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ARTICLE

Fullerenes in asphaltenes and other carbonaceous materials: natural constituents or laser artefacts?

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The presence of fullerenes as natural constituents of carbonaceous materials or their formation as laser artifacts during laser desorption ionization (LDI) mass spectrometry (MS) analysis is reinvestigated and reviewed. The results using asphaltenes samples with varying composition as well as a standard polycyclic aromatic hydrocarbons (PAH) and fullerene samples as models have demonstrated that indeed C_n ring fullerenes are not natural constituents but they are formed as common and predominant artifacts upon laser radiation, and a series of incorrect assignments based on LDI-MS data of several carbonaceous materials seems unfortunately to have been made. When the present results are evaluated also in the light of the vast literature on LDI-MS of carbonaceous materials, the formation of fullerene artifacts seems particular important for LDI-MS analysis of alphaltenes and other carbonaceous samples with considerably high levels of PAH and vary according to the type of laser used, and the intensity of the laser beam.

Introduction

The characterization of the myriad of molecules that form most complex chemical mixtures such as those found in crude oils [1,2], bitumen [3], carbonaceous rocks [4,5] and eventually even in extra-terrestrial bodies such as meteorites [5] has been a most challenging task for the analytical chemist. Sample manipulations such as solvent or thermal extractions or derivatization reactions are always a great concern due to eventual contamination, whereas the applied technique should be robust and extensively investigated for each type of matrix so as to avoid incomplete sample description or the misleading information from artefacts. Mass spectrometry (MS), due to its superior speed, selectivity and sensitivity as well as the increasingly reduced need for sample manipulation and the power to deal with mixtures via concomitant ion separation and measurement has been increasingly used for complex mixture analysis [6,7,8,9]. For instance, for the comprehensive characterization of crude oil, arguably the most complex chemical mixture found on earth, MS has become the central technique able to characterize thousands of its constituents [1,2] with little manipulation.

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Among crude oil constituents, asphaltenes forms an important class, but this fraction of petroleum is curiously not defined in terms of its structural features but a solubility criterion is used [10]. That is, asphaltenes are defined as the fraction of petroleum insoluble in low MW n-paraffin, such as *n*-pentane or *n*-heptane [11]. The composition and structure of the asphaltenes have been for decades, and still remains a topic of considerable debate in crude oil chemistry. In general, studies have suggested that asphaltenes comprises molecules with a vast range of functional groups, including alkyl rings and chains, aromatic and poly heteroaromatic rings, carboxylic acids, basic nitrogen groups and porphyrins [12,13,14]. A major controversy is also related to the predominance of either archipelago or island-like structures [15,16,17] such as the two model molecules of Figure 1, but numerous experiments using a vast array of techniques such as time-resolved fluorescence depolarization, laser desorption laser ionization (tandem) mass spectrometry, optical spectroscopy, molecular orbital calculations, NMR spectroscopy, and atomic force microscopy seem to have established that asphaltene molecules consist predominantly of single aromatic cores with alkyl side chains in molecules with an average weight around 700 Da [18].

Figure 1. Examples of compounds with island (A) and archipelago (B) structures assigned to asphaltene constituents.

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As for crude oils, asphaltene composition has indeed shown to be extremely complex and difficult to characterize [19]. To make its characterization even more challenging, asphaltene composition also varies as a function of the crude oil origin, the type of residuum from which they have been precipitated, either from atmospheric or vacuum distillation, the precipitant used as for instance *n*-pentane or *n*-heptane and/or the precipitation conditions such as sample-to-precipitant ratio, addition of solvents, temperature, agitation and digestion time [20].

MS characterization of asphaltenes has employed different ionization methods including field ionization (FI) [21], field desorption ionization (FDI) [22], electrospray ionization (ESI) [23], atmospheric pressure chemical ionization (APCI) [24], atmospheric pressure photoionization (APPI) [25], LDI [26,27,28], two-step laser desorption laser ionization (L^{2}MS) [29], and SALDI [18]. Although challenging, the comprehensive knowledge of asphaltene composition has been highly pursued in geochemistry studies since these molecules have been the source of major problems associated with crude oil processing [30,31]. Another longstanding controversy is the actual range of MW distribution of asphaltenes which predictions varying from less than 1000 Da to as high as 100.000 Da [32], but nowadays this question seems to have settle down and asphaltenes are believed to display an average MW of ca. 700 Da [18].

LDI techniques have been perhaps the most extensively applied but are those that have also generated most controversy, since a diverse range of MW including high MW have been predicted [**Error! Bookmark not defined.**]. It has been determined, however, that the high MW was due to formation of gaseous asphaltene aggregates [33]. A seminal controversy also arose when Buseck and co-workers [34a] used LDI-FT-ICR-MS, thermal desorption ionization-MS and electron ionization, in conjunction with high resolution transmission electron microscopy (HRTEM), to analyse Sungite - a carbon-rich Precambrian rock from Russia - and Fulgurite [35b] as well as a glassy rock formed by the lightning strike in the ground, concluding for the natural occurrence of fullerenes C_{60} and C_{70} in these specific carbon-rich rocks. In other seminal work that has attracted considerable attention, Becker and co-workers [35], using LDI-MS as well, have also concluded for the presence of fullerenes in meteorites, reporting the presence of C_{60} and C_{70}, as well as a unique distribution of remarkably stable clusters of C_{100} to C_{400} separated all by *m/z* 24 units in the mass spectra. In all, the authors concluded that "*these large extraterrestrial carbon clusters are either the first indication of higher fullerenes or are an entirely new range of aromatic carbon-rich molecules*".

More recently, Traldi and co-workers [36] used LDI-MS to analyse asphaltenes from crude oils and detected a Gaussian-like homologous ion series separated as well by *m/z* 24 units, which they have interpreted as well as latter by others [37] as evidence not for fullerenes but for PAH, that is, more specifically a series of polycondensed aromatic compounds with varying numbers of condensed rings with additional C=C bonds (Figure 2). MS measurements with high resolution and accuracy would have allowed the authors to characterize the +24 *m/z* series distinguishing all-carbon species (fullerenes) from PAH with their characteristic mass defects form the H atoms, but such data was not reported. For asphaltenes, controlled conditions such as laser power, surface concentration and pulsed ion extraction, have been reported to avoid aggregation whereas LDI-based techniques have been reported to provide consistent and reliable results for asphaltene MW and composition [27,33,38].
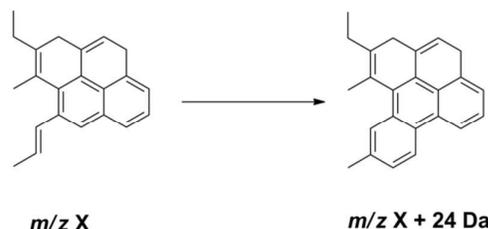
Figure 2. Structures proposed by Traldi and coworkers [36] for the homologous series of ions separated by *m/z* 24 units and detected during LDI-MS of asphaltenes.

The initial goal of this work was to perform a petroleomic investigation via (MA)LDI-MS of asphaltenes from crude oils with contrasting levels of thermal evolution hence with expected contrasting compositions. But surprisingly, we just observed very similar (MA)LDI-MS profiles for all samples with a well behaved Gaussian-like series of ions separated by *m/z* 24 units [39]. As discussed below, contrasting interpretations for such profile were found in the literature, not only for asphaltenes but also for samples ranging from coal, rocks, bitumen and meteorites. We decided therefore to perform, as this paper reports, a systematic evaluation of the LDI-MS reliability in asphaltene analysis and to re-evaluate the substantial body of literature data on LDI-MS of asphaltene and other carbonaceous materials on the light of our results to conclude for the natural presence of fullerenes or their formation as laser artifacts.

Experimental

Samples and Reagents

Samples of crude oil from different reservoirs in Brazil were provided by CENPES/ PETROBRAS. For the asphaltenes precipitation approximately 80 mg of crude oil were stirred in glass flasks during 24 hours in a 5 mL of 40:1 (v/v) mixture of heptane (HPLC grade, Honeywell Burdick) and crude oil [38]. In total, 20 different samples were evaluated. The insoluble asphaltenes were filtered using a Millipore 0.45 μm pore diameter PTFE filter and dissolved in toluene (HPLC grade, J.T Baker, Mexico City, Mexico) at concentrations of ca. 0.2 mg/mL. These solutions were spotted onto a steel target plate

Analyst

(MTP 384 polished steel target; Bruker Daltonics, Bremen, Germany) and allowed to air-dry. For MALDI, a saturated solution of DHB (98%, Sigma-Aldrich Co., St. Louis, MO, USA) was used as matrix and spotted upon the sample. For LDI, the asphaltene solutions in toluene were spotted and directly analyzed after toluene evaporation and asphaltene crystallization. A synthetic standard of fullerene C_{60} (Aldrich, MO, USA) and a standard of a polycyclic aromatic hydrocarbon (PAH) mixture (AccuStandard, CT, USA) containing the 16 EPA target PAHs (acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, benzo(a)pyrene, chrysene, dibenz(ah)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene and pyrene) were also subjected to LDI-MS and spotted using 1 μg/mL toluene solutions.

Mass Spectrometers

The MALDI-MS and LDI-MS analysis was performed in a Bruker Autoflex III MALDI-TOF(/TOF)-MS operated in the linear mode and equipped with a smart beam laser using Flex Control 3.3 software (Bruker Daltonics). The MS were collected within the mass range of m/z 100 to 3500 in the positive ion mode. The instrument settings were as follows: ion source 1 at 18.99 kV, ion source 2 at 16.64 kV, lens at 7.19 kV, and a pulsed ion extraction of 40 ns. The instrument was externally calibrated with the peptide standard supplied by Bruker Daltonics. The laser power was tested in a range of 10-90% to evaluate the optimal condition.

FT-ICR-MS analyses were performed in Thermo Scientific 7.2 T Fourier transform ion cyclotron resonance mass spectrometer (Thermo Scientific, Bremen, Germany). A scan range of m/z 200–1000 was used, and 100 microscans (transients) were collected in each acquisition. The average resolving power (R_p) was 400,000 at m/z 400. Time-domain data (ICR signal or transient signal) were acquired for 3000 ms. Microscans were co-added using Xcalibur version 2.0 (Thermo Scientific).

The molecular formulas of the APPI(-) signals in the mass spectrum of asphaltene samples were assigned using the Xcalibur 2.0 software as well and verified by its isotopic pattern of ^{13}C. Then, Excel spreadsheets were used to sort and group the formulas and compounds to build class diagrams (Supp. Information). All the interpretations and data treatment were made using homemade software for petroleomics.

Results and discussion

First, both MALDI-MS using DHB as the matrix as well as matrix-free LDI-MS data was acquired. Note that the presence of polycondensed aromatic molecules have been shown to make asphaltenes to work as a "self-matrix" for LDI-MS and

indeed, for the samples tested herein, a matrix was unnecessary. Figure 3 shows, as an example and for comparison, a characteristic MALDI and a LDI mass spectrum of a typical asphaltene sample. Surprisingly for all samples, despite the contrasting crude oil composition from which they were precipitated, the spectra were all very similar regardless the level of thermal evolution of the crude oil from which the asphaltenes samples had been precipitated.
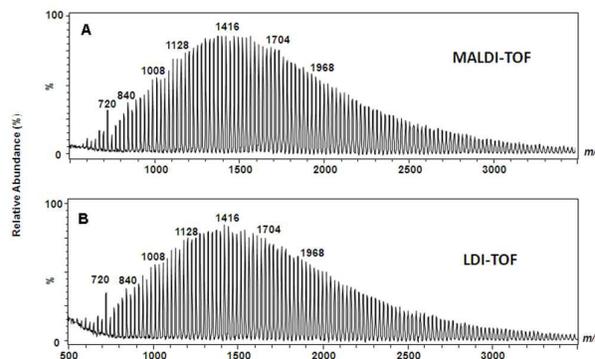
Figure 3. A) MALDI(+)-MS using DHB as matrix and B) LDI(+)-MS of a typical asphaltene sample.

Note that both spectra in Figure 3 show very similar and continuous Gaussian-like profiles but the ion of m/z 720 clearly shows up. These well behaved m/z 24 (24 Da for singly charged ions) apart ion series is also quite contrasting from those typical much packed continuous ion series commonly displayed by crude oil samples, which are often centered around m/z 400 [1,2]. See Figure S1 for a full range LDI mass spectrum within the m/z 200 – 2500 range.

Next, to investigate the nature of such homologous ions in Figure 3, their MS/MS data were acquired. Figure 4 shows, as examples, the MS/MNS for the ions of m/z 720, m/z 840, m/z 1008, m/z 1176. Their fragmentation patterns were dominated by fragment ions separated by 24 m/z units due to C_2 losses, a most typical pattern that firmly points to fullerenes [40,40, 41,42,43].
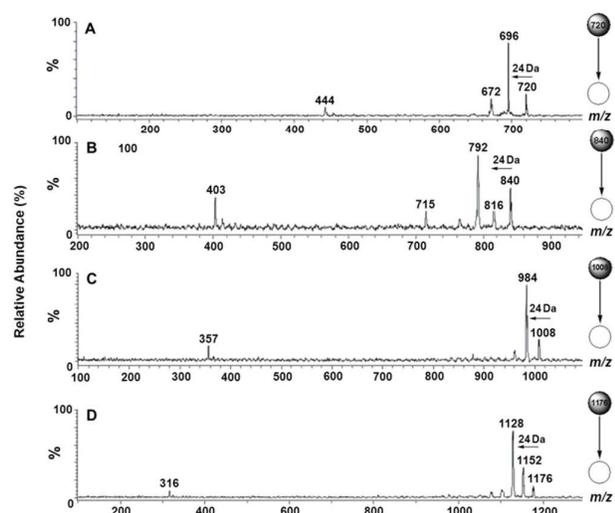
Figure 4. LDI-MS/MS of the ions of A) m/z 720 (C_{60}) and B) m/z 840 (C_{70}) C) m/z 1008 and D) m/z 1176 sampled for a typical asphalthene sample.

Next, we investigated the effect on the LDI-MS data of varying the fluency (intensity) of the laser beam (Figure 5). With the laser at 60% of fluency or less (Fig. 5A), only a noise spectrum was obtained. When the laser power was increased to 70% or even better at 80%, high ion abundances were attained and then the typical +24 m/z Gaussian distribution was formed (Figs 5B, C). Note that a similar behavior during LDI-MS was recently described elsewhere [44].

Next, to investigate the effect of the laser beam in creating artifacts for (MA)LDI-MS analysis, asphaltene samples were analyzed by APPI(-) in an FT-ICR mass spectrometer. APPI is a very mild photon ionization technique using ca. 10 eV photons and is known to work quite efficiently for fullerenes particularly in the negative ion mode [45].
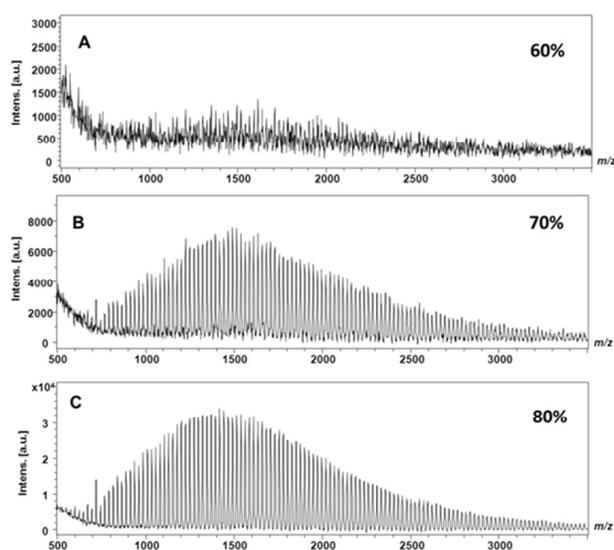
Figure 5. LDI-MS of a typical asphaltene sample acquired by varying the laser fluencies from 60% to 80%. A) 60%, B) 70% and C) 80%.

First, the APPI(-)-MS of the same asphaltene sample was acquired (Figure 6A) and a rather typical and complex ion profile of crude oil mixtures centered around m/z 400 was observed. Note also the absence of the fullerene ion series, more specifically of the C_{60} ion of m/z 720 which is an abundant ion under LDI-MS. The main classes detected and attributed in the mass spectrum in Figure 6A were the NO (75.3 %), NO_2 (21.3 %) and NO_3 (3.3 %) classes (See sup. information). Figure 6B shows then the APPI(-)-MS of a C_{60} solution from which an abundant molecular anion of C_{60} fullerene was detected. This result demonstrates the efficiency for APPI(-) of fullerenes under the chosen conditions.
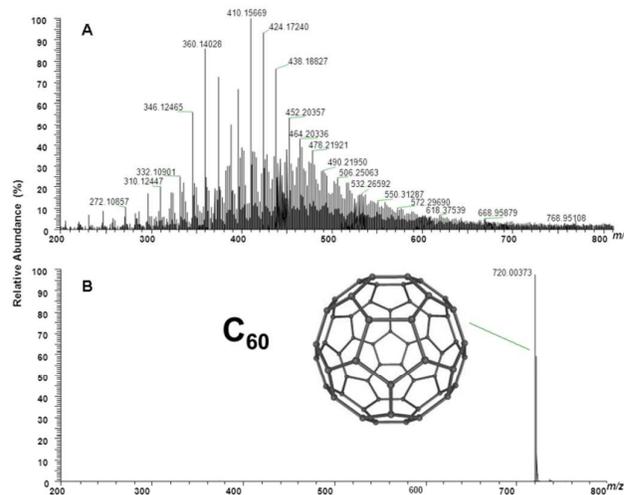
Figure 6. APPI(-)-MS of (A) an asphaltene sample and (B) of C_{60} fullerene synthetic standard.

To further test whether the natural polycyclic aromatic hydrocarbons present in asphaltenes were being indeed transformed into fullerene-artifacts upon laser irradiation, a standard mixture containing 16 PAH molecules (Figure 7) was subjected to LDI(+)-MS**.** As now expected, indeed a similar profile as that of Figure 3 of fullerene ions separated by m/z 24 units was clearly observed. Note that Boorum and co-workers [45] have reported formation of C_{60} fullerene from LDI(+)-MS of selected $C_{60}H_{30}$ and $C_{80}H_{40}$ PAH molecules but not from $C_{48}H_{24}$. This observation led the authors to conclude that the major mechanism for fullerene formation upon laser irradiation is H-removal followed by ring closure and not by ring condensation. Since the constituents of the standard mixture used are PAH with C_{16} (pyrene) or lighter molecules, and since fullerenes with MW up to 3,000 Da or more were detected (Figure 7), it seems that under our LDI conditions substantial ring fusion indeed occurred.
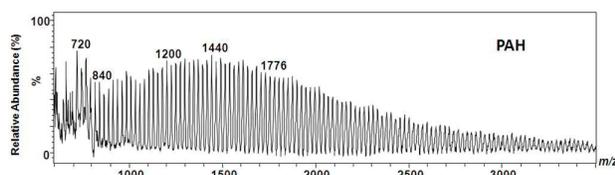
Figure 7. LDI(+)-MS spectra of a standard mixture containing 16 different PAH molecules.

It seems therefore that fullerenes are not major natural constituents of asphaltenes but photochemical reactions favored by laser radiation are transforming the natural PAH asphaltenes molecules into ring C_{2n} fullerenes which are detected as predominant artifacts during LDI(+)-MS.

Reviewing the literature about the detection of fullerenes in geological material using LDI-MS or related techniques, many studies were found. For example, as already mentioned, Buseck [34], and Jehlicka and co-workers [46] have concluded for the presence of C_{60} fullerene in carbonaceous rocks and bitumen. But such conclusion as reported by Buseck and co-workers [34] has been a subject of a large debate since later it was argued that the detected fullerenes were not endogenous but indeed formed as LDI artifacts [47]. As the present results indicate, an erroneous conclusion for the natural occurrence of fullerenes in shungite rocks has indeed been made. Becker and coworkers [35] may be also erroneously concluded using LDI-MS for the presence of fullerenes in meteorites, since a Gaussian distribution of fullerenes very similar with that seen in the spectrum of Figure 3 was reported. But we note that Zare [48] and coworkers have correctly interpreted such a series as due to fullerenes formed as LDI-MS artifacts from insoluble organic matter present in meteorites

Yacaman and co-workers [49] have also been able to form fullerenes from asphaltenes by electron radiation. Using high resolution transmission electron microscopy, the authors were able to produce fullerene onions by electron beam irradiance onto an asphaltene sample. They proposed that electron irradiance kicks off aliphatic chains (and hydrogen) leaving the aromatic rings free to accommodate into new fullerene structures. Laser irradiation could therefore produce similar results transforming asphaltenes into fullerenes.

Incorrect structural assignments in LDI-MS of asphaltene samples have therefore been unfortunately common [36, 37]. For instance, Traldi and co-workers [36] analyzed asphaltenes from two different Italian oil fields, and observed similar LDI-MS spectra the same homologous ion series separated by m/z 24 units. They initially attributed this series to electrical noise but analysis in another instrument gave the same profiles which lead them to conclude that asphaltene species differing by 24 Da were detected. They proposed that such molecules would be natural PAH constituents of asphaltenes differinfg by additional rings formed by reactions promoting "C=C attachments" (Figure 2). Rogalski and coworkers [37] also observed similar +m/z 24 profiles during LDI-MS of asphaltenes

missassigning them again as natural PAH asphaltene molecules.

Rogalski also used LDI-MS to infer MW distribution of asphaltenes, but again fullerenes were clearly detected [37]. Rogalski and co-workers, when investigating the formation of molecular aggregates of asphaltenes by LDI-MS, obtained again the same +m/z 24 profile with ions of m/z 720 and m/z 840 being the most abundant, but they cited the work of Traldi and co-workers [36] to support such ions as due to detection not of fullerenes but of natural PAH asphaltene-like molecules (Figure 2). Romão and co-workers [45] recently observed as well the formation of a +m/z 24 series for asphaltenes fractions during LDI-FT-ICR-MS analysis, but they incorrectly attributed the covalently bonded fullerene formations due to in source *"clusterization reactions"*.

Martinez-Haya and coworkers [50] also analyzed asphaltenes by LDI-MS. In fact, they used a combined IR and UV approach since such L^2DI [27,51] technique was believed to avoid gas phase asphaltene aggregation which was responsible for the erroneous high MW observed before by LDI-MS [52]. But again, fullerenes seem to have been formed but, citing Traldi and co-workers, the +m/z 24 series of ions was incorrectly attributed to natural PAH asphaltene constituents such as those exemplified in Figure 2 [36].

Apicela and co-workers [53] evaluated the influence of experimental parameters in LDI-MS of asphaltenes particularly in regard to correct MW determination concluding that laser power and surface concentration are the main parameters to control. They also performed LDI-MS of PAH, fullerene, and polyacenaphthylene. For the LDI-MS of PAH, a +m/z 24 series was also observed but two other ion series of +m/z 12 and 26 were detected. They interpreted these series as due to CH_2, C_2 and CH_2CH_2 "bridges".

Predominant fullerene formation as artifacts during asphaltene LDI or MALDI-MS analysis seems, however, not to be inevitable and has sometimes not been observed. For instance, Apicela and co-workers [53] performed asphaltene analysis by LDI-MS using a nitrogen laser and reported spectra with the classical crude oil-like Gaussian profile. Indeed asphaltene ions centered around m/z 400 seem to have been formed, with no evidence of fullerene artifacts.

What appears to a crucial clue to understand fullerene-artifact formation or not during LDI-MS of asphaltenes comes from the work of Rogalski and co-workers [37]. Their asphaltene fraction extracted with THF displayed once again the fullerene series mistakenly attribute to natural PAH asphaltene constituents but, interestingly, if extractions were done with more polar solvents such as N-methyl-2-pyrrolidone, dimethylformamide and dimethyl sulfoxide, the fullerene distribution was no longer seem and the LDI-MS displayed the normal crude oil-like pattern. Fullerene formation upon laser radiation seems therefore to depend on the actual constitution of the asphaltene sample and is most likely favored when PAH level or certain PAH classes are highly abundant. The easy ionization of fullerenes by LDI-MS can also

lead to ionization suppression of the remaining and intact but undetected "real" asphaltene molecules.

Another crucial parameters is the type of laser used for desorption. Zare and co-workers used a two-step laser based or single photon lasers and reported minor artifacts from fragmentation, aggregation, and multiple charging for asphaltene-model compounds [27,51,54] Interestingly, a recent study [55] also reported very minor fullerene formation for LDI-MS analysis of fullerenes using an Infrared (IR) laser. Via travelling wave ion mobility MS [52,55,56], both the asphaltene as well as the fullerene-ion series could be separated. This result seems to indicate therefore that IR lasers could be also an adequate choice for LDI-based fullerene analysis and that fullerenes artifacts can indeed be avoided.

Conclusions

As has been mistakably assumed and vastly reported, from several LDI-MS data on different carbonaceous materials, neither C_{2n} ring fullerenes nor the class of PAH proposed by Traldi (Figure 2) are natural constituents of asphaltenes. The molecules normally detected by LDI-MS are indeed C_{2n} fullerenes but they are in fact formed as artifacts of the laser radiation. Such artifacts are therefore common and seem to be particular favored for asphaltene samples or other carbon-rich materials most particularly for those with considerably high levels of PAH. For asphaltenes, formation of fullerene artifacts has been shown to depend on the precipitation or extraction methods used, and this trend can now be understood in terms of contrasting levels of PAH. The type of laser and its fluency (intensity), as recently demonstrated for IR, single photon or two-step lasers, also seems to greatly influence the extent of fullerene formation during LDI-MS analysis.

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