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Past to Future: Application of Gel Permeation Chromatography from Petroleomics and Metallopetroleomics to New Energies Applications: A Minireview

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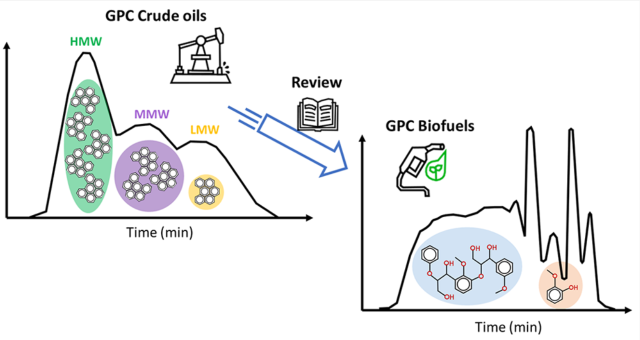
ABSTRACT: In the field of petroleomics and metallopetroleomics, the gel permeation chromatography (GPC) technique coupled with high-resolution detection technologies has made significant contributions as an analytical and preparative tool for over five decades. This bibliographic minireview highlights the study of the supramolecular and structural behavior of heavy crude oil and its fractions, as well as their reactivity to various processes by use of GPC. The preferred mobile phase is tetrahydrofuran (THF), whereas the stationary phase is polystyrene−divinylbenzene copolymer to avoid compound retention in the column. Other techniques such as HPTLC, RPLC, and NPHPLC have been used to provide multidimensional separations complementary to GPC. The high molecular weight (HMW) fraction, due to its greater polarity, reactivity to polymerization, and resistance to hydrodemetallization processes, has been the focus of interest for years. GPC coupled with high-resolution techniques has proven to be reliable for the detection of organic and inorganic species in bio-oils, making it a valuable tool for researchers and industry professionals in the context of feedstocks changes and new energy production.



1. INTRODUCTION

In the early 1970s, researchers began exploring the potential of gel permeation chromatography (GPC) as a mean to simplify the complex petroleum matrix. Pioneering studies, such as those conducted by Coleman et al.,[1] demonstrated the first petroleum GPC separation based on molecular volume in three different cuts of Texas crude oils. They employed Styragel columns prior to mass spectrometry (MS) and nuclear magnetic resonance (NMR) analyses. This research revealed absorption effects for certain polycyclic aromatics within highly condensed ring systems and provided insights into separation mechanisms, with the aromaticity of the systems playing a more significant role than the alkylation degree of the molecules. Importantly, the study uncovered previously undetected intermolecular interactions within these condensed structures, specifically the agglomeration of polar compounds associated with asphaltenes composition, which could not be directly observed through conventional analysis of the entire vacuum residue.[1] Ever since, various research teams have investigated new chromatographic conditions, such as stationary phase, mobile phase, temperature, and flow rate, to enhance separation, as well as evidencing new hydrodynamic volume features from the crude oils and more details about aggregation phenomena. Hence, many studies focused on petroleum samples, with GPC being a main asset to decipher the complexity of these matrices and provide further insights, particularly on asphaltenes samples. These samples are the most polar and complex fraction of petroleum, containing most of heteroatoms (N, O, S) but also metals such as nickel (Ni) or vanadium (V).[2−4] For many years, GPC has been used as a fractionation tool prior to other analyses and only hyphenated to classical liquid chromatography (LC) detectors, such as refractive index (RI) or ultraviolet (UV) detection, which do not specifically detect metals found within each fraction. The pioneering work of Reynolds et al.[5,6] showcased the interest of online hyphenation to a dedicated metal-detection system, i.e., inductively coupled plasma (ICP) or direct current plasma (DCP) atomic emission spectroscopy (AES) systems, for characterizing nickel and vanadium metal-

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loporphyrins. However, this approach was not widely adopted by other research teams until the 2010s along with the broader introduction of more sensitive ICP-MS systems in various laboratories. A micronebulizer was developed by the team of Bouyssiere in order to inject organic solvent such as xylene or tetrahydrofuran (THF) in the ICP-MS plasma without causing segregation of species linked to volatility.[7−9] Following this development, Caumette et al.[10] developed the first analytical GPC-ICP-MS hyphenation, which demonstrated the numerous benefits of GPC-ICP-MS, and how GPC could complement classical normal-phase LC separation by revealing other physicochemical properties of the petroporphyrins, such as their hydrodynamic volumes. Concurrently, a hyphenation was performed with μGPC columns (developed by Shodex) that can operate at the flow rate of the developed micronebulizer.[11] Subsequently, Acevedo et al.[12] evaluated the potential of μGPC-ICP-high-resolution mass spectrometry (HRMS) on different fractions from asphaltene solutions (A1, A2, and trapped compounds (TC)), whose isolation has been described by Gutiérrez et al.[13] as an alternative to regular GPC to reduce solvent consumption and analysis time. This methodology helped the authors to infer that both nickel and vanadyl porphyrins were found intercalated or trapped within asphaltene aggregates and to infer a strong ability from asphaltenes to trap external compounds (i.e., forming aggregates). It was also worth noting that no specific interactions between metalloporphyrins and asphaltenes were needed to form aggregates, thus providing further understanding of asphaltenes aggregation phenomena.

Although the use of GPC is a strong asset to simplify the matrix, the resulting fractions remain highly complex due to the thousands of molecules present within one single fraction of the crude oils, thereby challenging the resolution limits of classical ICP-MS systems. To tackle this challenge, the rising capabilities of ICP-HRMS instrumentation have been a significant advancement in the characterization of these matrices, as evidenced by Desprez et al.[14] in 2014. The use of high-resolution mass spectrometer detection has enabled the unambiguous quantification of Ni and V porphyrins and, in particular sulfur compounds, whose signal detection was complicated due to polyatomic interferences induced by the organic matrix (mainly C, O, N) in classical ICP-MS analyses. Gutierrez et al.[15] expanded upon this research by examining various factors that might influence the GPC-ICP-MS analysis of asphaltenes. They optimized the conditions to investigate asphaltene aggregation in solution. After 254 days, they observed an increase in relative intensity for higher molecular weight compounds, suggesting that metalloporphyrins participate in the aggregation process and not all of them exist as free entities (monomers) in the solution.[15] To increase the understanding of these aggregations phenomena, ultrahigh resolution mass spectrometry, particularly Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), is an outstanding tool to characterize these aggregates at the molecular-level. The unmatched resolution and mass accuracy of FT-ICR MS have already demonstrated their full potential in petroleomics studies.[16−23] However, the offline analysis of some of the fractions obtained by GPC is not straightforward as some limitations have been raised, such as dynamic range limitations or low ionization efficiencies.[24] To address these constraints, drawing inspiration from earlier studies by Sato et al.[25] with GPC APPI-MS hyphenation, subsequent research was conducted 12 years later on the online hyphenation of GPC to linear trap quadrupole orbitrap (LTQ-Orbitrap),[26,27] and FT-ICR MS.[28] The combination of chromatographic separation, which minimized selective ionization and provided unequivocal molecular assignment, has led to significant advances in the understanding of the aggregation of asphaltene molecules. Indeed, nonpolar intermolecular forces between saturated and long chains were found to be more contributing to aggregation than $\pi-\pi$ interactions. In this work, we propose to review and discuss the main works that have been performed during these last five decades from initial fractionations of crude oils using GPC, to its hyphenation respectively to ICP-HRMS and FT-ICR MS (Figure 1). The objective is to provide further insights about the future of GPC and its eventual application for new energies based on the knowledge that has been gained through petroleomics studies.

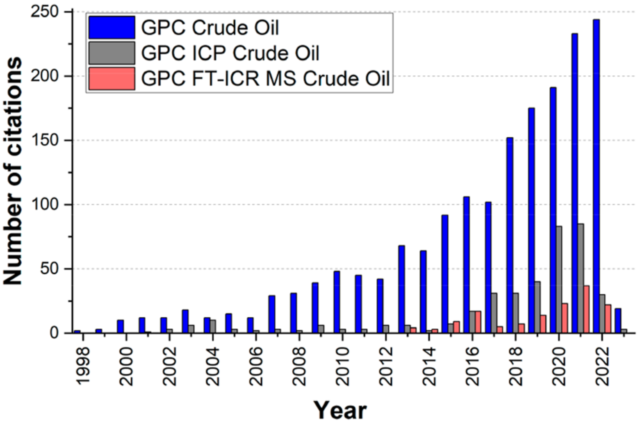

Figure 1. Article citation number per year from Web of Science with the stated keywords.

2. BASIC DEFINITIONS OF GPC

Gel permeation chromatography (GPC) is a technique designed for the analysis of polymers, rather than for samples with complex matrices as crude oil. GPC is a type of liquid chromatography (LC) technique used to separate and analyze polymers based on their (1) hydrodynamic volume, which is related to the size and shape of the polymer molecule, and (2) molecular weight, related to the number of repeat units in the polymer chain. The technique involves the use of a column packed with a porous gel material that acts as a molecular sieve. During GPC, a solution containing a mixture of polymer samples is injected into the column and is carried through the column by a solvent that flows through the pores of the gel. As the polymer molecules pass through the column, they are separated based on their size and molecular weight. Smaller molecules enter the pores of the stationary phase more easily and are therefore more retained, while larger molecules are excluded from the pores and elute more quickly.[29]

2.1. Retention Mechanism. A classic assumption in GPC is that the stationary phase does not interact chemically with the analyte, and the separation is based solely on the size and shape of the polymer molecules. However, this is not necessary the real case, and some interactions could happen leading to chemical retention. There are several factors that can affect elution in GPC, including the composition of the eluent, the characteristics of the column (such as the number of columns, dimensions, material, and pore size), the flow rate, and the characteristics of the analyte.

2.2. Molecular Weight Determination. In order to estimate the molecular weight of analytes in a sample using GPC, it is important to note that the technique does not directly provide this information. Instead, the retention time of the analytes, which is directly related to their hydrodynamic volume, must be used to obtain an estimation of their molecular weight. This can be done by comparing the retention time with a calibration curve obtained using standards of known molecular weight, being polystyrene standard often used for this purpose. It is important to recognize that the accuracy of the estimation depends on the reliability of the calibration curve, which may not be perfectly suited to the sample being analyzed.[29,30]

2.3. Complex Samples Analysis Using GPC. When analyzing samples that differ from standard polymers, the effective size observed for a sample in GPC may be affected by various phenomena, such as aggregation. If the analytes in the sample have a strong interaction, they could form aggregates. Aggregation can lead to overestimation of effective size by increasing the size of species in the sample.[15] The dilution of the sample and the choice of the solvent are critical in this phenomenon. The main limitation of the GPC is the lack of suitable standards for the calibration of the GPC instrument, as there is no widely accepted standard for polymeric components in specific samples, such as crude oil.[31] Additionally, the ideal concept of nonchemical interaction between the analytes and the stationary phase is not completely perfect, as in the case of "the peri-condensation effect" described later in the text.[32] This can make it difficult to obtain accurate molecular weight and size distribution. However, it is possible to use orthogonal techniques that allow confirming or correcting the results obtained by GPC, such as vapor pressure osmometry (VPO), Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), and simulates distillation (SD), among others. Despite the limitations of the GPC technique in accurately determining the molecular weight of complex samples, it offers a valuable tool as a comparative method for samples from different origins or after different processes. This qualitative and semiquantitative contribution is particularly important in the case of crude oil and its fractions. For over 50 years, GPC has been widely used as a practical technique for comparing processes and samples from a supramolecular perspective. With the help of GPC, the study of large compounds, such as crude oil, asphaltenes, and their aggregates, has been possible on a large scale, making it an essential tool for researchers in the field of petroleomics.

3. OPTIMIZATION OF THE GPC CONDITIONS (TRADITIONAL DETECTORS)

3.1. GPC for Light Fraction. The online characterization of petroleum samples by GPC with a detector such as UV and RI have been widely used for decades now. For example, Ferguson and O'Brien worked on experimental conditions of GPC employing polystyrene−divinylbenzene (PS-DVB) columns with pore sizes of 10,000 Å, 1000 Å, 500 Å, and 100 Å for different crude oils.[33] They compared two different mobile phases at a flow rate of 1 mL·min^{-1}: methylene chloride and a mixture of tetrahydrofuran and methanol (9/1 v/v). They showed that methylene chloride was an unsuitable mobile phase due to its high volatility, which resulted in the adsorption of polar species into the columns. Conversely, the THF/MeOH 9/1 v/v mixture led to less adsorption and was considered as a better choice. Larsen et al.[34] used three microstyragel columns, one with a pore size of 500 Å and two with a pore size of 100 Å, samples were 1,000-fold diluted in toluene, and the flow rate was fixed at 1 mL·min^{-1}. In this work, the molecular weight distribution of deasphalted oil, resin, pitch, and furfural raffinate from a deasphalted residue have been determined by GPC. Field desorption mass spectrometry was used to detect molecular weights below 3000 Da in the raffinate and below 5000 Da in the deasphalted oil and resin samples. The authors highlighted a potential pitfall in GPC analysis, wherein highly polar components could associate, leading to an overestimation of molecular weight. To mitigate this issue, Larsen et al.[34] suggested the use of higher temperatures or more dissociative solvents during the GPC analysis to promote the disassociation of polar components, thereby yielding more accurate molecular weight measurements. Borgund et al.[35] investigated acid extracts derived from crude oil using a PS-DVB column, and THF, stabilized with potassium hydroxide (KOH) pellets, was used as the mobile phase, maintaining a flow rate of 0.2 mL·min^{-1}. The GPC analysis revealed that the acid extracts predominantly contained compounds with intermediate molecular weights, ranging between 400 and 800 g·mol^{-1}. Then, Albaugh and Talarico conducted a study on six petroleum samples originating from different countries using GPC with five distinct columns and mobile phases, including tetrahydrofuran (THF), benzene, and trichlorobenzene, at flow rate of 1 mL·min^{-1}.[36] Their findings indicated that multiple columns were necessary to cover the entire molecular weight range present in petroleum samples. The GPC-RI analysis revealed variations in molecular weight distributions among the different petroleum samples. Moreover, they observed that condensed aromatics were eluted later than their predicted molecular weight when THF was used as the mobile phase. To address this issue, Greinke and O'Connor used quinoline as mobile phase at a flow rate of 1 mL·min^{-1}. Under these conditions, polycyclic aromatic hydrocarbons (PAHs) such as coronene and pyrene were found to elute more closely to their actual molecular sizes.[37] Islas-Flores et al.[38] used a Waters Styragel HR 4E column (7.8 × 300 mm) with a flow rate of 1 mL·min^{-1} of THF. In this work, they used GPC as a qualitative tool to compare the resin fractions previously obtained. They also report that polar and polyaromatics compounds eluted later than expected retention times. In another work, the same authors with the same columns and conditions analyzed SARA (saturate, aromatic, resin, and asphaltene) fractions and reported that as expected the resin fractions have compounds with bigger molecular weights and wider structural differences.[39] Guieze and Williams highlighted the advantages of GPC for analyzing heavier fractions, such as distillation residues, compared to GC-MS, which is more suitable for analyzing volatile fractions.[40] In this work, GPC was used to obtain molecular weight information and the smaller molecular weight portion of the residues were compared to GC-MS analysis; results were similar. Espada et al.[41] employed GPC using PLgel columns and a mobile phase of 1,2,4-trichlorobenzene at 145 °C to determine the molecular weight of fractions with boiling points ranging from 105 to 565 °C. Their results showed that molecular weight increased with boiling point and GPC yielded similar results to high-temperature (HT) GC while being a more straightforward technique. Similar results were found by Boczkaj et al.[42] They were able to determine boiling points of different fractions using GPC-RI. The results were compared and similar to GC analysis. According to Barman, GPC can also be used for the quantification of sulfur.[43] In this work, Barman used THF as mobile phase with 1 mL·min^{-1} as flow rate and columns with varying pore size (100 Å to 100,000 Å). He showed that with a

Table 1. Overview of Some Experimental Conditions for GPC Analysis of Crude Oils and Related Fractions

year	reference	column	stationary phase of the column	solvent(s)	flow rate (mL·min^{-1})
1969	Coleman et al.[1]	Styragel	PS-DVB polystyrene−divinylbenzene	benzene	1.7
1972	Albaugh et al.[36]	Styragel		THF, benzene, and 1,2,4-trichlorobenzene	1
1980	Greinke et al.[37]	Styragel		quinoline	1
1981	Ferguson et al.[33]	Styragel		CH_2Cl_2 and THF/MeOH 9/1	1
1986	Larsen et al.[34]	μ-Styragel		toluene	1
1996	Barman et al.[43]	Ultra-Styragel		THF	0.5 1 2
1998	Miller et al.[47]	Ultra-Styragel		THF and THF with 0.1% TCNE	1
2005 2006	Islas-Flores et al.[38,39]	Styragel		THF	1
2007	Borgund et al.[35]	Styragel		THF	0.2
2008	Behrouzi et al.[48]	Mixed-A and Mixed-D		NMP, THF, and toluene	0.5
2011	Espada et al.[41]	PLgel mixed		1,2,4-trichlorobenzene	1
2012	Zhang et al.[49]	Styragel		THF	1
2014	Zhang et al.[50]	Styragel		THF	1
2015	Boczkaj et al.[42]	Phenogel		THF	1
2018	Azinfar et al.[51]	Styragel		THF	0.1

pore size of 1,000 Å, the sulfur can be dissociated from the naphtha sample and quantified. He also showed that the flow rate did not affect the resolution of the chromatogram. Although this literature minireview does not aim to be exhaustive, it is interesting to highlight that PS-DVB, or polystyrene−divinylbenzene, has remained the preferred stationary phase for GPC in petroleomics from its inception to the present (see Table 1). Moreover, Table 1 reveals that THF is the most commonly used mobile phase. THF is of great importance in petroleomics since it prevents the metals Ni and V from being adsorbed in the GPC column.[44] Also asphaltene aggregates seem not to break down in this medium according to theoretical,[45] and experimental studies, even at low concentration of 25 ppm.[46] Considering the results of the previous works, the choice of solvent and the choice of pore size of the columns seems to be the most important parameters that influence the retention times and profile shape.

3.2. Analysis of Asphaltenes and Heavy Fractions.

Heavy fractions of petroleum and asphaltene are not volatile and cannot be separated by GC. In contrast, the GPC with adequate columns and mobile phase followed by detection seems to be a good technique to separate such samples. In their work, Miller et al.[47] analyzed colloidal and noncolloidal asphaltenes. They first prepared asphaltenes by taking vacuum residue (>537 °C) from Mayan crude oil, adding an excess of heptane (40:1), and stirring overnight. The precipitate was then dissolved in a minimum amount of toluene, followed by the addition of another excess of heptane (40:1). The heptane-soluble part is the maltene, while the asphaltene represents the precipitated part of the second extraction. In this work, they further extracted the asphaltene for seven more days with heptane. The solubilized part represents the noncolloidal asphaltene and the solid part represent the colloidal asphaltene. Consequently, the composition of asphaltenes depends on the extraction method. The different fractions were analyzed using GPC with Ultra-Styragel columns of various pore sizes (100,000 Å, 10,000 Å, 1,000 Å, and 500 Å). The flow rate was set at 1 mL·min^{-1} using either THF or THF with 0.1% of tetracyanoethylene (TCNE) as the mobile phase. The size of noncolloidal asphaltene was found to be about one-third of the colloidal part when THF was used as the mobile phase. With THF and TCNE as mobile phase, the size of noncolloidal asphaltene remained similar, while the size of colloidal asphaltene increased to about six times the initial size, compared to using only THF. These results showed that asphaltenes have a substantial capacity to self-aggregate and should be considered as an aggregation of molecules rather than individual molecules, potentially resulting in very high molecular weights.

Even for ultrahigh molecular weight, GPC could be used to determine molecular weight. For instance, Montanari et al.[52] employed GPC to analyze asphaltene samples before and after thermal hydrocracking treatment, demonstrating a decrease in molecular weight of these samples. Azinfar et al.[51] used three Styragel columns (HR-1, -2, and -3) with a flow rate of 0.1 mL·min^{-1} of THF to analyze heavy crude oils and residues. They generated a calibration curve using GPC and distillation to obtain boiling point distributions for any sample. GPC can also be used as a complementary technique, as demonstrated by Li et al.[53] They used thin-layer chromatography (TLC) silica plates to separate coal tar pitch and petroleum distillation residue based on polarity with pyridine, acetonitrile, toluene and pentane as eluent solvents. Then, they extract the eluted fraction using 1-methyl-2-pyrrolidinone (NMP). All fractions were subsequently analyzed using GPC with PS-DVB columns and NMP as the mobile phase. The results indicated that the more retained material on the silica plate had the bigger size, and molecular weight increased with decreasing mobility on the plate. However, further work is still needed to examine the very high molecular weight range. For example, Herod et al.[54] used different sets of GPC columns capable of separating molecular weights up to 20,000 Da, 200,000 Da, and 15,000,000 Da, polystyrene equivalent. They set the flow rate at either 0.45 or 0.5 mL·min^{-1}, depending on the column set used. The authors analyzed coal liquids, petroleum vacuum residues, soots, biomass tars, and humic substances. The molecular weight ranges of petroleum vacuum residues determined by GPC were found to be similar to those obtained using vapor pressure osmometry (VPO), where aggregation was avoided. However, all samples contained some excluded material of high molecular weight (>3000 Da), which was considered at the time to come

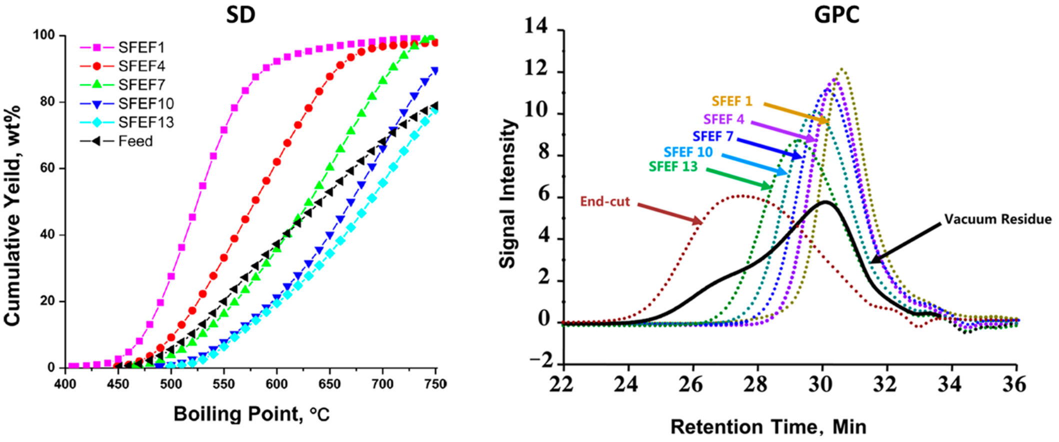

Figure 2. Simulated distillation (SD) and gel permeation chromatograms (GPC) of supercritical fluid fractions of vacuum residue sample.[49]

from the arrangement of smaller compounds. In the work of Woods et al.,[55] after an initial SARA separation on Canadian crude oils, each fraction was analyzed by GPC using THF as the eluent to determine their average molecular weights. As expected, the aromatic fraction had the lowest molecular weight (∼400 Da), followed by the saturate fraction (500 Da) and the resin fraction (∼700 Da). They concluded that for each crude oil, the asphaltene fraction had an average molecular weight at least two or three times higher, ranging between 2,000 and 3000 Da. Behrouzi et al.[48] analyzed asphaltene from a Mayan crude oil using two PS-DVB columns, Mixed-A (packing size of 20 μm) and Mixed-D (packing size of 5 μm), with a flow rate set at 0.5 mL·min^{-1} of NMP, THF, and toluene separately. The results demonstrated some limitations in analyzing asphaltenes due to varying outcomes depending on the solvent used, as well as the chemical structural differences between the analytes and the polystyrene used to calibrate the columns, and the discrepancies with other techniques such as FT-ICR-MS. Zhang et al.[49,50] studied Venezuela Orinoco vacuum residue in two separated works. They first used supercritical fluid extraction and fractionation (SFEF) to prepare multiple fractions, which they then analyzed using GPC with a Waters Styragel HT-5 column and THF as the mobile phase at a flow rate of 1 mL·min^{-1}, with a RI detector. They also analyzed the different fractions by mass spectrometry and compared the results. The first SFEF fraction contained low molecular weight compounds (∼400 g·mol^{-1}) and was mostly composed of saturated molecules. The following fractions contained more aromatics compounds, followed by resins with an increasing molecular weight of ∼600−700 g·mol^{-1}. For these previous fractions, the molecular weights determined by GPC were similar to those given by mass spectrometry. The end cut, nonextractable by SFEF, was mostly composed of asphaltene with a molecular weight of ∼2,000 g·mol^{-1} according to the GPC and only 1,000 g·mol^{-1} according to the mass spectrometry. The increasing molecular weight trend of the fractions observed by GPC was also observed in the increasing boiling point of the fractions followed by simulated distillation (SD) (see Figure 2). To better understand the high and very high molecular weights of such samples, GPC can be used as a preparative technique to analyze the specific fractions or can be coupled with elemental or molecular mass spectrometry detection such as ICP-MS or FT-ICR MS, respectively.

3.3. Preparative GPC.

The fractionation of complex samples is a critical step in their analysis, and the ability of GPC to separate samples based on their size makes it an ideal tool for this purpose. In 1969, Coleman et al.[1] reported for the first time the use of preparative GPC for crude oil fractionation. Despite the coherence of their results, which showed higher carbon number and boiling point in the larger size fractions (early eluted), it was also clear that the technique presented some limitations of adsorption in the stationary phase of the most peri-condensed structures. In this regard, the team of McKay et al.[32] utilized a preparative GPC system with a cross-linked polystyrene column and CH_2Cl_2 as the mobile phase to observe seven polyaromatic ring systems in the acid concentrate of a petroleum distillate. Their results demonstrated the "peri-condensation effect", where peri-condensed structures with higher molecular weight elute later than low molecular weight structures (contrary to the GPC principle), and cata-condensed structures elute before peri-condensed structures with similar mass. This phenomenon occurs because the retention time of the column depends highly on the way the analytes are solvated by the solvent.

Furthermore, if the mobile phase-analyte interaction impacts the hydrodynamic volume, the solvent to use will affect the outcome of the analysis. This behavior was shown by Cogswell et al.,[56] who analyzed acid concentrates from crude oil using a similar stationary phase but with CH_2Cl_2 and THF as the mobile phase. The data obtained from vapor-phase osmometry (VPO) revealed that CH_2Cl_2 led to a higher association compared to THF. These particular features of GPC as a preparative and analytical technique imply some significant limitations, as observed by Rudzinski et al.[57] in the analysis of the molecular weight of PAHs from Mayan crude oil using GPC and APCI/MS. The masses obtained from APCI/MS were lower by 160−240 Da compared to GPC.

Despite these limitations, some attempts have been carried out to make GPC a quantitative tool for molecular weight determination. For instance, Rodgers et al.[58] developed a model that linked GPC data to the actual molecular weight, considering factors such as the H/C ratio from elemental analysis, degree of aromaticity from ^{1}H NMR, number of methyl groups from IR spectroscopy, and number-average molecular weight (M_n) from GPC-IR. The team validated the model for standard compounds ranging from 84 to 535 Da using different techniques. Subsequently, they carried out multiple analytical GPC runs to get enough fractions from a real crude oil residue sample to analyze them and test the model. They found an average molecular weight (M_w) of 639 Da using their model, close to the

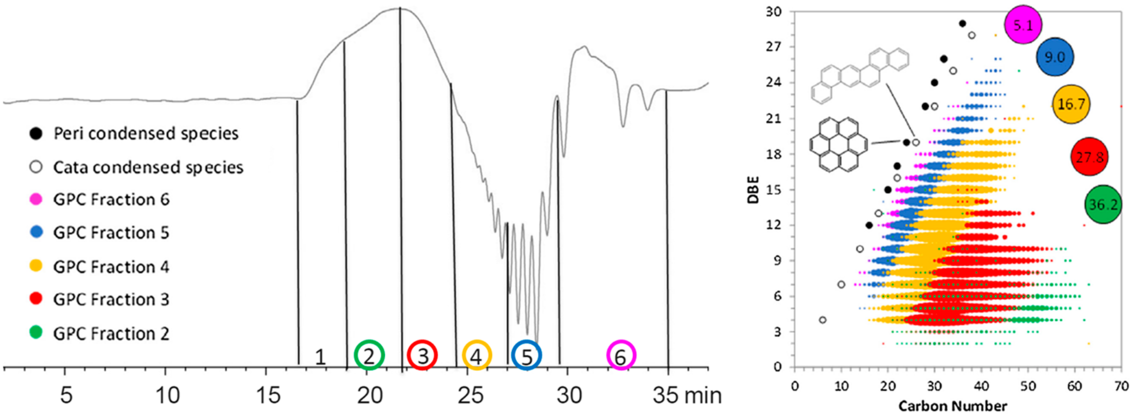

Figure 3. Preparative GPC chromatogram and FT-ICR MS analysis of each fraction. Adapted with permission from ref 62. Copyright 2023 Elsevier.

value of 630 Da obtained using field-ionization MS. However, the model may not be suitable for asphaltenes with a higher molecular weight distribution and a tendency to form aggregates. As an alternative approach, Tanaka et al.[59] used LDI-MS to determine the average molecular weight of asphaltenes in preparative GPC fractions. They proposed a two-step process: first, finding the number-averaged molecular weight of six asphaltene subfractions using LDI-MS, and second, calculating a weighted average based on the yields of these subfractions to minimize the impact of differences in ionization capacities among the asphaltene constituents.

Preparative GPC has been used to explore the chemical structure of crude oil fractions. Ignasiak et al.[60] used preparative GPC with PS-DVB column and chloroform as mobile phase. They obtained five fractions, which after analyzing compositional, spectral, and thermal properties, suggested similar chemical structures across the entire molecular weight range for asphaltenes (1,200−17,000 Da, obtained by VPO). The difference between the fractions appeared to be mainly the number of linked units, which should affect the physical properties of asphaltene association−dissociation. However, in terms of reactivity at 300 °C, the highest molecular-weight fraction has the most pronounced polymer-forming propensity, likely due to the absence of smaller molecules that act as chain terminators in this fraction. Similar trends have been observed in more recent results from preparative GPC fractions. Moulian et al.[61] studied the behavior of vanadium porphyrins present in asphaltenes analyzing preparative GPC fractions with HPTLC and the detectors UV and LA-ICP-MS. They found that vanadyl-free porphyrin standards eluted almost completely (99%) compared to the partially eluted vanadium across the entire molecular weight range of the GPC fractions. Since this interaction remains strong throughout the whole range of molecular weight, the chemical structure difference of these fractions might be linked only to the number of units that conforms them. In the same year, Panda et al.[62] published results on six different preparative GPC fractions from the Arabian heavy oil, analyzed using FT-ICR MS. The results showed that early eluted fractions had a higher number of carbon atoms (higher #C) compared to the later fractions, consistent with the GPC principle. Furthermore, in structures with similar #C, the more retained fractions had higher DBE values, which agrees with the "peri-condensation effect" discussed by McKay et al.;[32] see Figure 3.

Furthermore, significant insights into the stability and aggregation behavior have been obtained from the analysis of preparative GPC fractions. Dettman et al.[63] investigated the GPC fractions of Athabasca asphaltenes before and after thermal treatment. The results indicated that potential aggregates in the matrix might not originate from low molecular weight fractions but from high molecular weight species due to their boiling point (>750 °C, measured by HTSD). This implies that, in the supramolecular system, species with higher molecular weight will tend to self-associate preferentially compared to those in the low molecular weight range. Finally, Ali et al.[64] reported that, under the preparative GPC experimental conditions of two different asphaltene samples after hydrotreatment, the highest molecular weight asphaltene fractions of both samples exhibited highly similar structural features, whereas the lowest molecular weight fraction did not. This observation suggests that hydrotreatment can result in the conversion of low molecular weight asphaltenes to distillates, while the high molecular weight fractions (>6000 Da) remain unaltered and can cause severe coking during downstream processes, owing to the presence of highly refractory asphaltenes.

4. GPC HYPHENATION WITH ICP-MS

When targeting petroporphyrins, particularly metalloporphyrins, the use of ICP techniques such as ICP-AES or ICP-MS can be a powerful tool for elucidating metallic and sulfur species. For many years, ICP has proven to be highly effective in characterizing inorganic species found in petroleum samples, especially when coupled with additional separation system. However, this configuration presents numerous challenges beyond conventional organic conditions, including the selection of a suitable mobile phase and the use of splitters to ensure plasma stability and resistance to oxygen found in many solvents. The following section provides a comprehensive review of the literature on GPC-ICP coupling for the analysis of petroporphyrins and metalloporphyrins, discussing the advantages and limitations of this technique for the analysis of crude oil samples.

4.1. Methodologic Developments. Reynolds et al.[5,6] pioneered such hyphenation early in 1988, using two Ultragel columns in series made of cross-linked polyacrylamide (50 and 1,000 nm pore size) and xylene as the mobile phase. Subsequently numerous studies have been conducted in the field of metallopetroleomics (see Figure 4). The benefits of using different columns with different pore sizes were highlighted and expanded to 3 columns made of PS-DVB in series by Caumette

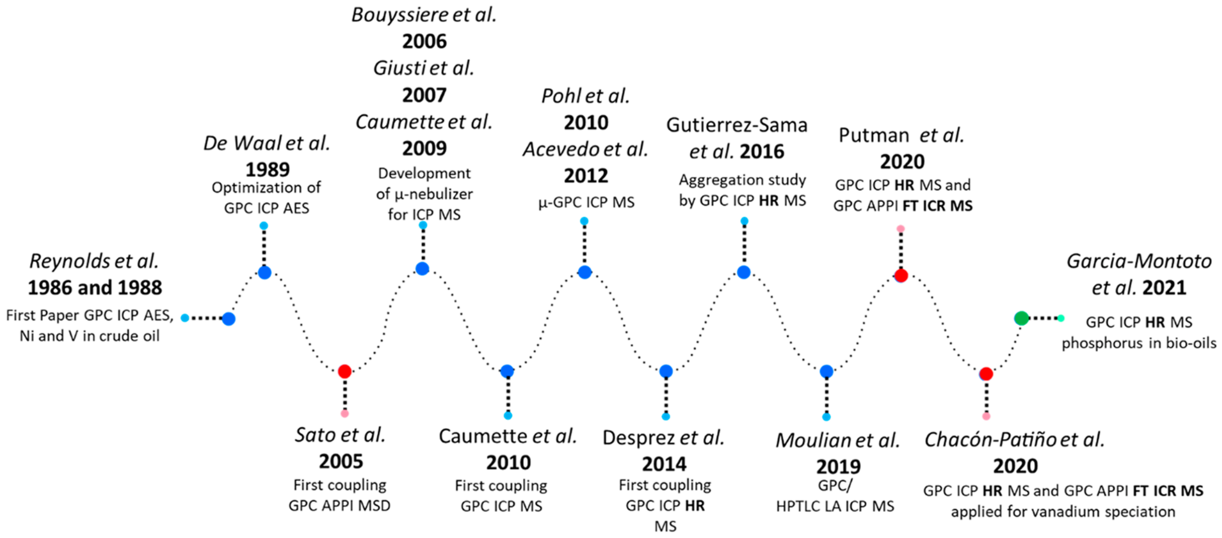

Figure 4. GPC ICP-MS and FT-ICR MS time line development.

et al.[10] who also switched the mobile phase to THF. For the analysis of an asphaltene sample, the dilution level of the samples significantly impacted the distributions of vanadium compounds. Gascon et al.[46] confirmed that high-molecular-weight (HMW) vanadium containing compounds remained in the asphaltene samples, even at high dilution factors. It was then suggested that vanadium complexes within this HMW fraction were bound by physical or weak physicochemical bonds to heavy aggregates. Additional separation information was obtained by implementing off-line or online normal-phase liquid chromatography as a bidimensional approach to characterize both the hydrodynamic volume and the polarity of the metalloporphyrins. Alongside advances in chromatographic separation and resolution, the development of higher-resolution mass spectrometry systems coupled to ICP such as sector field mass spectrometry or triple-quadrupole mass spectrometry, has facilitated more advanced characterizations. These systems provide sufficient resolution to resolve isobaric interferences, which previously hindered the analysis of sulfur compounds using classical ICP-MS. Sulfur compounds are known to cause significant challenges in refining processes and are subject to strict environmental regulations. In 2014, Desprez et al.[14] used for the first time the hyphenation GPC ICP-HRMS, to determine molecular weight distribution of sulfur, nickel and vanadium containing compounds. This analysis provided crucial insights into the distribution of sulfur within the fractions based on the crude oil's origin and various distillation cuts (e.g., gasoline, diesel, vacuum gas oil). The researchers found that the most refractory species to hydrodesulfurization were more likely to correspond to high-molecular weight aggregates, as higher steric hindrance could hinder the efficient removal of these sulfur-containing species during hydrotreatment. A correlation was observed between the GPC retention time and the boiling temperatures of the oils and cuts, demonstrating the principles of the Boduszynski model,[65] which states that there is a continuum in the molecules as a function of molecular size, number of heteroatoms, and boiling point. They also evidenced a correlation between the viscosities of the crude oils and the trapped sulfur compound percentages. On the other hand, some research teams have also evaluated the ability of ICP-MS/MS as an alternative to ICP-HRMS for sulfur speciation.[66,67] Chainet et al.[66] demonstrated the capability of ICP-MS/MS to overcome the polyatomic interferences that typically obstruct the analysis of sulfur, enabling both the quantification and the speciation of sulfur, nickel, and vanadium compounds in vacuum residues and their hydrotreated fraction. In fact, it was shown that the GPC profiles obtained on both ICP-HRMS and ICP-MS/MS were found to be very similar for each metal of interest. Moreover, the detection limits obtained for total quantification were comparable or better to those obtained for ICP-HRMS but only for lighter species ($Z < 40$). Although these interferences were addressed using the reaction/collision cells and selecting appropriate isotopes, the complexity of the matrix and the potential contaminants that could cause strong polyatomic or isobaric interferences remains a significant challenge, which high-resolution mass spectrometry is more likely to overcome.

Recently, Garcia-Montoto et al.[68] conducted a methodological development to assess the use of a 3D-printed total consumption microflow nebulizer to replace traditional micronebulizer. In addition to being silicon-free, which enables Si analysis and prevents Si contamination and background issues identified by Pohl et al. in 2010,[69] this nebulizer also showed very good performances in terms of preventing clogging or blockages while operating at desired flow rate (65 $\mu\text{L}\cdot\text{min}^{-1}$). After careful optimization, this nebulizer has been used in GPC-ICP-HRMS setup and proved to be as efficient as traditional nebulizer, as the observed elution profiles were very similar. Furthermore, there were some evidence of analyte retention from some species on the silica capillary used with classical nebulizer that could be avoided by use of this newly developed nebulizer.

4.2. Aggregation Studies.

The physicochemical properties guiding asphaltenes behavior in solution and especially their aggregation tendencies have been a very appropriate case-study for several GPC-ICP-HRMS studies. As an example, Gutierrez et al.[3,15,70] focused on the potential of GPC-ICP-HRMS to go further into the understanding of aggregation phenomenon. The researchers assessed numerous factors that could influence the size distribution of these molecules, potentially resulting in considerable discrepancies in the determination of hydrodynamic volume. Such variations hinder the investigation of the native aggregation state of asphaltenes containing petroporphyrins. Among them, the set of columns and the flow rate proved to be two main parameters influencing the size distribution behavior of these samples. Once optimal parameters have been carefully selected, the evolution of asphaltenes solutions over time has been evaluated for V and Ni aggregates. A strong increase of the high-molecular weight (HMW) fraction has been observed on a THF diluted sample, hence evidencing the formation of larger Ni and V aggregates over time with an even more pronounced aggregation behavior for Ni porphyrins. As a matter of fact, it was also showed that the use of pure THF was more suitable for the analysis of these types of samples, as all components in the crude oils might not be fully solubilized in xylene which could lead to adsorption on stationary phase hence retained in the columns, as reported by De Waal et al.[44] Combining these results with those obtained by Gascon et al.[46] showing that, even at high dilution factor, this HMW part of the GPC chromatogram is corresponding mainly to some aggregated molecules.

Gonzalez et al.[71] also evidenced the formation of higher entities such as asphaltenes clusters which would originate from the association of asphaltene nanoaggregates. To this end, the asphaltene samples were first fractionated to obtain A1, A2, and TC fractions as described elsewhere[13] and the resulting fractions have been analyzed by GPC-ICP-HRMS and compared to molecular dynamics simulation. The effect of thermal stressing was also investigated by heating the solutions to 200 °C. Their studies showed that these clusters were mainly found at shorter retention times, supporting the hypothesis that larger HMW aggregates contribute to the formation of such clusters.

A few years later, Castillo et al.[72] developed a protocol to adsorb asphaltenes onto SiO_2 nanoparticles (NPs) and used GPC-ICP-HRMS to examine the differences in terms of hydrodynamic volumes distribution for sulfur and vanadium before and after adsorption, while also varying the concentration of nanoparticles in asphaltene solution. Their work evidenced the adsorption of large asphaltene nanoaggregates onto nanoparticles through van der Waals forces, resulting in a significant increase of relative abundance of the LMW fraction and respective decrease of the HMW fraction. this was attributed to the disaggregation of the adsorbed aggregates onto NPs in solution. Thus, it was remarkable to note that SiO_2 nanoparticles were able to adsorb these problematic species in crude oils and could be used as a pretreatment for crude oils to remove them hence preserving their stability. Subsequently, Castillo et al.[73] also assessed the effect of the solvent on the adsorption and aggregate size observed for asphaltenes and their subfractions A1 and A2 on SiO_2 NPs. They found out that the dilution factor of the asphaltenes fraction was not influencing the size distribution profile, but the solvent media was playing a crucial role. Indeed, it was shown for the first time that there is no critical aggregation concentration and that solvation and solubilization were not the only phenomena that would stabilize the asphaltene in solution. Furthermore, they also demonstrated that larger aggregates were found when using poorly solvating solvent since there are more asphaltene-asphaltene interaction forces taking place whereas the use of a polar solvent would be more likely to stabilize the aggregates (avoiding asphaltene−asphaltene cluster formation), promoting an interparticle interaction (asphaltene nanoaggregate−silica nanoparticle) rather than a typical adsorption process.

The fate of aggregates during hydrodemetallization and hydrodesulfurization has also been studied by Barbier et al.[74] and Garcia-Montoto et al.[75] for crude oil feedstocks, which includes blends of atmospheric residue and vacuum residue that have been hydrotreated under different processing conditions. The main conclusions from these experiments highlight the refractory nature of HMW V and Ni-species that were not removed during hydrotreatment, in contrast to sulfur species. This observation suggests that the catalyst is less selective toward Ni and V aggregates. The researchers propose that this behavior might be linked to the location of Ni and V compounds deeper within the nanoaggregates. Considering that the outer shell surrounding these species would make it more difficult for Ni and V to reach the catalyst.

In parallel, Moulian et al.[76] assessed silver triflate (AgOTf) as a complexation agent to limit vanadyl aggregation in atmospheric residues through the complexation of a free pair of electrons from oxygen atoms in asphaltene molecules with silver ions. Therefore, it was clearly shown that a significant decrease of HMW region for vanadyl porphyrins was observed with increasing concentrations of AgOTf, correlating to a reduction in aggregate size. No difference was observed for sulfur distribution, meaning that sulfur compounds were not affected by AgOTf addition as they were not involved in the reaction. Some differences were spotted in terms of hydrodynamic volume loss, suggesting that more than one vanadium molecule could be chelated to aggregates, and variations depending on the origin of the atmospheric residue, which revealed that the aggregates were likely connected to different families and sizes of V molecules. Zheng et al.[77] extended this study to asphaltenes solutions and their corresponding fractions from extrography or preparative-GPC. Different behaviors were observed for sulfur species, depending on the fraction analyzed: the fractions obtained in heptane/toluene (1:1) and toluene/THF/MeOH (10:10:1) experienced much higher levels of aggregation compared to acetone fraction. After addition of AgOTf, the HMW fractions from preparative-GPC and the toluene/THF/MeOH (10:10:1) fraction from extrography were the most impacted leading to disruption of the interactions between the Ni and V porphyrins and S-compounds within asphaltene nanoaggregates. In contrast, the MMW and LMW fractions and the acetone fraction from extrography were less impacted by AgOTf. Hence, the benefits from fractionation to understand the mechanisms behind silver complexation were demonstrated, as these differences were not visible through the analysis of the whole asphaltene solution. Molecular dynamics has also been used to evaluate the aggregation mechanism of asphaltene and their A1/A2 subfractions, providing information about aggregate size distribution.[45,78,79] Key findings from these theoretical studies include the observation of no disaggregation of the asphaltenes in THF and the fact that THF, as a highly polar solvent, is more likely to limit the absorption of asphaltenes onto the stationary phase, which again proved the interest of using such solvent to study asphaltenes stability in a state as native as possible.

4.3. Mapping of Crude Samples.

Pohl et al.[11] employed μGPC coupled with ICP-HRMS to investigate the inorganic chemistry of crude oils by analyzing the distribution of various elements such as Co, Cr, Fe, Ni, S, Si, V, and Zn in both crude oil and vacuum distillation residue. The results of the study revealed that the distribution of the elements is heavily dependent on the origin of the sample. Similarly, Caumette et al.[10] reported in the same year the applicability of a bidimensional chromatography GPC × NPHPLC-ICP-MS technique for assessing the metal distribution in different types of crude oil based on the polarity and molecular weight parameters. The implementation of a fractionation method prior to GPC analysis proves beneficial for sample speciation. Gascon et al. developed liquid−liquid and liquid−solid extraction-based methods for fractionating asphaltenes and maltenes, enabling the separation of various fractions with varying molecular weight ranges containing nickel (Ni), vanadium(V), and sulfur compounds.[80,81]

4.4. Other Chromatographic Separations. The potential of other stationary phases based on silica with smaller dimensions (Advanced Polymer Chromatography, Waters) has also been evaluated by Gutierrez et al.[70] with the aim to reduce the analysis time and solvent consumption. Although the set of samples studied showed some differences, the observed profile of trapped vanadyl porphyrins differed significantly from the analytical GPC results. This difference was attributed to the shear rate in the column caused by the high pressure, which was due to the use of solid silica as stationary phase instead of gel. The high shear rate may have caused disaggregation, which in turn may prevent the potential use of these columns as an alternative to regular GPC columns.

The benefits from additional separation have also been evaluated by Bernardin et al.[82] who performed off-line SEC × RPLC-ICP-MS/MS on SARA fractions. In this study, an extended optimization process was conducted to maximize the efficiency of the separation and establish suitable conditions for ICP-MS/MS hyphenation. In particular, SEC × RPLC was preferred over RPLC × SEC because aggregates separated by SEC could be broken during RPLC separation. Another significant challenge emerged when using a gradient of solvents in RPLC prior to ICP-MS detection. Theoretically, this prevents the quantification of compounds since the composition of the plasma would be strongly affected by the nonhomogeneous mobile phase throughout the analysis, leading to discrepancies in ionization efficiencies due to differences in viscosities and boiling temperatures. The obtained results were presented in 2D-contour plots, enabling the visualization of mappings that represented both the hydrodynamic volume of the compounds on the first dimension and their polarity on the second dimension. This also enabled direct comparison between the different SARA fractions. The use of RPLC further facilitated the characterization of the highly complex HMW fraction, an area where GPC encounters limitations in resolution. To this end, Vargas et al.[83] developed a new chromatographic method for the separation and quantification of Ni, V, and S compounds based on silica gel columns prior to GPC-ICP-HRMS analysis. The protocol relied on the use of different combinations and proportions of solvents (hexane, dichloromethane, acetone, methanol, water) to obtain six different fractions depending on the polarity of the compounds. The advantages from this preliminary separation were clearly demonstrated, as the comparison of the GPC-ICP-HRMS profiles for the different metals between two oils of distinct origins revealed no significant differences. However, some differences were observed in the profiles of their respective obtained fractions. Concurrently, the development of new fractionation techniques, such as extrography fractionation that has been pushed forward by Chacón-Patiño et al.,[84−86] facilitated a more comprehensive characterization of asphaltenes through preliminary fractionation prior to GPC-ICP-HRMS analysis. Acevedo et al.[87] characterized these extrography fractions using various analytical techniques, including FT-ICR MS, quartz crystal resonator (QCR), atomic force microscopy (AFM), and GPC-ICP-HRMS. Different elution profiles were observed depending on the solvent system used for the different fractions. The heptane/toluene and toluene/THF/methanol fractions were found to contain larger nanoaggregates eluting in the HMW region, while the acetone fraction mainly contained smaller aggregates eluting in the LMW region. Another remarkable observation from their study was the significant difference in profiles between the whole samples and their corresponding fractions, indicating that extrography separation was stringent enough to partially disrupt nanoaggregation behavior. The combination of all obtained information helped the authors assume that aggregation was more likely driven by supramolecular network, as suggested by Gray et al.,[88] rather than by $\pi-\pi$ interactions. The evidence demonstrated that "island"-enriched samples should form fewer, well-organized deposit aggregates, while "archipelago"-enriched samples should form larger aggregates with a less-organized structure. More recently, Lacroix-Andrivet et al.[89] used this methodology to evaluate the long-term pressure aging of sulfur species contained in bitumen samples. To this end, preparative GPC was first used to collect three fractions (LMW, MMW, HMW) which were eventually reinjected and characterized by GPC-ICP-HRMS, resulting in three distinct distributions. The comparison of these distributions before and after aging did not evidence any significant difference, which reveals the limits of this technique to go further into the understanding of such aging mechanisms at the molecular level. In fact, the analysis of these preparative fractions by FT-ICR MS also highlighted three distinct distributions, with the molecular composition of the LMW fraction found to differ before and after aging, exhibiting an increase of the O_zS_y species.

5. GPC HYPHENATION WITH MS

The hyphenation of GPC to ICP-MS marked an important step in understanding asphaltene aggregation. However, the recent hyphenation of GPC with FT-ICR MS has provided further complementary information. Back in 2005, Sato et al.[25] performed a first online hyphenation between GPC, UV, and low-resolution MS, by assessing APPI (Atmospheric Pressure Photoionization) as an ionization source prior to detection by MSD (Mass Selective Detector) to evaluate the molecular weight distribution of an asphaltene sample. Despite optimizing the "fragmentor" parameter to prevent fragmentation of heavier species, which would lead to lighter shifts in molecular weight distributions, the comparison of GPC-UV and GPC-APPI-MSD distributions for a given retention time clearly showed that APPI-MSD was not appropriate to detect HMW species. The starting point for GPC-UV corresponded to an equivalent of 20,000 Da, while the starting point for GPC-APPI-MSD was about 2000 Da. This raised several concerns regarding the molecular weight distribution, particularly due to the lack of appropriate standards to correlate the retention time of asphaltene-like samples for accurate molecular weight reference, as the calibration is based on polystyrene standards. After the addition of polyaromatics and porphyrins into the calibration curve, the molecular weight of an asphaltene obtained from MS measurements was eventually estimated at 450 Da. Schrader's team successfully hyphenated GPC with high resolution mass spectrometry, specifically linear trap quadrupole orbitrap (LTQ-Orbitrap), to study asphaltenes as an effort to overcome limitations previously observed with low-resolution mass spectrometry.[26,27] A few years later, Putman et al.[28] addressed the limitations of off-line analysis using FT-ICR MS, including dynamic range limitations and significant biases in ionization efficiencies.[24] To overcome these challenges, they pioneered the hyphenation of GPC with ultrahigh resolution mass spectrometry, specifically using the 21 T FT-ICR MS. In their approach, the TIC (total ion chromatography) obtained via 21 T APPI-

FT-ICR MS was compared with the S and V GPC-ICP-MS chromatograms to evaluate differences in molecular weight distributions shifts. As an initial step, a porphyrin standard was injected using both GPC-ICP-MS and GPC-FT-ICR MS to evaluate the bias between both elution times and ensure accurate comparisons. Compromises were made for transient acquisition length, as longer transients provide higher resolution but result in reduced sensitivity and lower acquisition rates, which are two crucial features when performing LC-MS experiments to obtain sufficient signal-to-noise ratio to define chromatographic peaks. A mass resolving power of 2,500,000 at m/z 400 was used to record all spectra to provide sufficient resolution to maintain a 1.1 mDa mass split at m/z 1,000 that is necessary to resolve $^{13}C_1{}^1H_3{}^{32}S_1$ from $^{12}C_4$. Even though APPI was used as an attempt to limit differences in ionization efficiencies, some differences were still observed: in particular, the difference in monomer−ion yield (relative ionization efficiency) was found to increase with elution time and decreased aggregate size. The extracted ion chromatograms (XIC) for key species such as $N_4O_1{}^{51}V_1$ revealed that aromaticity (related to double bond equivalents, DBE) and H/C ratio were inversely related as the earliest eluting porphyrins were less aromatic. The retention times ranges corresponding to larger aggregation phenomena were mostly composed of aliphatic species whereas most condensed polycyclic aromatics were identified at longer retention times. Aside from key information about hydrocarbons, online hyphenation also provided valuable information about heteroatomic classes that were not accessible by direct infusion, i.e., $N_xO_yS_z$ classes. For O_1S_1, O_1S_2, and O_2S_1 classes, the most abundant species in the high DBE region corresponded to condensed aromatic compounds with very little alkylation. It was inferred that a change in sulfur moiety, from thiophenic/aromatic to sulfudic/aliphatic, was observed with larger elution time (i.e., less aggregation). The benefits from chromatographic resolution were again clearly evidenced for the comparison of DBE vs carbon number plots depending on the integrated retention range. Some significant differences were observed for a given heteroatomic class depending on the elution time. Globally, the most aliphatic species were found to elute earlier in the largest aggregates whereas aromaticity was found to increase at longer elution times leading to bimodal distributions. However, local trends for almost all studied heteroatomic species highlighted that the very first-eluting species in the largest aggregates are more aromatic, near to TIC limit and are followed by more alkylated compounds.

A dedicated study was conducted for vanadyl porphyrins found in asphaltenes and their extrography fractions by Chacón-Patiño et al.[90] As shown per their study, the acetone fraction, that contains the smaller aggregate sizes, experienced the highest ionization efficiency associated with the production of monomeric ions whereas the more stable and larger aggregates in Tol/THF/MeOH fractions were poorly ionized, thus not preferentially observed by APPI-MS (see Figure 5). The study also suggested that London forces (through polarizable functionalities and alkyl-side chain moieties) were more likely to explain the self-assembly of asphaltene nanoaggregates to produce massive clusters, rather than π-stacking interactions, as already described by Gonzales et al.[71]

6. CHALLENGES AND PERSPECTIVES

Recently, it has been demonstrated that APPI-FT-ICR MS could only detect 37% of the total vanadium-containing compounds in asphaltenes.[90] Despite advancements in FT-ICR MS instruments, such as the 21 T FT-ICR MS, limitations related to ionization sources and efficiencies in molecular mass spectrometry still pose significant drawbacks for accurately and representatively characterizing samples. On the other hand, the optimization of GPC-UV/ICP-HRMS methods has reached a plateau. The most appropriate conditions have been identified to characterize asphaltenes in their native state, minimizing the retention of species on the stationary phase. However, these methods only provide general trends regarding hydrodynamic volume rather than the molecular weight of the species. Consequently, it is crucial not to rely solely on a single technique or detector but to combine results from multiple techniques, simultaneously providing elemental, spectroscopic, and molecular information. The key to characterizing these increasingly complex matrices lies in the combination of separation systems and multidetectors. In the context of the energy transition, the search for new energy sources is shifting toward biomass-based feedstocks, such as bio-oils. These bio-oils originate from many sources, including for example animal fat, vegetable oils, and used cooked oils or from the pyrolysis of wood/harvest biomass leading to pyrolysis bio-oils whose composition is a mix of lignin, hemicellulose, and cellulose fragmented compounds.[91−93] Owing to their nature, bio-oils contain a higher proportion of oxygenated species, heteroatomic species and metalloids/metallic species than petroleum ever did, hence complicating their analysis and separation. This has led to a significant reevaluation of the methods that have been employed for the past 50 years.[93,94] In light of the need to analyze new sample types, it is crucial to consider various factors, such as selecting suitable stationary phases (e.g., silica-based). This consideration is particularly important because oxygenated compounds and some heteroatomic species might experience significant retention on the columns, which could impede their elution and quantification.[95,96] Andrianova et al.[97] performed GPC-UV analysis using different columns based on different chemistries, including hydroxylated polymethacrylate-based gel (HPMA), glucose rings bonded to divinylbenzene-based (GDVB), and polystyrene−divinylbenzene (PS-DVB), with a THF-based mobile phase to characterize lignin-compounds. Both GDVB and HPMA columns were found to be unsuitable

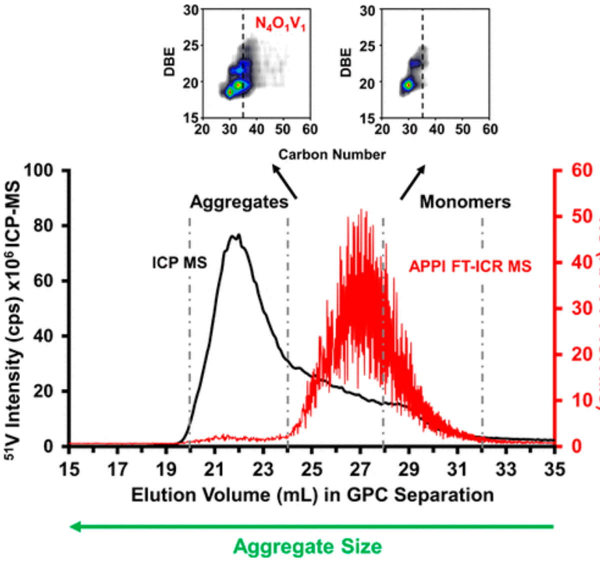

Figure 5. Contrast GPC ICP-MS vs GPC FT-ICR MS following vanadium containing species.[90]

for these matrices due to non-GPC interactions, potentially caused by hydrogen bonding between the stationary phase and the lignin compounds. Conversely, PS-DVB appeared to be more appropriate, but a dedicated GPC calibration employing at least two structurally different sets of standards is necessary. The molecular weight distribution obtained by GPC was compared to that obtained by LDI-MS, revealing a shift toward lower molecular weight when using LDI-MS. This is likely due to ionization suppression of larger species, as previously described in petroleomics studies. GPC-UV has been employed in characterizing bio-oil products in comparative studies. For instance, Oudenhoven et al.[98] utilized this technique to compare the fast pyrolysis bio-oil derived from raw and leached pinewood feedstock. They found that the size distribution was not significantly impacted by this parameter. Conversely, Harman-Ware et al.[99] employed GPC-UV to compare the molecular weight distribution of noncatalytic and catalytic fast pyrolysis oils. Their observations indicated that the catalytic process promotes the formation of smaller compounds in the bio-oil. Moreover, Van Aelst et al.[100] employed GPC-UV to investigate the reductive catalytic fractionation (RCF) product of pinewood. They proposed a fractionation procedure based on solubility (similar to the one suggested by Gascon et al.[80,81] for crude oil fractions) and monitored these fractions using GPC. One notable observation from this research was that as the solvent polarity in the fraction increased, the size of the molecular families in the bio-oil also increased. This finding is particularly interesting for determining the efficiency of pyrolysis processes based on residual lignin fragments after the depolymerization process of RCF. The observations by Van Aelst et al. correspond with other studies where lignin fractions exhibited a linear correlation between their size and polarity.[101−103] This correlation is expected, considering the polyphenolic structure of lignin, where larger sizes result in a higher number of hydroxyl groups in the fraction.[100]

As a first attempt to characterize specifically heteroatomic species, Garcia-Montoto et al.[104] first evaluated the potential of GPC-ICP-HRMS with PS-DVB-based columns as a tool to characterize phosphorus species and evaluate their aggregation behavior in bio-oils originating from different sources. Their study revealed the presence of very-high molecular weight species (MW > 10,000 Da) without any fractionation, revealing the presence of micelles formed through the agglomeration of phospholipids which could be analogous to asphaltene aggregations in solution. Moreover, some aggregation of phosphorus-containing compounds was also evidenced during the pyrolysis process with the identification of aggregates whose molecular weight was estimated to about 91,000 as well as aggregation phenomenon after a three months storage. If these results are compared with all the work performed for petroleomics studies over these last few years, it can be inferred that appropriate prefractionation of the samples might also lead to the identification of even larger aggregates as previously shown through the analysis of fractions from extrography or other separation techniques. As a matter of fact, the aqueous phases from a fast pyrolysis bio-oil and a pretreated wheat straw have been characterized by Dubuis et al.[105] by the means of online SEC-UV-FT-ICR MS and off-line SEC × RPLC-UV/IT-ToF. The SEC separation enabled the grouping of carbohydrates, heterosides, and aromatic species which were confirmed by online ESI(−)-FT ICR MS, that lead to 140 fractions which were eventually collected prior to their reinjection and analysis by RPLC-UV/IT-ToF. The analysis of these fractions doubled the number of molecular species identified hence providing extended coverage of the matrix. A study performed by Castellvi Barnés et al.[106] relied on GPC to determine the molecular weight distribution for a fast pyrolysis pine oil and liquefaction pine oil but also as a prefractionation tool. In that configuration, it was possible to remove the liquefaction solvent which prevented adequate ^{13}C NMR experiments, but some issues were experienced in choosing an adequate eluent to fully dissolve the oils. Indeed, the choice of appropriate mobile phase and solvents for fractionation is also an important challenge, as these bio-oils have very different solubility behaviors, whereas we need to select a common mobile phase for all kind of samples which eventually would also be compatible for mass spectrometry analysis. Moreover, some other strongly impactful factors for the analysis of bio-oils are the storage and temperature which need to be carefully selected and assessed as significant evolutions of the bio-oils over time or depending on the temperature (both for storage and analysis) can take place, hence preventing comparisons of the results obtained for a given sample if performed at different times. For all of these reasons, it is crucial not only to develop new chromatographic and fractionation methods to simplify these matrices, but also to provide online hyphenation to highly resolutive and sensitive systems such as ICP-HRMS and FT-ICR MS, which seem to be the two most powerful and capable tools to decipher these matrices respectively at the elemental and molecular levels.

7. CONCLUSIONS

In conclusion, this bibliographic minireview has highlighted the significant contributions of GPC technique throughout history and its coupling to high-resolution elemental and molecular detection technologies for petroleomics and metallopetroleomics. Over 50 years of research, GPC has been used as an effective analytical and preparative tool to better understand the supramolecular and structural behavior of heavy crude oil and its fractions, as well as its reactivity to different processes. The trend observed throughout 50 years of research highlights the use of tetrahydrofuran (THF) as the mobile phase and polystyrene−divinylbenzene copolymer as the stationary phase to avoid retention of compounds in the column, with flow rate being a minor variable, leading to optimized chromatographic conditions that are suitable for both elemental and molecular mass spectrometry detection. One of the key applications derived from this methodology is the aggregation phenomenon studied for asphaltenes samples by means of GPC-ICP-HRMS and GPC-APPI FT-ICR MS focusing on vanadium compounds. The benefits from additional separation to perform multidimensional separations complementary to GPC have been evaluated by use of other techniques such as HPTLC, RPLC, and NPHPLC, in which the general trend observed is a greater polarity in the compounds of higher molecular weight, which is supported by experiments in adsorption with SiO_2 NPs followed through GPC where there is selective adsorption of high molecular weight (HMW) species. These HMW compounds are actually a cluster of aggregates that are capable of undergoing disruption by external agents that can affect supramolecular structures such as AgOTf, as observed for asphaltenes by GPC ICP-HRMS. The HMW fraction has been the focus of much interest, as it presents the greatest reactivity to polymerization and is more refractory to hydrodemetallization processes. Even with the state-of-the-art Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), effective and representative ionization of this fraction is still not possible, hence raising the need for multidetection approaches to provide an extended characterization of the full sample, which tend to be even more complex in the future in the context of the energy transition. Indeed, the use of GPC coupled with high-resolution mass spectrometry techniques has proven to be a promising approach for the detection of organic and inorganic species present in bio-oils, making it a valuable tool for researchers and industry professionals in the context of feedstocks changes and new energy production.

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Wladimir Ruiz received his B.S. in Chemistry from Central University of Venezuela in 2021. He is currently conducting a Ph.D. in Analytical Chemistry at the University of Pau and Adour Countries in collaboration with TotalEnergies. His research involves the fractionation and inorganic speciation of pyrolysis bio-oils and new sources of energy, using liquid chromatography hyphenated to ICP-HRMS.

Rémi Moulian earned his Ph.D. in Analytical Chemistry from the University of Pau and Adour Countries in 2020, where he focused on inorganic characterization of crude oil and asphaltene through liquid chromatography and HPTLC hyphenated with ICPMS. Currently, he works as a Post-Doctoral researcher at the University of Rouen. In his research, he applies HPTL UV and MS to the sugar characterization in pyrolysis bio-oils.

Ryan P. Rodgers is a Professor, International Chair Leader (UPPA), and member of iC2MC who specializes in elemental and molecular analysis of complex matrices at the National High Magnetic Field Laboratory (Florida State University). He received his Ph.D. in Analytical Chemistry from Florida State University in 1999. After a postdoctoral appointment at Oak Ridge National Laboratory, he returned to the Ion Cyclotron Resonance Program at the National High Magnetic Field Laboratory as an Assistant Scholar-Scientist and courtesy faculty member in the Chemistry Department. Currently, he serves as the Director of the Future Fuels Institute, FSU Distinguished Scholar, Research Faculty III, and he is a past Associate Editor of *Energy and Fuels*.

Pierre Giusti obtained his Ph.D. in analytical chemistry from the University of Pau and the Adour Countries in 2006. He is currently the Manager of the Molecular Separation and Identification Service in the R&D Analytical Platform of TotalEnergies Downstream Processes and Polymers. As a cofounder of the iC2MC Laboratory, he also obtained the position of CNRS Research Director in January 2021. His research interests are the elemental and molecular analyses of complex matrixes in the field of energy.

Brice Bouyssiere received his Ph.D. in Chemistry and Microbiology of Water from the University of Pau and the Adour Countries (UPPA) in 2002, followed by a Habilitation à Diriger les Recherches in "Total

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Alan G. Marshall, Chief Scientist for Ion Cyclotron Resonance, obtained his Ph.D. from Stanford University in 1970. He coinvented Fourier transform ICR mass spectrometry and leads its development. His research involves FT-ICR instrumentation, complex mixture analysis, and protein structure mapping. Marshall has published extensively and presented over 2,000 seminars/posters. He has received numerous awards and fellowships, including three from the American Chemical Society and the International Society for Mass Spectrometry.

Caroline Barrère-Mangote obtained her Ph.D. in MS and NMR at Aix-Marseille University in 2011. Then, she worked as a post doc at Normandy University on the development of innovating analytical methods for polymers and petroleum samples by (IM)MS. Since 2014, she has been the LC Laboratory Manager in the R&D Analytical Platform of TotalEnergies Downstream Processes and Polymers. Her area of research includes fractionation and elemental speciation in petroleum samples, pyrolysis oil (plastic or bio), or biocrude.

Julie Guillemant obtained her Ph.D. in Analytical Chemistry from the University of Lyon in 2020. From 2020 to 2022, Julie held a postdoctoral position at the University of Pau and the Adour Countries, where she developed hyphenations between chromatographic and molecular techniques for the analysis of complex matrices. Since 2022, she has been the Elemental Laboratory Manager in the R&D Analytical Platform of TotalEnergies Downstream Processes and Polymers, focusing on elemental determination of biofuel and plastic feedstocks.

■ ABBREVIATIONS

μ-GPC, micro gel permeation chromatography; AES, atomic emission spectroscopy; AFM, atomic force microscopy; AgOTf, silver triflate; APPI, atmospheric pressure photoionization; DBE, double bond equivalents; DCP, direct coupled plasma; ESI, electrospray ionization; FT-ICR, fourier transform ion cyclotron resonance; GC, gas chromatography; GDVB, glucose rings bonded to divinylbenzene-based gel; GPC, gel permeation chromatography; HMW, high molecular weight; HPMA, hydroxylated polymethacrylate-based gel; HTGC, high temperature gas chromatography; HTSD, high temperature simulated distillation; ICP, inductively coupled Plasma; IR, infrared spectroscopy; IT-ToF, ion-trap and time-of-flight; LC, liquid chromatography; LDI-MS, laser desorption ionization mass spectrometry; LTQ-Orbitrap, linear trap quadrupole orbitrap; LMW, low molecular weight; MeOH, methanol; MMW, medium molecular weight; MS, mass spectrometry; MSD, mass selective detector; MW, molecular weight; NMR, nuclear magnetic resonance; NPHPLC, normal phase high performance liquid chromatography; NPs, nanoparticles; PAH, polycyclic aromatic hydrocarbon; PS, polystyrene; PSDVB, polystyrene–divinylbenzene copolymer; potassium hydroxide, KOH; NMP, 1-methyl-2-pyrrolidinone; QCR, quartz crystal resonator; RCF, reductive catalytic fractionation; RI, refractive index; RPLC, reversed-phase liquid chromatography; SARA, saturate, aromatic, resin, and asphaltene; SD, simulated distillation; SEC, size exclusion chromatography; SFEF, supercritical fluid extraction and fractionation; TC, trapped compounds; TCNE, tetracyanoethylene; THF, tetrahydrofuran; TIC, total-ion chromatogram; Tol, toluene; UV, ultraviolet; VPO, vapor pressure osmometry; XIC, extracted ion chromatograms

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