Petroleomics by EASI(±) FT-ICR MS

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An ambient ionization/desorption technique, namely, easy ambient sonic-spray ionization mass spectrometry (EASI), has been applied to crude oil samples. From a single droplet of the sample placed on an inert surface, EASI(±) is shown to promote efficient desorption and ionization of a myriad of polar components via the action of its cloud of very minute supersonic bipolar charged droplets. The gaseous $[M + H]^+$ and $[M - H]^-$ ions concurrently formed by EASI(±) were analyzed by Fourier transform mass spectrometry (FT-ICR MS), and a total of ~6000 acidic and basic components have been attributed. EASI(±) FT-ICR MS of crude oils is show to be almost as fast as ESI(+)/ESI(−) FT-ICR MS, providing similar compositional information of polar components and spectral quality comparable to that of a commercial nonochip-based robotic ESI device. EASI(±) requires no sample workup thus eliminating risks of contamination during sample manipulation and memory effects because of carry over in pumping ESI lines. More importantly, EASI(±) is a voltage-free ionization technique therefore eliminating risks of redox processes or duality of ionization mechanisms that can be observed in voltage-assisted processes. Data visualization via typical petroleomic plots confirms the similarity of the compositional information provided by EASI(±) compared to ESI(+)/ESI(−). The ambient EASI(±) FT-ICR MS method requires no voltage switching in changing the ion polarity mode, offering a workup, heating and voltage-free protocol for petroleomic studies performed at open atmosphere directly on the undisturbed crude oil sample.

Petroleum is considered the world's most complex natural mixture, and its myriad of constituents offers one of the most challenging samples for chemical analysis. Since its infancy and commercial birth, mass spectrometry (MS) has been intimately tied to the analysis of crude petroleum and distillates.[1] The use of MS, particularly in its high resolution or tandem versions or in combination with different separation techniques, such as in GC-MS or LC-MS, has provided quite comprehensive characterization of petroleum distillates. Until recently, however, the compositional complexity of the crude sample and its heavy distillates exceeded the requisite resolution and mass accuracy of state of the art mass spectrometers and this restriction limited the compositional information during direct MS analysis of crude oils.[2] This restriction was removed, however, with the development of atmospheric pressure ionization (API) techniques, most notably electrospray ionization (ESI), and of ultrahigh-resolution and ultrahigh-accuracy Fourier transform ion cyclotron ressonance mass spectrometry (FT-ICR MS). The combination of ESI with FT-ICR MS has allowed direct characterization of crude oils without pre-separation methods with unambiguous assignment of heteroatom-containing organic components of crude oils that contain in excess of 20 000 distinct elemental compositions. This field is now well-established and known as petroleomic MS.[3] Petroleomic MS characterization of crude oils has highlighted compositional trends providing data on most important geochemical parameters such as type and geochemical origin, maturity, acidity, corrosion levels, biodegradability, and processing information related to distillation cuts, extraction methods, catalytic processing, and other crucial crude oil properties.[4]

Recently, a series of ambient MS techniques have been introduced,[5] allowing very rapid and simple MS chemical analysis on open atmosphere directly from samples in their natural environments or matrixes with no pre-separation and little or no sample workup. These techniques were pioneered by desorption electrospray ionization[5] (DESI) and direct analysis in real time (DART),[6] but a set of related techniques are currently available

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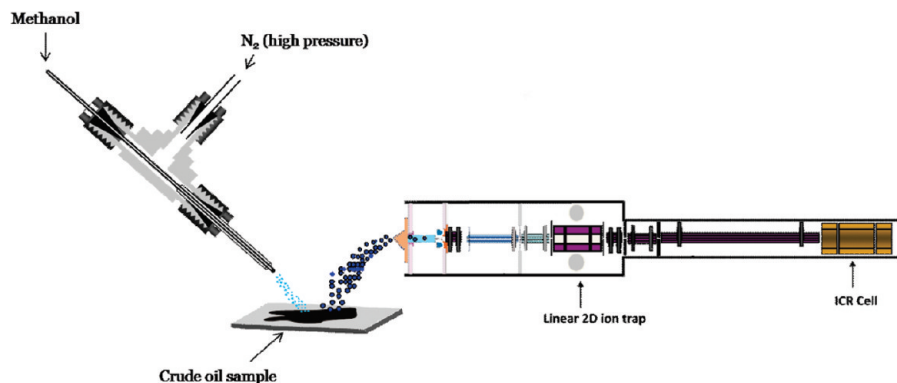‡ Petróleo Brasileiro.
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Figure 1. Schematics of petroleomics by EASI(±) FT-ICR MS. A tiny droplet of the undisturbed crude oil is placed on an inert polyethylene film surface. A myriad of polar components (ca. 6000) are desorbed by the supersonic stream of minute bipolar charged methanol droplets (light blue dots), ionized as both [M + H]$^+$ and [M − H]$^-$ ions (dark blue dots) and transferred (either [M + H]$^+$ or [M − H]$^-$ ions depending on the polarity of operation) inside the mass spectrometer for ultrahigh resolution and ultrahigh accuracy FT-ICR MS measurements.

including atmospheric solids analysis probe (ASAP),[7] electrospray-assisted laser desorption ionization (ELDI),[8] extractive ESI (EESI),[9] desorption atmosphere pressure photon ionization (DAPPI),[10] and easy ambient sonic-spray ionization (EASI).[11] Collectively, these direct desorption/ionization methods present compelling advantages in terms of simplicity via elimination of sample pre-separation and preparation, with reduced risks of contamination or perturbation of sample composition and potentially high sample throughput. EASI is one of the simplest and most easily implemented of such techniques. Because it is based on supersonic spray ionization (SSI),[12] EASI relies solely on the forces of a high-velocity supersonic nebulizing gas to generate gaseous ions without the assistance of any voltage, radiation or heating. The SSI spray provides a dense cloud of bipolar (both negatively and positively charged) droplets that promote analyte desorption and ionization typically as both [M + H]$^+$ and [M − H]$^-$ ions, eliminating the need to switch high voltages in going, for instance, from EASI(+) to EASI(−). EASI is also known to deposit less energy into the gaseous ions than ESI.[13] Since EASI is a voltage-free and heat-free technique, it also avoids thermal, electrochemical or discharge processes[14] that are known, for the high-voltage ESI[15] and derived techniques such as DESI,[16] to induce redox interferences or competitive different ionization mechanisms leading to diverse ionic forms such as, for instance, [M + H]$^+$ and M$^+$ that may disturb petroleomic data processing.

A myriad of samples and mixtures have been tested for ambient MS analysis,[17] but there is no report on the use of ambient ionization techniques for petroleomic studies. The ability of ambient MS to perform efficient desorption and ionization would be tested to its topmost limit if applied to crude oils, one of the most complex and challenging chemical mixtures. Herein we describe the first petroleomic MS investigation via ambient MS, and compare its performance and advantages to conventional petroleomic studies.

EXPERIMENTAL SECTION

ESI(+)/ESI(−) FT-ICR MS. A sample of a Brazilian crude oil (10 mg) was dissolved in 10 mL of toluene and then diluted with 10 mL of methanol. For analysis in both the positive and negative ion modes, pure methanol was used as the spray solvent. Solvents and additives were of HPLC grade, purchased from Sigma-Aldrich, and used as received. Direct infusion automated chip-based nano-ESI-MS were performed on a Triversa NanoMate 100 system (Advion BioSciences, Ithaca, NY) in both the positive and negative ion modes. Samples were loaded into 96-well plates (total volume of 100 μL in each well) and analyzed by a 7.2T LTQ FT Ultra mass spectrometer (ThermoScientific, Bremen, Germany). General ESI conditions were as follow: gas pressure of 0.3 psi and capillary voltage of 1.55 kV and a flow rate of 250 nl min^{-1}. Mass spectra were the result of over 100 microscans processed via the Xcalibur 2.0 software (ThermoScientific, Bremen, Germany).

EASI(±) FT-ICR MS. A droplet of the crude oil was placed on a solid and inert surface (polyethylene film) and bombarded by the EASI(±) supersonic spray (200 psi) of bipolar charged droplets. More details of the EASI source and its operation are provided elsewhere.[18] Basically, a custom source (Figure 1) was used, and the EASI solvent was infused at a flow rate of 10 μL min^{-1}. The supersonic spray ionization was assisted only by compressed N$_2$ at 200 psi, and the EASI source used a simple Swagelok T-element with appropriate ferrules and tubing for the gas flow and a fused-silica capillary at the supersonic spray exit. A very large number of polar components (~6000) have been detected, resolved via EASI(±) FT-ICR MS in the crude oil sample. The ICR cell is limited to

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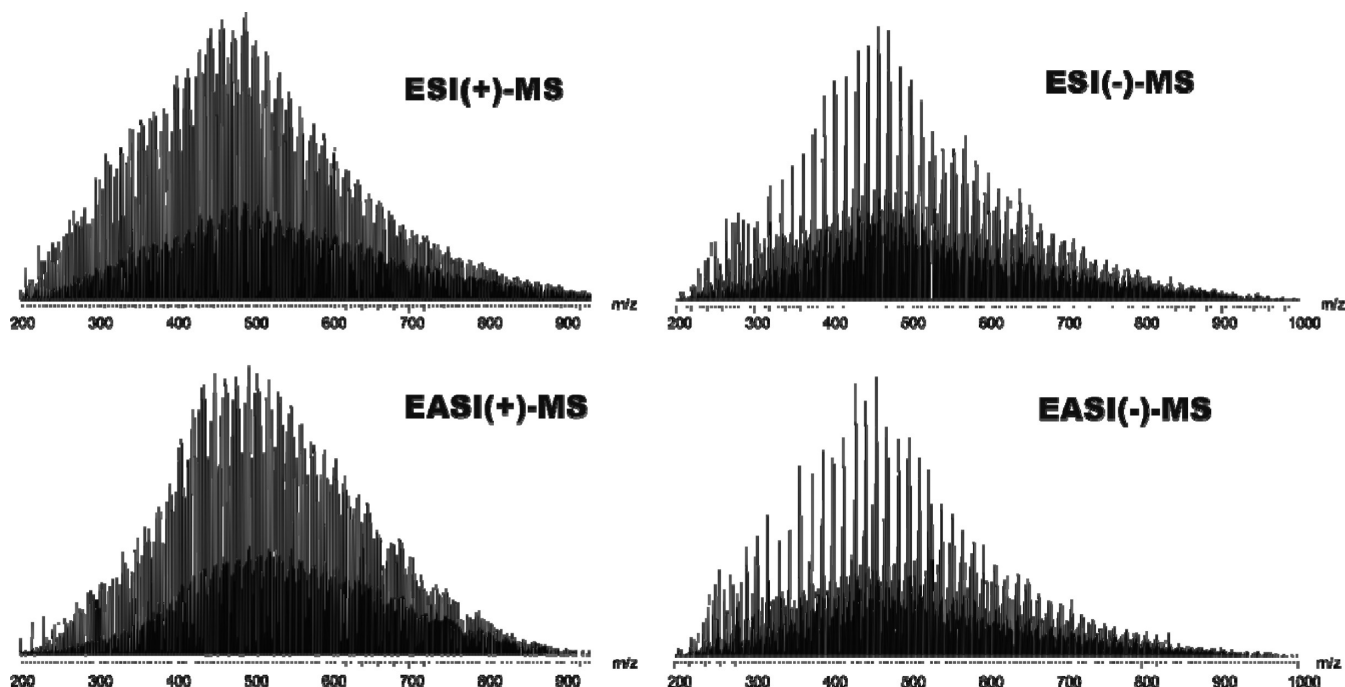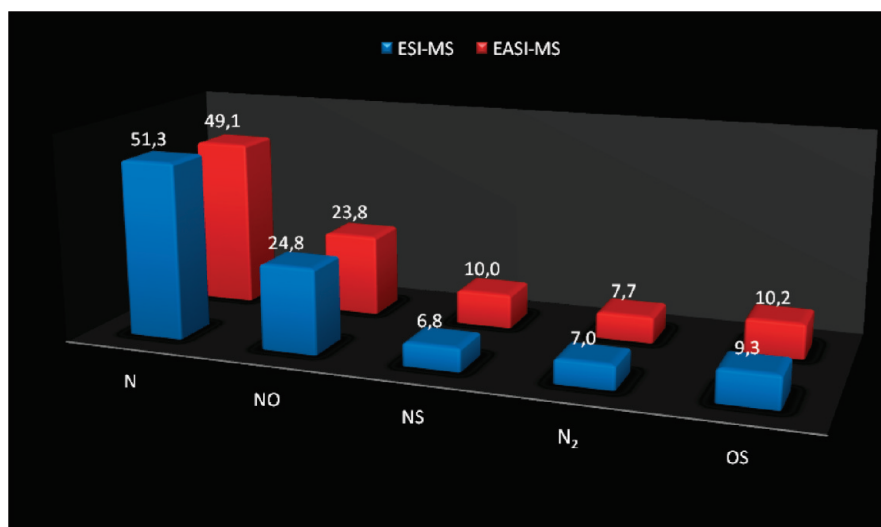
Figure 2. ESI(+)/ESI(−) FT-ICR MS and EASI(±) FT-ICR MS of a representative raw crude oil sample. Note the similarity of the two spectra as evidenced, for instance, by similar M_n and M_w. The average mass resolving power in both spectra ranges from ∼300 k < $m/\Delta m_{50\%}$ < 450 k.
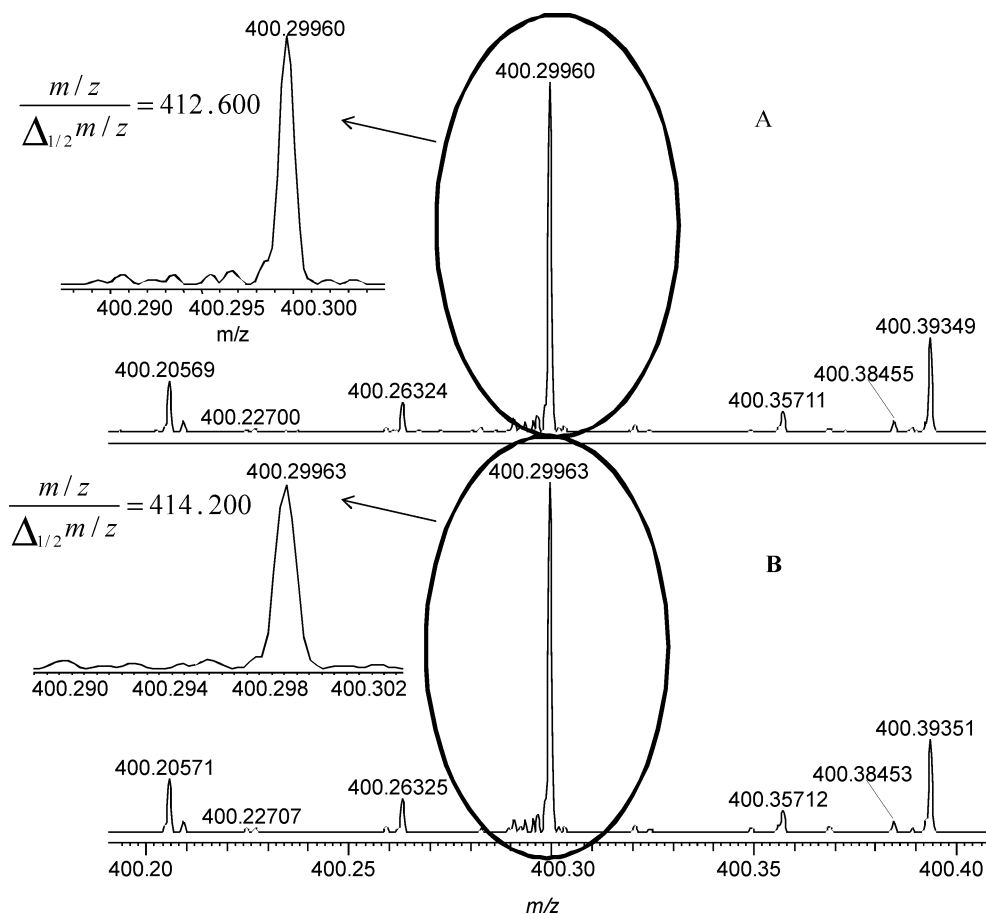
Figure 3. Expansion of the A) ESI(+)-MS and B) EASI(+)-MS data of Figure 1 illustrating the similar resolution, accuracy and signal-to-noise ratio of both spectra.

hold ∼1 000 000 ions per scan, thus 6 min (nano-ESI) or 10 min (EASI) of spectra acquisition was required to sum 100 time domain transients so as to obtain the best compromise for signal-to-noise ratio and speed of analysis.

Figure 4. Profiles of major classes of crude oil components (%) as obtained by ESI(+)-FT MS and EASI(+)-FT MS.

RESULTS AND DISCUSSION

Figure 2 compares both EASI and chip-based ESI FT-ICR MS data in both the positive and negative ion modes. Note that, overall, spectra with similar chemical profiles and signal-to-noise ratios were obtained, as confirmed by data analysis (see below). This similarity is an interesting finding since, despite the myriad of chemicals, no substantial discrimination seems to occur during direct desorption and ionization via EASI(±) from the crude oil sample as compared to the solution-based robotic nanochip ESI(+)/ESI(−) processes. The very minute *bipolar* droplets produced by supersonic spraying the methanol solution perform a superb job of picking up a myriad of chemicals in a very short contact time directly from the raw crude oil likely via a "splashing" mechanism similar to what has been proposed for DESI.[5]

Figure 3 illustrates an expansion around m/z 400 for both EASI(+) FT-ICR MS and ESI(+)-FT-ICR MS, which allows one to compares the overall performance of both techniques. Note, in both expansions, very similar spectrum quality with nearly the same mass resolving power (412.600 for ESI and 414.200 for EASI), excellent *signal-to-noise* ratios, the same number of assigned peaks, and the similar accuracies for the m/z measurements. We are currently testing the similarity of EASI(±)-MS and ESI(+)/ESI(−) data for different types of crude oils, and very similar data has always been obtained.

Petroleomic Data Processing. The MS data were processed for formula attribution from the measured m/z values using a custom algorithm developed specifically for petroleum data processing: the PetroMS software.[19] Approximately, 6000 components were assigned (elemental composition) from each spectrum, and Figure 4 shows the relative distributions of these components by heteroatom classes. Note the very similar profiles for the major classes of polar constituents present in crude oils. This similarity confirms the ability of EASI(±)-MS to reproduce ESI(+)/ESI(−) profiles, even those provided by the finest robotic chip-based ESI devices.[20]

Classical Petroleomic Plots. To help summarize, visualize, and interpret petroleomic data, because of its huge complexity,
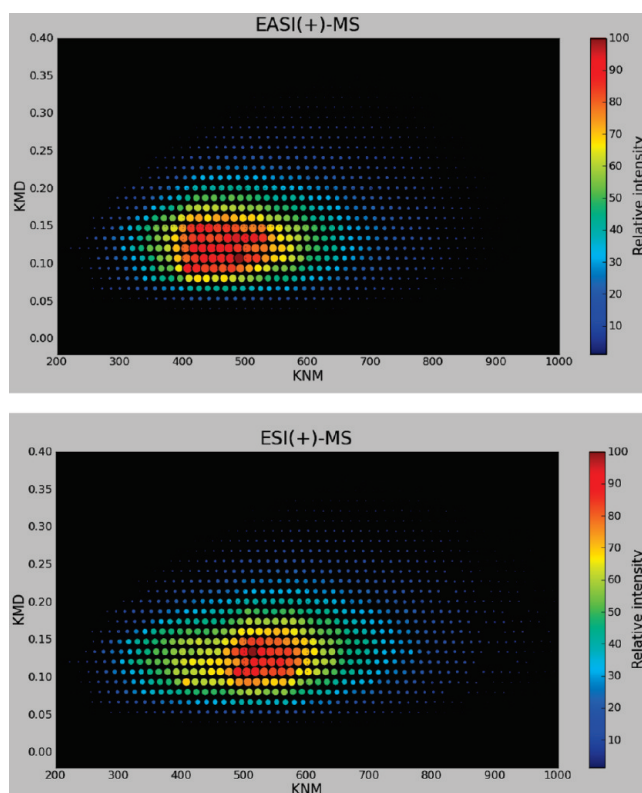
Figure 5. Kendrick plot for the class of N polar components from both ESI(+) FT-ICR MS (upper) and EASI(+) FT-ICR MS data (bottom).

classical plots are commonly used, such as the Kendrick[21] and van Krevelen[22] diagrams. For the Kendrick plot, a Kendrick

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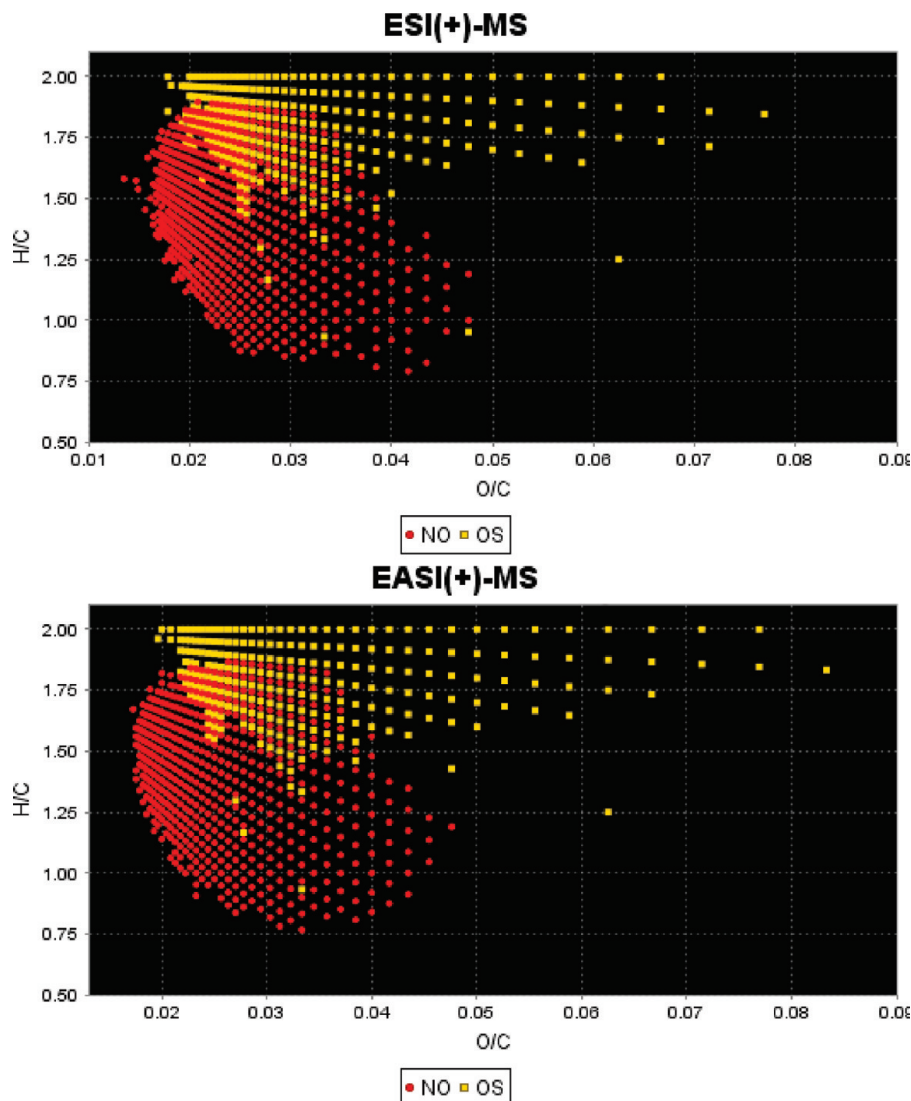
Figure 6. van Krevelen diagram (H/C versus O/C ratios) for the classes of N and OS polar components from both ESI(+) FT-ICR MS and EASI(+) FT-ICR MS data.

nominal mass (KNM) is calculated by converting the mass of all CH_2 groups from 14.015 65 to 14.000 00. In this way, the mass defect of the repeating CH_2 units are canceled, and homologous series, that is homologue series of compounds with the same constitution of heteroatoms and number of rings plus double bonds (DBE) but different numbers of CH_2, will have the same Kendrick mass defect (KMD) and will fall on a single horizontal line. Unsaturation (double bonds or rings = DBE level) will increase KMD by 0.01340 Da (i.e., the KMD for two H atoms) hence the same class differing from DBE level will fall in characteristic parallel lines separated by 0.01340 KMD with the higher the DBE, the higher the line. When different classes are plotted together, classes with different elemental composition such as N (pyridine homologues) and NO (amides or quinoline homologues), but the same DBE will display slightly different KMD and produce close parallel lines. The 3D Kendrik plot provides, therefore, the mass distribution according to specific classes as a function of the unsaturation level.

The top point on its 3D surface (the red areas in Figure 5) shows the extent of unsaturation and at which masses it concentrates. This information is used as an important geochemical tool for determining maturation.[4]

In van Krevelen plots,[22] elemental composition of the oil components are compared by projecting net atomic ratios, such as H/C, O/C, and N/C, onto two (or three) axes. The H/C ratio separates compounds according to degree of unsaturation, whereas the O/C or N/C ratios separate them according to the amount of O and N. It provides therefore visual comparison of classes as a function of heteroatom content and unsaturation level, being also a classical plot used to follow crude oil processing. As an example, this plot can be used to monitor hydrotreatment of crude oils or biodegradation levels via the relative abundance of the O, O_2, and O_3 classes.[4]

Figures 5–8 compare such classical petroleomic plots obtained via EASI(+)-MS and ESI(+)-MS for the same crude oil sample. Figures 5 and 6 shows Kendrick and van Krevelen plots; Figure 7 shows another widely used petroleomic plot for carbon number versus DBE. Figure 8 displays a 3D counterplot of the Kendrick

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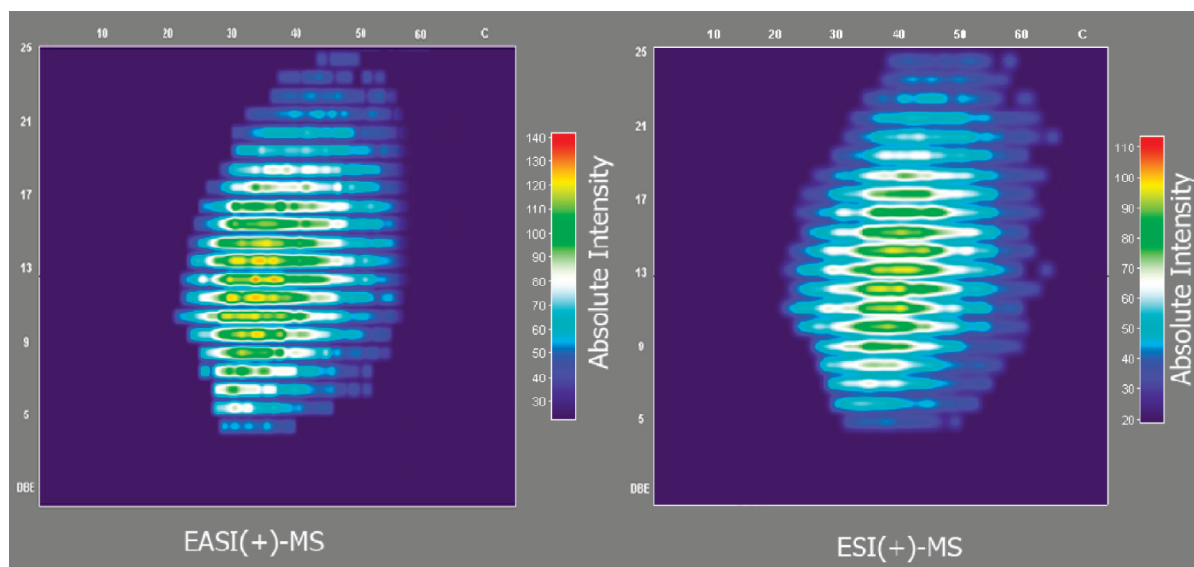
Figure 7. Plots of carbon number vs DBE for the NO class of polar components from both EASI(+)and ESI(+) FT-ICR MS data.
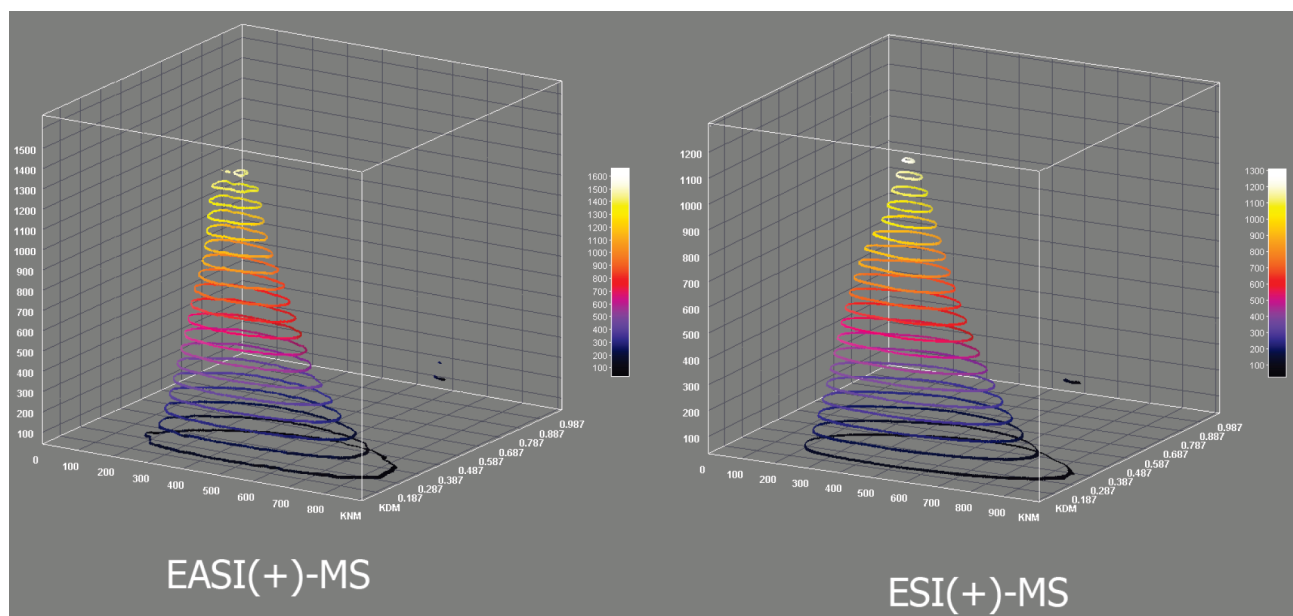
Figure 8. 3D Kendrick counterplots (KNM × KMD × abundances) for the class of *N* polar components from both EASI(+) and ESI(+) FT-ICR MS data.

plots of Figure 5 aimed to facilitate visualization of the abundances of homologous series.

In these sets of classical petroleomic plots, similar profiles are observed for both ESI(+)-MS and EASI(+)-MS, which again points toward the ability of the very minute bipolar EASI(±) charged droplets to pick up, ionize and then transfer to the gas phase, concurrently as both $[M + H]^+$ and $[M - H]^-$ ions, the immensity of components directly from an undisturbed single droplet of the crude oil sample. The similarities indicate that these tasks are performed in a matter as efficient as that observed from the monopolar charged ESI(+) plus ESI(−) droplets produced by electrospraying methanolic solutions of the oil via the finest commercial robotic ESI nanochip systems.

CONCLUSION

The first petroleomic study by ambient mass spectrometry has been described. Crude oil, because of its high diversity and complexity, represents a test for ambient MS of its topmost limit of desorption and ionization efficiency of a complex chemical mixture. Despite the myriad of chemicals that must be picked up from the oil surface at exceptionally short contact times, the very minute bipolar EASI(±) charged droplets have been shown, on average, to perform an excellent desorption/ ionization job for a single droplet of crude oil placed on an inert auxiliary surface. Acquiring EASI(±) FT-ICR MS data was slightly more time-consuming (10 vs 6 min), it provided similar profiles of polar components with similar spectra quality as compared to a solution-based commercial nanochip-based

robotic ESI(+)/ESI(−) device, as exemplified by classical petroleomic plots. EASI(±) is based on SSI(±), which is known to be one of the softest ionization techniques, depositing less energy into the gaseous ions than ESI.[12] EASI(±) also concomitantly generates mainly $[M + H]^+$ and $[M − H]^-$ ions, whereas ESI(+)/ESI(−) may also form molecular ions M^+ or M^- depending on the analyte molecules.[14] These features of EASI(±) seem advantageous for the analyzes of complex mixtures since analytical information will be concentrated in a single ionic species rather than fragments or the competitive molecular ions such as M^+ for $[M + H]^+$. More importantly, the EASI(±) FT-ICR MS data was obtained via a direct approach for the "undisturbed" crude oils thus eliminating any type of sample workup, and therefore the risks associated with sample manipulation such as contamination from solvents and glassware or chemical disturbance during sample preparation protocols. Additionally and contrary to ESI(+)/ESI(−), EASI(±) uses neither heating nor voltages, which eliminates risks of thermal, electrochemical, or discharge interferences (redox processes) that may occur during ESI(+)/ESI(−). EASI(±) displays therefore several features beneficial for petroleomic studies.