ESI–MS of derivatized NAs mixtures when scanning the quadrupole. A probable assignment for this fragment is loss of an EDC urea species from the NA with subsequent loss of neutral dimethylamine and cyclization of the remaining fragment. This fragmentation could occur reasonably in both *O*-acylisourea and *N*-acylurea species, but not reasonably in the amine-derivatized NA species.

3.2. General characteristics of CE–ESI–MS data of amine-derivatized NAs

For all cases described below, the data generated by CE–ESI–MS of amine-derivatized Aldrich NAs revealed the presence of up to 5 swaths of peaks representing compounds (carboxylic acids) between 200 and 800 amu when using the TOF mass analyzer. When scanning with the quadrupole, two swaths of peaks (and fragmentations) representing low-molecular weight NAs between 150 and 450 amu were visible. Each swath of peaks likely represented a distinct class of NAFCs. Due to differences in total number of $-CH_2-$ groups in NAFC molecules, there was clear mass resolution between groups of molecules with different carbon number—*i.e.*, the difference of 14 amu between classes in the Carbon series was easily noticed (Fig. 5). The migration time resolution of NAFC groups adjacent to each other in the Carbon series ranged from 0 for high molecular weight NAFCs to baseline separation for low-molecular weight NAFCs.

Within each molecular group of the Carbon series, there were varying levels of C–C bond saturation: For each double bond or ring structure, there was a mass deficiency of 2 amu. The numbers of peaks in this "Z-series" were difficult to discern

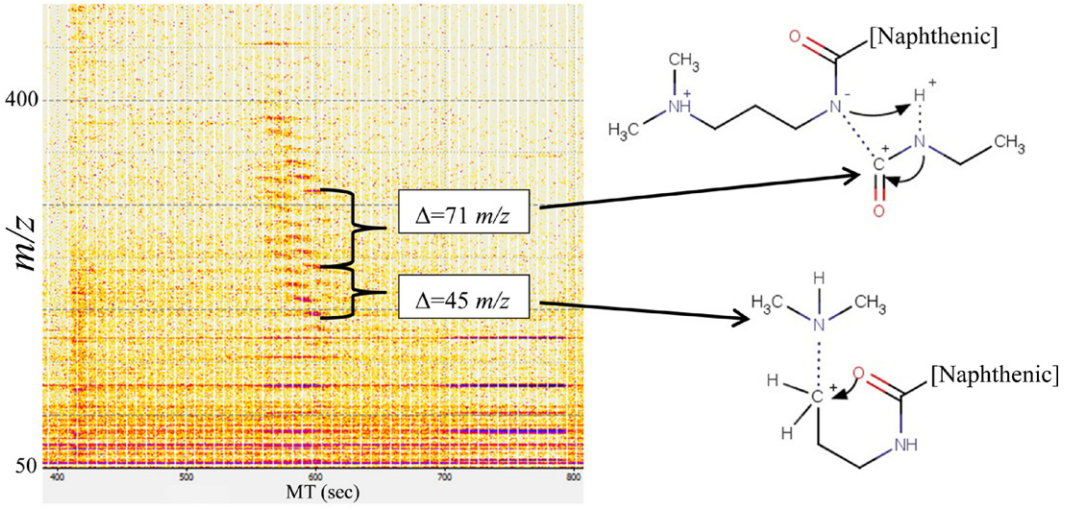

Fig. 4 – Capillary electrophoresis–electrospray ionization–quadrupole mass spectrometry data generated from carbodiimide-derivatized standard naphthenic acids mixture prior to aminolysis. The vertical axis has units of *m/z* and the horizontal axis has units of seconds (time). This mixture showed three swaths of peaks representing one group of parent ions and two groups of fragmentations. Tentative structural assignments for neutral losses are shown to the right.

at low mass resolution and the migration time resolution of adjacent NAFCs in the Z-series was low, but non-zero.

3.3. Derivatization with diamine using EDC/NHS in DMSO

Despite the commonalities between CE–ESI–MS datasets of amine-derivatized NAFCs in different derivatization conditions, there were several salient differences worth noting. Derivatization of NAs using EDC, NHS, and diamine in DMSO produced the largest number of *m/z* and total ion peak features of all methods described herein. The base peak electropherogram (Fig. 6) shows baseline separation between the derivatized NAs and the derivatization by-products as well as good resolution within NA classes.

An in-house algorithm written in *R* was applied to the CE–ESI–TOFMS data to identify the *m/z* values of peaks whose intensity exceeds a predefined threshold. This identification produced approximately 350 unique *m/z* values belonging to NAFCs. This large number of peaks is likely due in part to the presence of up to 13 reactions extraneous to the amidation of NAs that have been described in several reviews (Katritzky et al., 1998; Mikołajczyk and Kiełbasiński, 1981; Williams and Ibrahim, 1981). Two of these side reactions are likely to form new analytes with significantly different mobilities: ring-opening of NHS by primary amine and reaction of EDC with DMSO. Ring-opening of NHS forms a dipeptide-like molecule ($[M-OH]^+$ at *m/z* 187) that could participate in ester aminolysis, while EDC forms a reactive adduct with DMSO. It is unclear as to whether these reactions impart a greater selectivity to the target analytes in the sample or are entirely redundant in terms of information. High-resolution MS is expected to clarify the issue.

3.4. Derivatization using EDC under different conditions

A derivatization without NHS was performed in order to eliminate the possibility of ring-opening reaction between NHS and the primary amine. The resulting CE–ESI–TOFMS data revealed a smaller total number of peaks in the same swath pattern as with EDC/NHS in DMSO, and at lower intensity. Approximately 250 unique *m/z* peaks were found using the same identification algorithm. We replaced DMSO with DCM as the reaction solvent in order to reduce the formation of adducts between DMSO and EDC. CE–ESI–TOFMS of the resulting mixture of *N,N*-DMEDA derivatized NAs resulted in a separation profile with less background. Approximately 300 unique *m/z* values were matched between the two derivatization methods.

Additionally, CE–ESI–MS of the mixture derivatized with *N,N*-DMEDA in EtOAc revealed a virtually identical electropherogram to the reaction performed in DCM, with the exception of differing migration time. The swath of peaks with a higher *m/z* likely represents the parent ions of amine-derivatized NAs and the lower *m/z* swath of peaks is a combination of fragmentation products of amine-derivatized NAs and the stable *N*-acylureas which resisted aminolysis. The neutral loss of dimethylamine (45 amu) from *N,N*-DMEDA-derivatized NAs was confirmed by the neutral loss of diethylamine (73 amu) from *N,N*-DEEDA derivatized NAs, as shown in Fig. 4. Table 1 summarizes the principal differences in peak characteristics for the derivatized NAs in terms of derivatization solvent properties.

For EtOAc and DCM, a prominent inverse peak was consistently seen at 3.5 min, and can be used as the electroosmotic flow marker. For DMSO, the EOF marker peak was less prominent and often absent. DMSO is about twice as viscous as the BGE and has a lower dielectric constant than the BGE, causing an increase in local field strength experienced by the analytes for a longer period of time than with DCM or EtOAc, resulting in sharper (less broad) peaks.

3.5. Optimization of CE separation parameters

In addition to evaluating the EDC-based derivatizations of NAs in organic solvents, the effects of BGE composition on

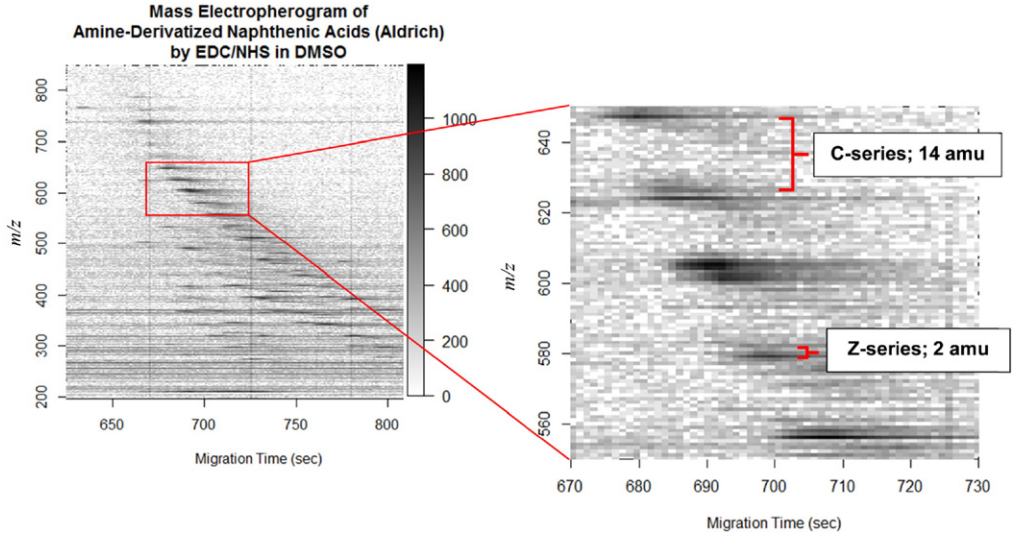

Fig. 5 – Sample mass-electropherogram for amine-derivatized naphthenic acids from NA standard (Aldrich). The zoomed inset on the right demonstrates the typical spacing of naphthenic acids data: Swaths of peaks with 14 *m/z* difference represent differences of $-CH_2-$ and within each carbon number class, there is a Z-series which represents increasing double bond equivalents (double bonds or rings), each producing a hydrogen deficiency of 2.

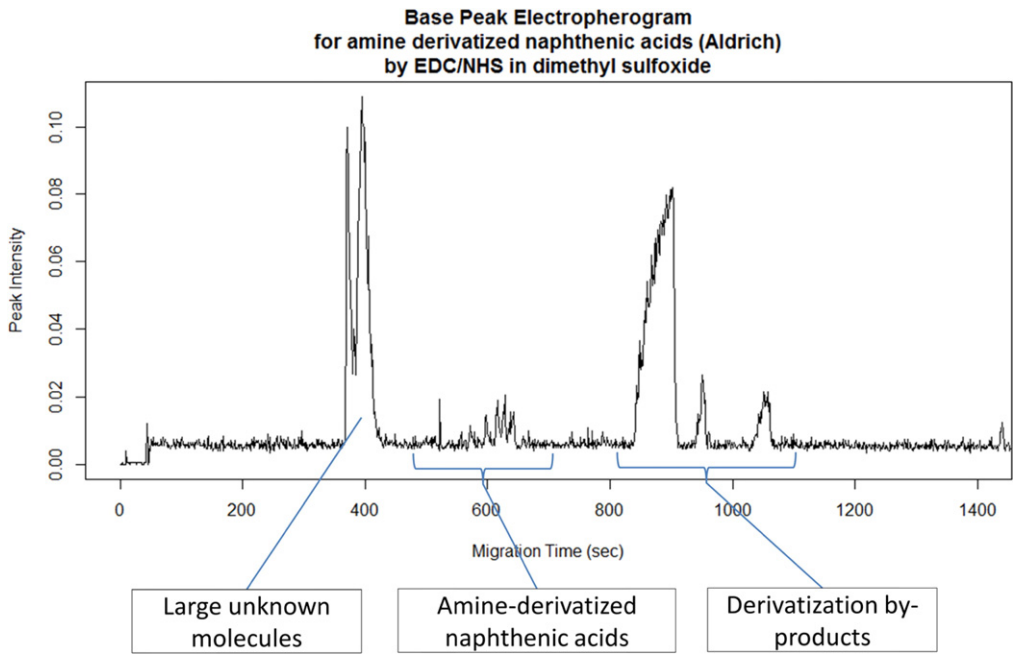

Fig. 6 – A sample base peak electropherogram (BPE) produced by analysis of amine-derivatized naphthenic acids, mediated by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide (EDC/NHS) in dimethyl sulfoxide (DMSO).

various separation parameters were assessed using a simplex-type design of experiment approach. The concentrations of formic acid and methanol in the BGE were varied and the optimum composition was assessed empirically based on a rough balance of 5 responses (in order of importance): greatest peak resolution(s), medium-small peak width, medium-high separation current, fast migration time of first peak and total analysis time 15 min or less. The data for the 5 responses (*z*) were fit to polynomial models with two BGE variables, % methanol (*x*) and % formic acid (*y*). For all responses, the effects of methanol were between 1 and 4 orders of magnitude weaker than the effects of formic acid. An increase in formic acid led to a decrease in peak width, a decrease in average peak resolution in the time domain (*i.e.*, decrease in "peak swath slope" which is used as a proxy for "chromatographic resolution"), and decreased analysis time (due to much faster electroosmotic flow). An increase in the percentage of methanol in the BGE increased total analysis time. It is unclear as to whether or not there is better separation resolution of isomeric NA species at higher methanol percentage. The effects of formic acid and methanol on the CE–MS data are qualitatively summarized in Table 2. Given that the optimum area included a range of methanol concentrations, we chose the highest % methanol within that area to ensure solubility of the analytes and to stabilize the electrospray. In this case, methanol evaporation from 30% would propel the system deeper into the global optimum region (*i.e.*, the region within the red triangle in Fig. 7). The BGE composition of 30% methanol and 2% formic acid in water was chosen as optimum.

Table 1 – Comparison of salient features of CE–ESI–MS electropherograms of derivatized NAs with derivatization solvent properties. DMSO stands out as high viscosity, high dielectric constant compared to ethyl acetate and dichloromethane and also produces different separation characteristics under the same BGE conditions.

	EDC/NHS + diamine in DMSO	EDC + diamine in dichloromethane	EDC + diamine in ethyl acetate
Average peak NA width	25 sec	65 sec	65 sec
Migration time range of NA peaks	100 sec	250 sec	250 sec
Derivatization solvent dielectric constant	4.7	0.89	0.6
Viscosity (mPa/sec)	2.20	0.45	0.46
EOF marker inverse peak (due to solvent)	Absent	3.5 min	3.5 min
Relative vapor pressure	Low	High	High

CE–ESI–MS: capillary electrophoresis–electrospray ionization–(low resolution) mass spectrometry; NAs: naphthenic acids; DMSO: dimethyl sulfoxide; BGE: background electrolyte; NHS: *N*-hydroxysuccinimide; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EOF: electroosmotic flow.

Table 2 – Effects of varying % volume of methanol and formic acid in the background electrolyte for separations of amine-derivatized NAs (EDC/NHS + DMSO).

	Effect of methanol % (*x*)	Effect of formic acid % (*y*)
Migration time of first peak	Weak positive correlation; weakly coupled to formic acid	Positive correlation
Peak width	N/A	Strong negative correlation
Average peak resolution	Very weak negative correlation	Strong negative correlation
Total analysis time	Weak correlation; weakly coupled to formic acid	Strong negative correlation

NAs: naphthenic acids; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS: *N*-hydroxysuccinimide; DMSO: dimethyl sulfoxide; N/A: not available.

3.6. CE–ESI–MS of NA fraction compounds derived from oil sands process waters

The optimized separation conditions were applied to a sample of NAFCs extracted from OSPW which were derivatized with *N*,*N*-DMEDA, mediated by EDC/NHS in DMSO. The mass-electropherogram revealed a single large swath of peaks between 400 and 500 sec migration time and extending from 1500 to 350 *m*/*z* (Fig. 8), as well as two smaller swaths of peaks, one swath with a significantly different migration time, but much less intense.

4. Conclusions

Amine-derivatized standard NA mixture shows considerable tolerance for changes in the composition of the BGE for CE separation. When optimizing the concentrations of formic acid and methanol, formic acid concentration has the largest impact on the electroosmotic flow, separation time, peak shape, and global resolution. It was determined that 30% methanol, 2% formic acid in water was an optimum BGE composition for the separation and analysis of amine-derivatized NAs, based on a rough global optimization of 5 separation criteria.

Different EDC/NHS derivatization schemes result in very different two-dimensional spectra, due to the presence or absence of reaction side-products. Reactions without NHS and DMSO as solvent resulted in spectra with a lower degree of complexity. Further studies are needed to determine if spectrum complexity is an advantage or disadvantage in the effort to characterize persistent water-soluble oil sands contaminants such as NAs. Our data confirms the presence of *m*/*z* 129 ion in CE–ESI–QMS coeluting with EDC-derivatized NAs (Woudneh et al., 2013) and the absence of *m*/*z* 129 for amidated NAs. We provide further evidence for the ring-opening reaction of NHS with a primary amine (Katritzky et al., 1998). We also have evidence to support the formation of *N*-acylurea by-products in the low-dielectric organic solvents DCM and EtOAc, supplementing a previous report (Rich and Singh, 1979).

Preliminary results for the EDC/NHS + diamine derivatization in DMSO on NAFCs derived from OSPW reveal the presence of a swath of compounds between 350 and 1500 *m*/*z*.

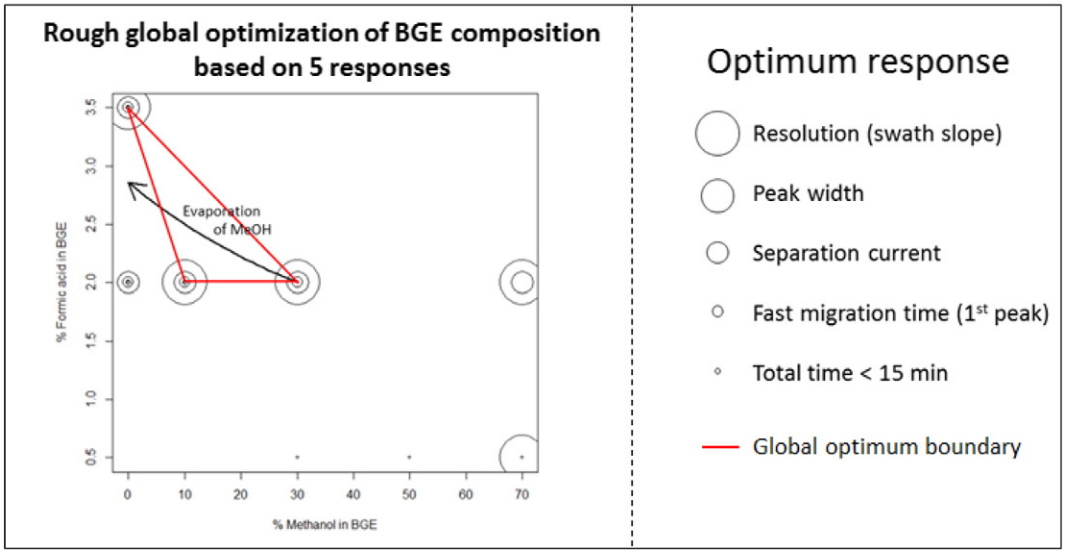

Fig. 7 – Diagram describing global optima for 5 CE separation responses. A circle means an optimum was reached for a response. The diameter of the circle indicates the relative importance (weight) of the response. The most circles at a particular BGE composition indicate a point in the optimum regions for all responses. The red triangle outlines the region of global optimality. The black arrow indicates the direction of the system from initial BGE composition toward global optimality upon natural methanol evaporation (starting at 30% and evaporating to 0%). CE: capillary electrophoresis; BGE: background electrolyte.

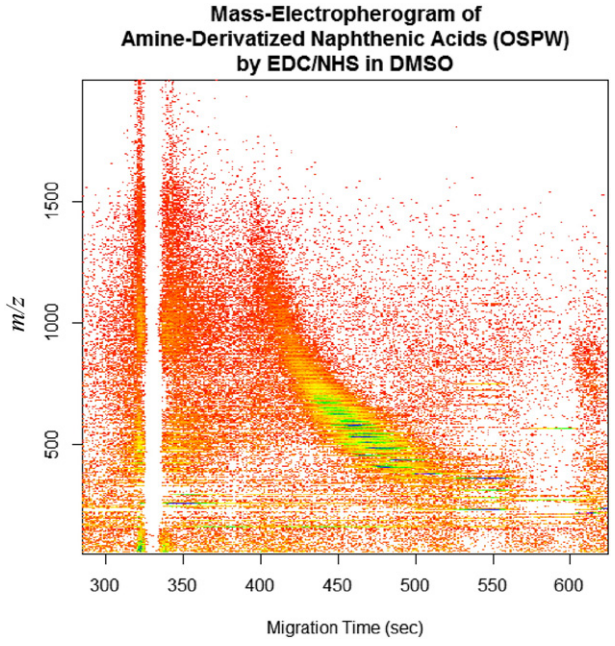

Fig. 8 – Capillary electrophoresis–electrospray ionization–time of flight mass spectrometry of derivatized naphthenic acid fraction compounds (NAFCs) derived from oil sands process waters (OSPW). The data shown here are mean-centered and normalized to standard deviation by *m/z* channel.

Further optimization of the reaction kinetics of derivatization and application to a wider range of environmental NA samples are needed in order to develop this method as a "turn-key" operation for the routine analysis of NAFCs. Application of high-resolution MS to the CE separations should allow for accurate chemical formula assignment in order to identify individual components present in NAFC samples.

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