Atmospheric Pressure Photoionization Proton Transfer for Complex Organic Mixtures Investigated by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Jeremiah M. Purcell,^{a,b} Christopher L. Hendrickson,^{a,b}
Ryan P. Rodgers,^{a,b} and Alan G. Marshall^{a,b}

^a National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida, USA

^b Department of Chemistry, Florida State University, Tallahassee, Florida, USA

To further clarify the role of dopant solvent in proton transfer in atmospheric pressure photoionization (APPI), we employ ultrahigh-resolution FT-ICR mass analysis to identify $M^{+\bullet}$, $[M + H]^+$, $[M - H]^-$, and $[M + D]^+$ species in toluene or perdeuterotoluene for an equimolar mixture of five pyrrolic and pyridinic nitrogen heterocyclic model compounds, as well as for a complex organic mixture (Canadian Athabasca bitumen middle distillate). In the petroleum sample, the protons in the $[M + H]^+$ species originate primarily from other components of the mixture itself, rather than from the toluene dopant. In contrast to electrospray ionization, in which basic (e.g., pyridinic) species protonate to form $[M + H]^+$ positive ions and acidic (e.g., pyrrolic) species deprotonate to form $[M - H]^-$ negative ions, APPI generates ions from both basic and acidic species in a single positive-ion mass spectrum. Ultrahigh-resolution mass analysis (in this work, $m/\Delta m_{50\%} = 500,000$, in which $\Delta m_{50\%}$ is the mass spectral peak full width at half-maximum peak height) is needed to distinguish various close mass doublets: ^{13}C versus ^{12}CH (4.5 mDa), ^{13}CH versus ^{12}CD (2.9 mDa), and H_2 versus D (1.5 mDa). (J Am Soc Mass Spectrom 2007, 18, 1682–1689) © 2007 American Society for Mass Spectrometry

Atmospheric pressure photoionization (APPI) forms positive ions through several mechanisms that include proton transfer reactions [1, 2]. As for electrospray ionization (ESI), a protic compound must be present in solution or the gas phase to facilitate efficient proton transfer. ESI solutions typically include an acid (positive ion) or base (negative ion) at ~1% by volume. Likewise, APPI can have an additional protic solvent added to solution. For example, acetonitrile or methanol has been used successfully to increase protonation of neutral analytes [3, 4]. Furthermore, toluene is often added as a dopant to increase ion yield by proton transfer and/or charge exchange reactions [5, 6]. Specifically for protonation, a compound with a higher proton affinity than the benzyl radical will form the desired $[M + H]^+$ ion [1, 3]. Conversely, charge exchange reactions can produce $M^{+\bullet}$ if the ionization potential of the toluene cation is higher than that of the analyte.

Anions can also be formed in the APPI source. Acidic species can deprotonate to form $(M - H)^-$ and positive electron affinity compounds can capture thermal elec-

trons and form $M^{+\bullet}$. Kostianen et al. studied negative ion formation mechanisms by investigating ionization efficiency and ion type (even- or odd-electron ion) for analytes of different polarity in various solvents [3, 7]. Traldi et al. reported negative ion formation by resonant electron capture from thermal electrons originating from metal surfaces and dopant [8].

Appropriately, these positive and negative ion formation mechanistic studies have involved various compounds mass analyzed sequentially. The molecular structure (i.e., polarity) of the selected compounds and solvent system govern the efficiency of positive and negative ion formation and the proton donor species could be theoretically and experimentally determined.

However, complex mixtures can contain many potential proton donors. Negative and positive ions can be formed simultaneously in the APPI source. Furthermore, negative-ion APPI Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry of a crude oil can produce >12,000 spectral peaks, more than 99% of which arise from deprotonated compounds (i.e., proton donors) [9]. In this work, we couple APPI with a 9.4 tesla FT-ICR mass spectrometer [10] for analysis of a petroleum sample to investigate proton transfer reactions with deuterated toluene, and thereby determine the extent of toluene's contribution to protonation (deu-

Address reprint requests to Dr. A. G. Marshall, Department of Chemistry, Florida State University, National High Magnetic Field Laboratory, 1800 E. Paul Dirac Drive, Tallahassee, FL 32310, USA. E-mail: marshall@magnet.fsu.edu

teration) of analyte ions in a complex mixture. The complexity of the petroleum sample and the presence of close mass doublets (see below) require the high-resolution and mass accuracy afforded by FT-ICR mass spectrometry [11] to resolve and assign molecular formulas to the various deuterated versus protonated compounds.

Relative to ESI, APPI has the advantage of access to much less polar analytes, such as thiophenes, furans, and polycyclic aromatic hydrocarbons. Another recently demonstrated advantage of APPI MS for nitrogen-class compounds is that APPI can differentiate between pyridinic versus pyrrolic nitrogen species in a single positive-ion mass spectrum, whereas electrospray ionization requires acquisition of both positive- and negative-ion mass spectra [12]. Here, we chose to analyze petroleum with high nitrogen content because those nitrogen compounds readily participate in proton transfer reactions (the subject of this paper).

Experimental

Solvents and Compounds

Model compounds and deuterated toluene (C_7D_8) were purchased from Sigma-Aldrich (St. Louis, MO). The aromatic nitrogen compounds were prepared in equimolar concentration (50 μ M) in deuterated toluene.

Crude Oil

Three Athabasca Canadian bitumen distillates were provided by the National Center for Upgrading Technologies (NCUT), Devon, Alberta, Canada. The bitumen distillation cuts were diluted in toluene (500 μ g/mL) or deuterated toluene and analyzed without further preparation. A Thermo Fisher Scientific (Lakewood, NJ) CHNS-O Flash EA elemental analyzer provided elemental weight percentages for the bitumen: carbon $84.1 \pm 0.06\%$ RSD, hydrogen $11.1 \pm 0.01\%$ RSD, nitrogen $0.23 \pm 0.01\%$ RSD, sulfur $3.4 \pm 1.1\%$ RSD, oxygen $0.88 \pm 1.2\%$ RSD.

APPI Source

The APPI source was supplied by Thermo Fisher Scientific. The vaporized analyte gas stream flows orthogonally to the mass spectrometer inlet (heated metal capillary) and the krypton vacuum UV lamp. The source was interfaced via a custom-built adapter to the first differentially-pumped stage of the 9.4 tesla FT-ICR mass spectrometer through a heated metal capillary [9]. A Harvard stainless steel syringe (8 mL) and syringe pump delivered solution to the heated nebulizer of the APPI source. In the APPI source, solvent flow rate was 50 μ L/min; the nebulizer heater was operated at 250 to 350 $^{\circ}$ C with carbon dioxide as the sheath gas at 50 p.s.i., and the auxiliary gas port was closed.

9.4 Tesla FT-ICR MS

All experiments were performed with a custom-built FT-ICR mass spectrometer equipped with a passively shielded Oxford 9.4 tesla superconducting magnet [10, 13]. In the first octopole, ions are accumulated (~ 1 s) [14] before transfer through a quadrupole (not operated in mass-resolving mode) into a second rf-only octopole where they are collisionally cooled (10–20 ms) with helium before transfer through an rf-only octopole to a 10 cm diameter, 30 cm-long open cylindrical Penning ion trap. Multiple (50 to 200) time-domain acquisitions were summed for each sample, Hanning-apodized ($\cos^2(\pi t/2T)$, in which T is time-domain data acquisition period), and zero-filled once before fast Fourier transform and magnitude calculation [15]. Measured ICR frequency was converted to mass-to-charge ratio (m/z) by the usual quadrupolar trapping potential approximation [16, 17]. Negative ion data were collected with similar parameters and appropriate polarity changes. All observed ions were singly-charged, based on the unit m/z separation between $^{12}C_n$ and $^{13}C_1^{12}C_{n-1}$ isotopic variants of the same elemental composition [18]. Therefore, mass spectral peak positions are reported in Da rather than as m/z .

Results and Discussion

Canadian bitumen tar sands have a relatively high nitrogen content. ESI FT-ICR mass spectrometry efficiently ionizes the acidic (pyrrolic) and basic (pyridinic) nitrogen classes in petroleum through proton transfer reactions [19–21]. Prior APPI analysis of a petroleum sample and nitrogen model compounds showed similar ionization trends (compared to ESI) for aromatic nitrogen compounds with additional radical molecular cation formation (primarily for other heteroatom class compounds). Aromatic nitrogen species preferentially form $(M + H)^+$ or $(M - H)^-$ in the APPI source and therefore provide a good test bed for investigation of proton transfer reactions in complex mixtures.

Nitrogen Class Compounds

Five aromatic nitrogen compounds (Figure 1) were chosen to model the proton transfer reaction. The structure of the nitrogen-containing ring determines the preferred ionization mechanism. For five-membered nitrogen rings, the hydrogen bonded to the nitrogen atom is acidic and preferentially deprotonates. On the other hand, the six-membered nitrogen ring species are basic (because of the electron lone pair on nitrogen) and efficiently protonate. Thus, two of the model compounds are basic, two are acidic, and the remaining compound (ellipticine) has both acidic and basic moieties.

The negative-ion APPI FT-ICR mass spectrum (Figure 2) of an equimolar solution of all five nitrogen compounds in deuterated toluene shows ions only for the acidic

Figure 1. Five aromatic nitrogen compounds chosen to model petroleum acidic and/or basic compounds. Five-membered ring nitrogen structures are acidic and six-membered ring nitrogen species are basic.

species. No radical anions ($M^{\bullet-}$) appear. Under continuous APPI source operation, the mass spectrometer was reconfigured for positive ion detection (Figure 3). All five nitrogen model compounds yield positive ions by APPI. The pyrrolic compounds (carbazole and 7H-dibenzo[c,g]carbazole) primarily form radical cations, $M^{+\bullet}$ (Table 1), whereas the pyridinic compounds (acridine and 7,9-dimethylbenz[c]acridine) protonate to form $[M + H]^+$. Ellipticine, which contains both a pyrrolic and a pyri-

dinic moiety, preferentially forms $[M + H]^+$. Table 1 lists the ion relative abundances for each compound, and the parenthetical values are the percentages of $M^{+\bullet}$, $[M + H]^+$, and $[M + D]^+$ for each compound. For the pyridinic species, the ratio of $[M + D]^+$ to $[M + H]^+$ was 0.10 to 0.15 (see upper left inset in Figure 3). In contrast, there was no detectable $[M + D]^+$ for the pyrrolic class compounds (which did form $[M + H]^+$ in low abundance). Interestingly, 7H-dibenzo[c,g]carba-

Figure 2. Negative-ion APPI FT-ICR mass spectrum of an equimolar solution of the model compounds of Figure 1 in deuterated toluene. Only the acidic compounds containing a pyrrole ring deprotonate to yield $[M - H]^-$ ions, none of which contained deuterium.

Figure 3. Positive-ion APPI FT-ICR mass spectrum of an equimolar solution of the model compounds of Figure 1 in deuterated toluene. All five compounds yielded positive molecular ($M^{+\bullet}$) or quasimolecular ($[M + H]^+$) ions. The compounds containing a six-membered pyridinic ring are sufficiently basic to readily protonate (or deuterate) (along with $\sim 1\%$ of radical molecular radical cations), whereas the more acidic compounds containing a five-membered pyrrolic ring form molecular radical cations, and $< 1\%$ protonation (or deuteration). For the even-electron species, the extent of deuteration was $\sim 15\%$ for acridine (see the mass scale expanded inset spectrum), $\sim 10\%$ for ellipticine, and $\sim 14\%$ for 7,9-dimethylbenz[*c*]acridine. Also, at nominal mass 268 (right mass scale-expanded inset), 7H-dibenzo[*c,g*]carbazole exhibits slight hydrogen-deuterium exchange.

zole exhibited minor hydrogen-deuterium exchange $[C_{20}H_{12}D_1N_1]^{+\bullet}$ (see Figure 3). Carbazole also participates in hydrogen-deuterium exchange, but in low relative abundance.

Bitumen Distillation Cuts

Three Canadian bitumen petroleum distillation cuts were analyzed by positive-ion APPI FT-ICR MS. Mass analysis (with ~ 100 ppb mass accuracy) for all three cuts showed that $\sim 90\%$ of the compounds contain at least one nitrogen atom. Furthermore, the N_1 class ("class" denotes $N_nO_xS_s$ heteroatom composition) ions consist primarily of protonated compounds ($> 97\%$). Figure 4 shows the heteroatom class distributions (both APPI and ESI) for the middle

distillation cut (475–500 °C). Note the absence of the S_1 , S_2 , HC, and O_1S_1 classes in the ESI data. Further evidence for the different ionization patterns for APPI versus ESI is provided by the elemental relative abundances shown in Table 2. Positive- (negative-)ion ESI preferentially ionizes basic (acidic) species, whereas both polar and nonpolar species can be generated by positive-ion APPI.

Unlike electrospray ionization sources [22, 23], atmospheric pressure chemical ionization [24], or field desorption ionization [25], the APPI source produces both positive and negative ions simultaneously. Broadband APPI positive- and negative-ion mass spectra (not shown) were obtained for the middle distillation cut. Both spectra were collected without ion source interruption by reversing DC voltage polarity for ion transfer and trapping.

Table 1. Positive-ion APPI FT-ICR MS ion relative abundances for the five aromatic nitrogen compounds of Figure 1

Compound	$M^{+\bullet}$	$[M + H]^+$	$[M + D]^+$
Carbazole	2.7 (87)	0.4 (13)	—
Acridine	0.4 (1)	39.0 (84)	7.0 (15)
Ellipticine	0.8 (5)	14.8 (85)	1.8 (10)
7,9-dimethylbenz[<i>c</i>]acridine	1.2 (3)	25.6 (83)	4.2 (14)
7H-dibenzo[<i>c,g</i>]carbazole	34.6 (99)	0.4 (1)	—

Parentetical values show the percentages of $M^{+\bullet}$, $[M + H]^+$, and $[M + D]^+$ for each compound.

Deuteration Versus Protonation

The Canadian bitumen middle distillation cut was dissolved in deuterated toluene and mass analyzed. The deuterated toluene sample produced a class distribution (not shown) identical to that for the toluene sample (Figure 4). Due to the sample complexity (~ 2000 molecular species within a 400 Da mass window), the ultrahigh resolving power of FT-ICR mass spectrometry was essen-

Figure 4. APPI positive-ion FT-ICR mass spectrum (upper right) and heteroatom class distribution (lower left, along with the corresponding class distribution from ESI FT-ICR MS) for a bitumen mid-range distillate. Each class represents the relative ion abundance of species that contain the stated heteroatom(s) in the assigned molecular formula. The error bars for the APPI data are standard deviation computed from three separate sample preparations and analysis.

tial to resolve and identify the deuterated species. **Figure 5** is a mass scale expanded segment from the bitumen broadband positive-ion mass spectrum. The 2.9 mDa mass difference between two compounds that differ elementally by ^{13}CH versus CD requires a minimum mass resolving power of 160,000 ($m/\Delta m_{50\%}$, in which $\Delta m_{50\%}$ is the mass spectral peak full width at half-maximum peak height). Furthermore, the 1.5 mDa mass difference between H_2 and D requires a minimum mass resolving power of 300,000. Additional mass doublets unique to APPI mass spectra arise by virtue of the presence of radical molecular cations and (de)protonated compounds: namely, 4.5 mDa separation [9] for ^{13}C of $\text{M}^{+\bullet}$ versus ^{12}CH of $[\text{M} + \text{H}]^+$ and 1.5 mDa separation for H_2 of $\text{M}^{+\bullet}$ versus D of $[\text{M} - \text{H}_2 + \text{D}]^+$. All of the above mass doublets could be resolved by FT-ICR mass spectrometry at an average mass resolving power of 500,000 for the seven assigned spectral peaks in **Figure 5**.

It is thus possible to quantitate the relative abundances of deuterated ($[\text{C}_{33}\text{H}_{49}\text{N}_1 + \text{D}]^+$) versus protonated

($[\text{C}_{33}\text{H}_{49}\text{N}_1 + \text{H}]^+$) ions identified in **Figure 5**. For the spectral segment in **Figure 5**, as well as the mass spectrum as a whole, the detected deuterated ions contributed $([\text{M} + \text{D}]^+ / [\text{M} + \text{H}]^+) \approx 5\%$ for the even-electron N_1 class compounds. For other less abundant protonated species, any deuterated species were below the detection limit.

Negative and Positive Ion Class Distributions

Unique elemental compositions were assigned to both negative and positive ion mass spectral peaks based solely on accurate mass measurement [26] combined with sorting of homologous alkylation series [27, 28] to yield 1844 negative-ion elemental compositions with an RMS mass error of 105 parts-per-billion, and 1801 positive-ion elemental compositions with an RMS mass error of 149 parts-per-billion. **Figure 6** displays the heteroatom classes for positive- and negative-ion APPI FT-ICR mass spectra. The elemental compositions for each ion class can be further sorted by DBE double-bond equivalents, in which DBE is the number of rings plus double bonds calculated from eq 1:

$$\text{Double Bond Equivalents } (\text{C}_c\text{H}_h\text{N}_n\text{O}_o\text{S}_s)$$

$$= c - h/2 + n/2 + 1 \quad (1)$$

Representative structures for the most abundant DBE components of the most abundant (N_1) class com-

Table 2. Elemental relative abundance for Canadian bitumen, calculated from ESI or APPI FT-ICR mass spectral peak magnitudes

	% C	% H	% N	% O	% S
(+) ESI	84	11	2.9	0.16	1.2
(-) ESI	79	11	0.0	7.2	2.0
(+) APPI	84	11	2.6	0.60	1.9
(-) APPI	81	11	0.37	5.7	1.5

Figure 5. Positive-ion APPI FT-ICR mass scale-expanded segment of a bitumen mid-range distillate in deuterated toluene. This figure emphasizes the ultrahigh resolving power required to resolve the deuterated species in complex petroleum mixtures.

Figure 6. Heteroatom class distribution for the positive and negative ions from a bitumen mid-range distillate. Generic structures are shown for the most abundant positive and negative species. DBE is the number of rings plus double bonds, and is calculated from eq 1. Because only $\sim 5\%$ of the even-electron N_1 class ions contain deuterium, the acidic neutrals in the original sample are likely proton donors to form the even-electron species from basic neutrals.

pounds are illustrated. Each molecular structure represents one of many possible isomers. Nevertheless, we can say that the nitrogen atom DBE 9 N_1 class positive ion resides in a six-membered pyridinic ring.

Need for Dopant

We also performed APPI (data not shown) for a crude oil sample in methylene chloride (i.e., no charge-exchange dopant), and observed a dramatic shift (relative to toluene as solvent) in favor of protonated compounds relative to radical cations for both N_1 and C_6H_5 classes. The sample was a processed petroleum that contained highly condensed polycyclic aromatic compounds ($20 < \text{DBE} < 40$), favoring charge-exchange (radical cation) reactions in the presence of toluene. However, APPI applications typically use a dopant to promote proton transfer, and toluene is a common dopant.

Conclusions

APPI of the nitrogen class compounds in a petroleum sample preferentially forms ions by means of proton transfer reactions. The Athabasca bitumen N_1 class positive ions consist of $>97\%$ protonated compounds and only $\sim 3\%$ radical molecular cations. Based on their elemental compositions, all of the negative ions form by deprotonation of precursor neutrals. Aromatic nitrogen model compounds (Figures 2 and 3) exhibit similar ionization trends in which most of nitrogen species are ionized through proton transfer reactions (Table 1). Although pyrrolic species more efficiently form negative ions and pyridinic species positive ions, pyrrolic species can also form radical cations ($M^{+\bullet}$). Interestingly, the more aromatic pyrrolic compound (7H-dibenzo[c,g]carbazole) exhibited higher ion abundance (radical cation) than carbazole, suggesting that a more condensed aromatic core structure can add stability to a radical cation. The carbazole positive ion is present at low spectral magnitude (Figure 3). Carbazole is a likely proton donor to the pyridinic species and thus would be detected as a negative ion in higher abundance (as seen in Figure 2).

Negative and positive ions can be formed simultaneously in the APPI source and, therefore, there are many potential proton donors including toluene (or deuterated toluene) solvent. However, in the present bitumen sample, deuterated toluene donated a deuterium to only 10% to 15% of the even-electron ions formed from pyridinic nitrogen model compounds. Presumably, the proton donors for the pyridinic nitrogen compounds (for the model nitrogen compound spectra) are the pyrrolic (acidic) nitrogen compounds from the sample itself (even though the toluene dopant is present at orders of magnitude higher concentration).

For the petroleum sample, only $\sim 5\%$ of the even-electron nitrogen class species were deuterated. The most abundant negative-ion species is the O_2 class,

likely carboxylic acids. All species detected in the petroleum negative-ion spectrum were $[M - H]^-$. Reasonably, the even electron nitrogen class species that were protonated (not deuterated) are protonated through reactions with acidic species present in the sample.

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