Multidimensional Analytical Characterization of Water-Soluble Organic Aerosols: Challenges and New Perspectives

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Abstract: Water-soluble organic aerosols (OA) are an important component of air particles and one of the key drivers that impact both climate and human health. Understanding the processes involving water-soluble OA depends on how well the chemical composition of this aerosol component is decoded. Yet, obtaining detailed information faces several challenges, including water-soluble OA collection, extraction, and chemical complexity. This review highlights the multidimensional non-targeted analytical strategies that have been developed and employed for providing new insights into the structural and molecular features of water-soluble organic components present in air particles. First, the most prominent high-resolution mass spectrometric methods for near real-time measurements of water-soluble OA and their limitations are discussed. Afterward, a special emphasis is given to the degree of compositional information provided by offline multidimensional analytical techniques, namely excitation–emission (EEM) fluorescence spectroscopy, high-resolution mass spectrometry and two-dimensional nuclear magnetic resonance (NMR) spectroscopy and their hyphenation with chromatographic systems. The major challenges ahead on the application of these multidimensional analytical strategies for OA research are also addressed so that they can be used advantageously in future studies.

Keywords: organic aerosols; air particles; water-soluble organic matter; 2D NMR spectroscopy; high-resolution mass spectrometry; EEM fluorescence spectroscopy; 2D chromatographic separation

1. Introduction

The study and characterization of atmospheric aerosols, as well as their effects on radiative climate forcing, atmospheric chemistry, air quality and visibility, and human health, are some of the most predominant research topics in atmospheric chemistry [1,2]. Atmospheric particulate matter (PM) can be directly emitted into the atmosphere (primary aerosols) from a diversity of natural and anthropogenic sources, including biomass burning, incomplete combustion of fossil fuels, volcanic eruptions, and wind-driven or traffic-related suspension of road, soil, and mineral dust, sea salt, and biological materials [1]. Nonetheless, atmospheric PM can also be formed in the atmosphere (secondary aerosols) through gas-to-particle conversion processes of gaseous species (i.e., nucleation, condensation, and heterogeneous and multiphase chemical reactions) [1,3]. Furthermore, primary and secondary aerosols may undergo chemical and physical transformations, being subjected to transport, cloud processing, and removal from the atmosphere [4]. This multitude of emission sources and formation/processing mechanisms contribute to the diversity and complexity of the chemical composition (i.e., carbonaceous and inorganic components) and physical properties (i.e., concentration, size distribution, and surface area) of atmospheric PM, which in turn influences climate and health effects, further adding a layer of complexity.

Although the composition of the inorganic aerosol component has been thoroughly described in the literature, in-depth knowledge of its organic counterpart is particularly
more difficult to achieve due to the structural diversity and complexity of this aerosol component. The organic aerosols (OA) fraction can be the predominant component of suspended PM mass, accounting for ~20–50% of the total fine particulate mass at continental mid-latitudes and reach 90% in tropical forested areas [5,6]. The interest of the atmospheric research community on this OA component has been fueled by the realization that an important fraction of the OA is water-soluble. In Northern Hemisphere midlatitudes, the ubiquitous water-soluble organic matter (WSOM) represents 10% to 80% of the total particulate organics [7–10], whereas lower percentage values (up to 13%) have been reported for Southern Hemisphere locations [11]. The aerosol WSOM plays a key role in cloud formation and properties [12,13], Earth’s radiative balance [14,15], and atmospheric chemistry [14,16]. Atmospheric deposition of aerosol WSOM can also affect carbon and nitrogen biogeochemical cycles in aquatic ecosystems [17–19]. Aerosol WSOM in fine inhalable air particles may also exert adverse health effects by generating reactive oxygen and nitrogen species [20,21] or by promoting a moderate pro-inflammatory status [7]. Understanding these dynamic processes involving aerosol WSOM depends on how well one can identify its organic constituents.

It is now well established that the atmospheric aerosol WSOM comprises a multitude of molecular structures, physical properties, and sources (primary and secondary formation) [14,15,22,23]. However, coping with aerosol WSOM complexity still is a major impairment in advancing our understanding of its molecular composition, as well as its physical properties, sources, transformation, fate, as well as climatic and health impacts. Understanding aerosol WSOM composition and significance is, therefore, a focus of major scientific and policy concern. This review aims at highlighting the use of high-resolution multidimensional analytical techniques for assessing the molecular- and bulk-level structural features of the water-soluble fraction of OA. This review also provides an overview of how the gathered structural data had been used to glean new insights on the major sources, formation, transformation, and transport of water-soluble OA, particularly in urban areas where their level and composition potentially have high health risks. The final section addresses the challenges ahead to strengthen and optimize the current knowledge on the chemical structures of this important atmospheric OA fraction.

2. Coping with Water-Soluble OA Complexity

As mentioned above, the water-soluble OA fraction covers a huge variety of molecular structures with different physicochemical properties and sources. Nevertheless, according to Nozière et al. [22], not all atmospheric issues require the identification of all organic compounds present in OA samples. Depending on the purpose of the investigation, different levels of organic compositional information can be distinguished [22,24]: (i) functional group analysis is typically employed when interested in understanding specific properties of OA (e.g., chemistry, optical properties or structural average parameters of OAs); (ii) resolve the chemical composition of OA into different organic components (e.g., hydrocarbon-like OA (HOA), oxidized OA (OOA), and biomass-burning OA (BBOA)) in real time is usually chosen for capturing the characteristic chemical and physical properties of OA in a rapidly changing environment; (iii) target analysis of molecular markers is typically used when monitoring known OA formation processes or sources; and (iv) identification of up to three specific organic compounds when studying unknown OA formation processes or sources. For over two decades, these different levels of OA analysis have been dedicated to the quantification and characterization of the aerosol WSOM component. These efforts have been accompanied by numerous laboratory and field studies focused on the characterization of WSOM sources, as well as its mechanisms of formation and rates of its atmospheric transformations. Currently, these issues are still poorly known, although the major sources of these compounds are considered to be biomass burning and secondary formation (involving both anthropogenic and biogenic volatile organic compounds). To further enhance the diversity and complexity of this OA fraction, the atmospheric WSOM and their precursor gases can also be modified in the atmosphere by ox-
idative processes and, thereby, becoming less volatile, more hygroscopic and, consequently, more water-soluble [25,26]. Several procedures and methodologies have been developed to study the chemical composition of the aerosol WSOM [8–11,23,27–34]. These studies are usually carried out using a combination of total organic carbon analysis, isolation and fractionation procedures, and characterization of molecular fragments and intermolecular bonds by different analytical techniques. Such studies have demonstrated that this fraction consists of a highly diverse suite of oxygenated compounds, including dicarboxylic acids, keto-carboxylic acids, aliphatic aldehydes and alcohols, saccharides, saccharide anhydrides, aromatic acids, phenols, but also amines, amino acids, organic nitrates, and organic sulfates [11,28,30,33,35–42]. Despite the advances in understanding the atmospheric importance of the aerosol WSOM component, there are still several fundamental questions related to the complexity of its chemical composition, mechanisms of formation, atmospheric fate, and reactivity [15,22]. Determining the molecular composition and structure of aerosol WSOM is warranted to further increase the current understanding of its role in various atmospheric processes. Furthermore, due to its dynamic nature, the establishment of general models for the structure of the water-soluble fraction of OA still is far from being fully accomplished [23], and further studies are needed to understand their actual impact on a regional and global scale. As reviewed by Nozière et al. [22] and Duarte and Duarte [43], a universal technique for OA analysis does not exist. Several different offline and online analytical methodologies have been developed and applied to mitigate the complexity of OA, as well as to unravel the structure and composition of this aerosol component. The following Section 3 addresses the state-of-the-art multidimensional analytical strategies applied thus far in the characterization of OA. Analytical strategies combining one- or two-dimensional chromatographic separation and a detector (e.g., diode array, fluorescence, nuclear magnetic resonance (NMR), or mass spectrometry (MS)) or involving two or more stages of MS (i.e., tandem MS) or frequency (i.e., NMR spectroscopy) or wavelength (i.e., excitation–emission matrix (EEM) fluorescence spectroscopy) dimensions are here defined as multidimensional.

3. Advanced Multidimensional Techniques for Aerosol WSOM Analysis

3.1. Online vs. Offline Techniques: Where Does Multidimensionality Stand?

Ideally, the analysis of atmospheric organic matter should provide, in a continuous way, a thorough chemical profile of the sample, simultaneously ensuring a continuous size and time resolution. Yet, nowadays, there is no technique capable of providing this sort of information. Therefore, analytical techniques can be broadly divided into two major groups: online and offline techniques. The online methodologies mainly ensure high-time resolution for insight into brief events and diurnal changes [44], whereas offline methodologies are commonly applied to attain high chemical resolution [43,45].

Online or in situ methodologies are typically used in field observations to provide information on the chemical and physical properties of OA on short timescales. When using these methodologies, the analysis is performed almost at the same time that the particles are sampled and with very fast acquisition rates. These online measurements avoid potential artifacts associated with offline analysis methods, such as evaporation and chemical reactions during long aerosol sample collection and prolonged time of analysis periods [43–45]. Online aerosol mass spectrometry (AMS) analysis alongside positive matrix factorization (PMF) and high-resolution time-of-flight AMS (HR-ToF-AMS) analyzers have allowed a more in-depth investigation into the sources and composition of OA and its evolution in the atmosphere (e.g., [6,37]). Using this online approach, organics have been deconvoluted into several categories based on source, volatility, and distribution of oxygen-containing functionalities, namely: HOA, low-volatility oxidized OA (LV-OOA), semi-volatile OOA (SV-OOA), and BBOA. In the case of aerosol WSOM, Paglione et al. [46] compared these AMS factors for OOA with the NMR features of aerosol WSOM samples collected in filters. The authors concluded that the very oxidized LV-OOA
from AMS correlates with the NMR factor showing the so-called “humic-like substances” (HULIS) features.

The question now lies on where multidimensionality stands in these online MS methodologies for the analysis of aerosol WSOM. The concept of multidimensional MS has been highlighted in the review work of Han et al. [47] on lipidomic analyses. According to these authors, a two-dimensional (2D) mass spectrum, showing cross-peaks (i.e., the individual fragments) of a given primary molecule ion in the first dimension with the second dimension representing the fragments of this given molecule ion, readily produce an informative 2D map of the structure of the given molecule as well as its isomers and isobars [47]. This 2D MS concept evolves into a multidimensional MS model when conducting comprehensive MS analyses by changing an array of instrumental variables (e.g., different ionization conditions and/or the effects of collision conditions on fragmentation processes) to acquire critical information on the molecular species present in a given sample and their abundance, in what collectively comprise a multidimensional MS spectrum [47]. It should also be mentioned that a detailed interpretation of this huge amount of MS data requires advanced computational technology capable of handling all this information. At this moment, the multidimensional MS spectrum is decomposed into multiple 2D MS, which are displayed by varying only one variable at a time while keeping the others constant under fixed experimental conditions [47].

This concept of multidimensional MS can also be applied in online aerosol WSOM analysis. For example, Brege et al. [26] employed HR-ToF-AMS for the characterization of fog and fine aerosol samples, using the relative intensity of peak m/z 60 (f60) and peak m/z 44 (f44) as markers of fresh biomass burning influence and oxygenated and processed dissolved organic molecules, respectively. The relative intensity of f44 and f60 can then be used to build a 2D map to distinguish between fresh and aged biomass-burning OA [48] or between fresh and aged WSOM from fog samples [26] (Figure 1). Following a similar approach, Ng et al. [37] analyzed 43 AMS datasets from multiple field studies of OA in the Northern Hemisphere. The authors focused their attention on the OOA components, which were deconvolved into LV-OOA and SV-OOA. Differences in the mass spectra of these two components were characterized in terms of two main ions: m/z 44 (CO2+, f44) and m/z 43 (mostly C2H3O+, f43). The relative intensities of f44 and f43 were used to develop a 2D mass spectral profile for assessing the aging of the LV-OOA and SV-OOA components in the atmosphere: the LV-OOA component has higher f44 and lower f43 than SV-OOA. The work of Xu et al. [49] is another example of the multidimensionality of data acquired from online HR-ToF-AMS analysis. The authors coupled a particle-into-liquid sampler (PILS) upstream of an HR-TOF-AMS for the direct and online characterization of water-solubility of OA in contrasting urban and rural environments in the Southeastern United States. A 2D map of the mass defect spectrum (calculated by subtracting the ion’s nominal mass from its exact mass) vs. m/z of HR-ToF-AMS organic ions (Cn+, CnH2N+, CnH3+, CnH4O+, and CnH5O > 1+) allowed us to visualize how the water-solubility of OA factors (resolved by PMF analysis) varies with time and location. Nevertheless, this online PILS- HR-ToF-AMS methodology has large uncertainties associated with estimating the ambient concentration of HOA or OA from sources with low water-solubility [49]. Although the multidimensional nature of the online HR-ToF-AMS approach has not been fully explored, this methodology was also employed for assessing the size-resolved nonrefractory chemical composition of submicron aerosol particles across ocean waters next to the Antarctic Peninsula [50]. Notwithstanding the high-time resolution in terms of bulk chemical information attained by the online MS strategies, these approaches provide limited information and an oversimplification of the structural complexity of organic molecules present in atmospheric WSOM, particularly when compared with offline analytical assemblies. In fact, the use of online MS strategies is still far from replacing the offline techniques for an in-depth structural characterization of water-soluble OA, being mostly employed to spot marked differences in terms of different sources and atmospheric aging of sample’s organic content.
3.2. Application of Off-Line Multidimensional MS Methodologies for Water-Soluble OA Analysis

Offline MS methodologies, especially when associated with chromatographic separation procedures, allow a high selectivity and molecular discrimination of the OA constituents [45,52]. These hyphenated techniques, which can be considered multi-method strategies, have been widely used in target analysis, aiming at the quantitative identification of specific organic compounds and chemical markers for OA source apportionment [45]. The most widely used methodology for the chemical characterization of OA compounds is gas chromatography coupled to MS (GC–MS) [45]. This powerful, sensitive and selective hyphenated assemblage allows the separation, identification, and quantification of specific organic species within atmospheric aerosols. Several studies using GC–MS for molecular identification and quantification of target compounds in OA have been the focus of several reviews [22,43,45,53,54], and the main classes of compounds include aliphatic acids, aromatic compounds, amino acids, multifunctional oxygenated compounds, polyols, sugars, nitrogenated compounds, and polycyclic aromatic hydrocarbons. However, the GC–MS usually requires time-consuming derivatization procedures, especially for the analysis of highly polar and less volatile compounds, such as those of aerosol WSOM [43]. Liquid chromatography coupled to MS (LC–MS) has become a robust alternative to GC–MS for the analysis of individual compounds in water-soluble OA. Although it is less quantitative, LC–MS is especially suited for the analysis of high-molecular-mass, thermally unstable and/or very polar molecules without derivatization [22,53]. The most recent examples of the application of LC–MS to molecular identification and quantification of target com-

Figure 1. Example of a 2D map showing $f_{44}$ vs. $f_{60}$ obtained from the online high-resolution time-of-flight (HR-ToF)-aerosol mass spectrometry (AMS) analysis of fresh and aged organic aerosols (OA), including biomass-burning OA (BBOA) and fog samples. Reprinted from the work of Brege et al. [26], under the Creative Commons Attribution 4.0 License.
pounds in OA have been reviewed by Nozière et al. [22]. In particular, LC–MS techniques have been used to investigate secondary OA (SOA) formation and aging from a range of precursors in laboratory studies, as well as to identify water-soluble organosulfates and their nitrated derivatives in ambient PM samples (see, for example, references [22] and [53] and references therein). Additional main classes of compounds studied using this technique include aliphatic, alicyclic and aromatic carboxylic acids, carbohydrate-like substances, and amino acids and other organo-nitrogen compounds. More recently, LC coupled to HR-MS has been used to study the photochemical evolution of the WSOM composition in combustion-derived aerosols (namely, wheat straw burning aerosol samples), translated by the photodegradation of low oxygenated species and the formation of highly oxygenated products [55]. However, several challenges limit the application of LC-HR-MS to aerosol WSOM analysis, such as the complex chemical composition of these samples and the consequent poor resolution of the chromatographic separations, the presence of compounds at low concentrations or compounds that are poorly ionized, and the lack of authentic standards for compounds identification/quantification.

One possible way to deal with this problem is to increase the chromatographic resolution by, including a second chromatographic dimension, as in comprehensive multidimensional chromatographic systems. Comprehensive 2D gas chromatography (GC × GC) coupled to time-of-flight mass spectrometry (ToF-MS) has been successfully applied to the analysis of ambient volatile organic compounds, OA, and analysis of reaction products in chamber experiments concerning SOA formation [22,56]. For example, GC × GC-ToF-MS has been successfully applied to the separation and detection of more than 15,000 peaks in atmospheric OA samples, of which up to 200 organic compounds have been identified, including \textit{n}-alkanes, \textit{n}-alkan-2-ones, \textit{n}-alkanoic acid methyl esters, acetic acid esters, \textit{n}-alkanoic acid amides, nitriles, linear alkylbenzenes and 2-alkyltoluenes, hopanes, polycyclic aromatic hydrocarbons (PAH), alkylated PAH and oxidized PAH [56]. As in GC separations, GC × GC is of limited application for the analysis of very polar and low volatile water-soluble OA. Nevertheless, comprehensive 2D liquid chromatography (LC × LC) can be a leading technique for attaining a suitable chromatographic resolution of such water-soluble organic compounds, allowing to overcome the lack of chromatographic resolution of the traditional one-dimensional LC separation of complex mixtures. These multidimensional separation techniques provide a characteristic 2D separation pattern of a specific class of compounds, and when coupled to MS detection, can be quite useful for associating sample fingerprinting with the different parameters causing aerosols variabilities, such as the aerosol sources and the actual meteorological conditions. For example, LC × LC coupled to electrospray ionization ToF-MS (ESI-ToF-MS) has been applied for the identification and quantification of carboxylic acids in rural and urban aerosol samples [57]. LC × LC has also been employed for unraveling the complexity of WSOM from fine urban atmospheric aerosols and identify its seasonal patterns [27,58]. In these studies, LC × LC, combining the use of two independent separation mechanisms (per aqueous liquid chromatography (PALC) vs. size-exclusion chromatography (SEC)), was coupled with a diode array (DAD) and fluorescence (FL) detectors to map the hydrophobicity vs. molecular weight distribution of the most hydrophobic acid fractions in WSOM and alkaline-soluble organic matter from fine urban aerosols collected over different seasons. As shown in Figure 2a,b for samples collected during summer and winter, the PALC × SEC method allowed the separation of WSOM hydrophobic acid samples into several fractions, with the UV absorption contour plots displaying different elution patterns [27]. Apparently, the obtained fractions exhibit different molar mass distributions and chemical characteristics in terms of hydrophobicity between the two different seasons (Figure 2c,d).
and alkaline-soluble organic matter from fine urban aerosols collected over different seasons. As shown in Figure 2a,b for samples collected during summer and winter, the PALC × SEC-DAD chromatograms of the WSOM hydrophobic acids from (a) summer and (b) winter urban aerosol samples (color normalized to the intensity of the highest peak), and three-dimensional representation of the peak regional maxima location of the PALC × SEC–DAD chromatograms of the WSOM hydrophobic acids from (c) summer and (d) winter samples. Reprinted with permission from the work of Matos et al. [27].

More recently, Spranger et al. [59] employed a 2D offline chromatographic method (also known as the heart-cutting approach, denoted as LC-LC) combining SEC and reversed-phase LC (RP-LC) to characterize the so-called HULIS in aerosol particles. This method involved the collection of five SEC fractions (first dimension (1D) effluent) and their subsequent injection into the RP column (second dimension (2D)) for further separation into 11 fractions with different polarities, after being concentrated and redissolved in a proper solvent for injection into the second separation dimension. In the following study, the same group of authors characterized those 11 fractions collected from the RP column by means of Fourier transform–ion cyclotron resonance mass spectrometry (FT-ICR MS) to resolve the high complexity of the aerosol organic material [60]. The authors reported that the number of assigned molecular formulas increased by a factor of 2.3 for the fractionated sample (18,144) compared to a bulk sample analysis without fractionation (7819), with more than 15,000 compounds being identified in the fractionated sample (including organosulfates, reduced N-containing compounds, and polyaromatic compounds) [60]. This 2D offline chromatographic approach coupled to an HR-MS detection method offers an astounding separation power, besides being a simple and easy implementation of 2D LC. However, this offline multidimensional separation method still has limitations in terms of the increased conceptual and instrumental complexity, the increased time of analysis when multiple fractions are selected for analysis in the 2D, phase-system incompatibility issues, and possibly reduced number of target unresolved analytes that can be addressed in the analysis [61].

On the other hand, in the LC × LC approach, such as that applied by Matos et al. [27] and Paula et al. [58], all fractions are subjected to two different separations, thus providing a comprehensive characterization of the sample. The LC × LC is a considerably faster
multidimensional separation method than LC-LC, with a high degree of orthogonality, thus proving a reliable fingerprint that allows an efficient comparison (often visual) of samples [27,58]. Combined with HR-MS detection methods, LC × LC is well suited for the untargeted analysis of the atmospheric WSOM samples, offering a thorough mapping of the complexity of these samples and enabling the identification of an unsurpassed number of organic compounds.

In addition to the hyphenated approaches, there are other MS-based techniques that have also been used for acquiring detailed structural information on water-soluble OA. An example of this application is the development of a methodology using matrix-assisted laser desorption/ionization (MALDI) ToF-MS for the analysis of bioaerosol particles [62]. Nowadays, however, the most emerging systems based on MS for the analysis of aerosol WSOM is FT-ICR MS, with soft ionization techniques (e.g., ESI, MALDI) (e.g., references [26,29,63]). In such analytical systems, the soft ionization provides intact molecular ions, and the HR-MS can provide up to 25 billion theoretical plates of separation, thus allowing a rapid and definitive molecular mass determination of intact polar macromolecules that are difficult to analyze by the traditional hyphenated MS techniques [64]. The application of these HR-MS techniques has increased, mainly aiming at the identification of the molecular elemental composition of thousands of individual organic components for further investigations on the influence of the aerosol sources on the composition of OA [43,45]. The detailed interpretation of these huge amounts of MS data generated by these HR-MS techniques requires data processing methods capable of handling all this information. Typically, the acquired data are represented in the form of van Krevelen diagrams, in which the H/C ratio is plotted as a function of the O/C ratio for each mass and corresponding molecular formula identified in a sample. As reviewed by Nozière et al. [22], these diagrams are plotted as three-dimensional figures with ion signal intensities included as an additional dimension, being often used to describe the evolution and differentiate potential sources of OA. Another approach to glean compositional information from HR-MS analysis of OA was introduced by Wei et al. [64], who developed a framework to consider the chemical and physical evolution of OA. The approach is based on a three-dimensional (3D) coordinate system (Figure 3) defined by the molecular descriptors of molecular weight (directly proportional to the number of C atoms in a molecule and inversely proportional to volatility), heteroatom mass (e.g., O, N, S, Cl), and double bond equivalents (which allows an improved description of chemical reactivity of the material through ozonolysis or hydroxyl addition). This coordinate system is then coupled to HR-MS data of OA, allowing to estimate their chemical and physical properties [64]. The most striking recurrent drawback of these representations, particularly of van Krevelen diagrams, is that formulas with different atom numbers but identical atomic ratios cannot be disambiguated [22]. Furthermore, both the van Krevelen diagrams and 3D coordinate system representations share a common downside: the compound molecular formula derived from HR-MS data only allows an attempt to propose possible chemical structures, with the assistance of a spectral database or a reference/standard. Thus, when such information is not available, it becomes highly recommendable to associate the information obtained from the HR-MS systems with that acquired by another “structural analysis” technique, namely the NMR spectroscopy [24,43].
Despite these constraints, it has been shown that protons in aliphatic structures (H-C) are the dominant moieties in aerosol WSOM samples (30–60% of the total $^1$H content), followed by oxygenated aliphatic compounds (H-C-O) and unsaturated aliphatic (H-C=C=) groups (5–27%), and only a minor contribution from aromatic groups (typically, below 15%) [24]. The $^1$H NMR data has also been used to deduce the sources of aerosol WSOM in different areas by making use of a source apportionment model, developed by De Cesari et al. [32], where the ratios of calculated carbonyl and aliphatic carboxylic groups (H–C–C=O)-to-total aliphatics (H–C–C=O/$\Sigma$Aliphatics) and oxygenated aliphatic groups (H–C–O)-to-total aliphatics (H–C–O/$\Sigma$Aliphatics) are plotted in a 2D coordinate system. For this model, the concentrations of organic hydrogen determined in the different spectral regions of the $^1$H NMR spectra were converted into carbon concentrations using hydrogen-to-carbon (H/C) ratios, whereas the contribution of H–C–C=O groups is indi-
rectly estimated by subtraction of benzylic groups (assumed to be 16.7% of the measured H–Ar signal) from the measured H–C–C= signal. In their study, Decesari et al. [32] used different sets of water-soluble OA samples to identify and establish fingerprint boundaries regions for three major aerosol sources, namely SOA, aged BBOA, and marine OA (as shown in Figure 4). Since this seminal work, the ¹H NMR source apportionment model has been applied by other researchers to water-soluble OA samples collected under different emission scenarios [38,39,41,65]. Moreover, this 2D model has been updated (Figure 4) with new fingerprint boundary regions, namely a rural OA region [39], a fresh pollen region [41], and a fresh BBOA region [65]. The fingerprint region of urban OA was firstly established by Cleveland et al. [38] with NMR data from samples collected in an urban industrial center and subsequently verified by Lopes et al. [8] and Duarte et al. [10,11].

1D solid-state cross-polarization with magic-angle spinning (CP-MAS) ¹³C NMR spectroscopy is another NMR-based technique successfully applied in the last fifteen years for investigating the distribution of carbon functional groups in the water-soluble fraction of OA [9,30,31,66,67]. This technique allows a semi-quantitative measure of the relative contribution of the different carbon functional groups to the OC present in the sample and, consequently, it can be used for investigating the composition and the structural variability of the water-soluble organic fraction according to different aerosol sources, sampling sites, and meteorological conditions [24,68]. Although intuitively, the analysis in liquid-state of the water-soluble OA could make more sense, 1D solid-state CP-MAS ¹³C NMR presents some advantages for the analysis of this fraction, especially when compared to 1D liquid-state ¹H NMR spectroscopy or even to 1D liquid-state ¹³C NMR spectroscopy. Some of these advantages have been reviewed by Duarte and Duarte [24,69] and include: the solid-state technique is not affected by the solvent, which can cause changes in the chemical shifts of the sample, hide some sample chemical resonances by the solvent signals, or even loss of some peaks, namely those of the carboxylic acids, due to the presence of rapidly exchangeable protons; the liquid-state NMR spectra may present lower resolution and sensitivity due to the low solubility of some organic components in the selected solvent, and the solid-state technique is a non-destructive method, allowing the sample to be used for further complementary structural analysis. On the other hand, a major limitation of the solid-state CP-MAS ¹³C NMR technique is the difficulty in obtaining high-quality NMR spectra due to the relatively low carbon content of ambient water-soluble OA samples. This fact requires combining several aerosol samples collected under a common feature (e.g., similar ambient conditions) in order to obtain a sufficient amount of sample for
analysis (it needs ~20–100 mg of sample, depending on the size of the NMR probe) [69]. Furthermore, to make the sample amenable to CP-MAS $^{13}$C NMR analysis, the organic material must be isolated from the inorganic ions (e.g., through solid-phase extraction, ion-exchange chromatography, or size-exclusion chromatography) and prepared as a solid (e.g., freeze-drying) prior to analysis [69]. It must be emphasized, however, that under these circumstances, the acquisition of a high-quality $^{13}$C NMR spectrum is made at the expense of pursuing studies on near real-time variations of the chemical composition of OA. Nonetheless, recently, and for the first time, Duarte et al. [23] applied advanced solid-state $^{13}$C NMR techniques, including nearly quantitative $^{13}$C multiple cross-polarization/magic angle spinning (multiCP/MAS), multiCP/MAS with dipolar dephasing, multiCP/MAS with $^{13}$C chemical shift anisotropy filter, and 2D $^{1}$H–$^{13}$C heteronuclear correlation (2D HETCOR), to acquire an accurate quantitative structural description of aerosol WSOM collected in an urban atmosphere. Two urban aerosol WSOM samples collected in two short periods of time (one week each) under different wintry weather conditions were investigated. NMR data successfully pinpointed the variability of whole aerosol WSOM composition, allowing to suggest source-specific structural characteristics for each sample in those two short periods of time [23]. A new structural model of urban aerosol WSOM was build based on the acquired 2D compositional data (Figure 5), showing the presence of three independent classes of compounds that vary both in content and molecular diversity within those two short periods of time: heteroatom-rich aliphatics (either chain or branched), carbohydrate-like moieties, and highly substituted aromatic units [23].

![Figure 5](image_url)

**Figure 5.** 2D $^{1}$H–$^{13}$C HETCOR NMR spectrum (a) and associated $^{1}$H slices (b) of the WSOM extracted from fine urban air particulate matter. Reprinted from *Atmospheric Environment*, 230, R.M.B.O. Duarte, P. Duan, J. Mao, W. Chu, A.C. Duarte, K. Schmidt-Rohr, Exploring water-soluble organic aerosols structures in the urban atmosphere using advanced solid-state $^{13}$C NMR spectroscopy, 117503, Copyright (2020), with permission from Elsevier.

In another study of Duarte et al. [9], a chemometric tool, called generalized perturbation-based 2-D correlation spectroscopy (2-D correlation spectroscopy), was used to highlight subtle changes in compound class distributions within urban water-soluble OA samples from different seasons, using the traditional solid-state CP-MAS $^{13}$C NMR and Fourier-transform infrared (FTIR) spectroscopy data as chemical probes [59]. This chemometric tool generates a synchronous 2D correlation map involving CP-MAS $^{13}$C NMR and/or FTIR spectra (Figure 6), which provide 2D information on how $^{13}$C NMR and/or FTIR bands correlate along a seasonal gradient (e.g., median of the air temperature within each season). Using this chemometric approach, Duarte et al. [9] demonstrated that the water-soluble compounds isolated from urban aerosol samples (Aveiro, Portugal) consist of at least two classes of compounds: one is rich in both carboxylic and hydroxyl functional groups, and it has an aliphatic character, and the other entails lignin-derived structures.
Another set of NMR-based multidimensional methodologies that have emerged for the structural analysis of water-soluble OA are the 2D liquid-state NMR techniques [33,70–73]. These techniques have proven to be very useful to solve the spectral information overlaid in 1D NMR spectra by separating the data into a second frequency dimension. The combination of the data from some of these 2D liquid-state NMR techniques, namely $^1$H–$^1$H homonuclear (COSY and total correlation spectroscopy (TOCSY)) and $^1$H–$^{13}$C heteronuclear (HSQC and HMBC) connectivity, improve the spectral resolution and provide a deeper insight into the C–H backbone of the organic compounds from the atmospheric aerosols [69]. Although the use of liquid-state 2D NMR techniques to water-soluble OA analysis seems to be still very limited, the existing studies [33,70–73] have demonstrated the potential of these high-resolution NMR techniques for a comprehensive description of the substructures present in aerosol WSOM, as well as to identify molecular fingerprints of the different aerosol sources and formation mechanisms. Conceivably, one of the most important drawbacks of these techniques is the time required for signal acquisition, which can reach several days, thus limiting its application, at least on a routine basis.

Nevertheless, as reviewed by Duarte and Duarte [43,69], the keys to developing accurate knowledge about the structural features of water-soluble OA also lies in the development of analytical procedures taking advantage of the synergistic application of all of the aforementioned NMR techniques (i.e., 1D solid-state NMR, 1D and 2D liquid-state NMR), as well as their association with other advanced analytical techniques, namely HR-MS. Furthermore, due to the inherent complexity of the aerosol WSOM fraction, the application of a chromatographic separation procedure prior to NMR analysis can directly provide major advances in the knowledge gained from the sample. Very few studies have applied LC combined with 1D and 2D NMR detection for the identification of individual unknown molecular components in aerosol WSOM samples. The studies published thus far using this analytical approach share a common feature: their main purpose is the identification of SOA tracers produced in laboratory chambers/reactors.
to simulate atmospheric chemistry [74–76]. To the best of the authors’ knowledge, the use of LC coupled to NMR for the analysis of field water-soluble OAs samples has yet to be carried out, a situation that is likely explained by the amount of sample needed to obtain a meaningful NMR signal. Nonetheless, taking into account the results of the recently developed \( \text{LC} \times \text{LC} \) method for resolving the chemical heterogeneity of aerosol WSOM samples [27,58], the development of protocols based on both \( \text{LC} \times \text{LC} \) and NMR techniques may provide valuable clues for unfolding the complexity of water-soluble OA.

3.4. Excitation–Emission Matrix (EEM) Fluorescence Spectroscopy for Water-Soluble OA Analysis

EEM fluorescence spectroscopy is a high sensitivity and non-destructive 3D technique, widely used to compare and discern the dynamics of chromophoric water-extracted organic matter from field- and chamber-generated air particles [10,34,63,77–85]. The acquisition of an EEM fluorescence spectrum involves the collection of sequential fluorescence emission (Em) spectra at successively increasing excitation (Ex) wavelengths. The Em spectra obtained are concatenated to produce a plot in which the fluorescence intensity is displayed as a function of Ex and Em wavelengths. EEM fluorescence spectroscopy combined with parallel factor analysis (PARAFAC) modeling has made it possible to further resolve the complex 3D EEM spectra into its dominant fluorescent components and quantify each component’s contribution to the total fluorescence [77]. In this regard, the reader is encouraged to refer to the tutorial of Murphy et al. [86] in the practical application of PARAFAC to fluorescence datasets, using complex organic mixtures fluorescence dataset. When applied to investigate the fluorescence features of chromophoric WSOM in atmospheric aerosols from different environments (urban, forest, marine, and pristine), EEM-PARAFAC aided in the classification and source identification of chromophores in atmospheric organic aerosols [10,34,63,77–85]. The acquired EEM-PARAFAC data are of utmost importance to shed light on the pivotal role played by the WSOM in the optical properties and photochemical reactivity of atmospheric OA.

An additional interesting application of multidimensional analytical approaches was shown by Chen et al. [82], where EEM and HR-MS were applied for the characterization of the chromophoric WSOM in urban, forest, and marine aerosols. Statistical analyses of PARAFAC and non-negative matrix factorization (NMF) were applied to EEM and HR-MS spectra to resolve the different components of WSOM that exhibit different chemical-structural and fluorescence characteristics. Using EEM-PARAFAC, the authors reported the presence of three types of water-soluble chromophores, two with fluorescence characteristics similar to those of the so-called HULIS (which the authors defined as HULIS-1 and HULIS-2) and one with fluorescence characteristics similar to those of protein compounds (designated as PLOM) [82]. The HR-MS analyses indicated that the chromophoric components of HULIS-1 and HULIS-2 were associated with highly and less-oxygenated structures, respectively. HULIS-1 was ubiquitous in all water-soluble organic aerosol samples, whereas HULIS-2 and PLOM were abundant only in terrestrial and marine aerosols, respectively [82]. This combined application of multidimensional EEM and HR-MS techniques, aid by chemometric tools, set the basis for new analytic strategies aiming at the chemical structural identification and further understanding of the origin and fate of chromophoric WSOM in atmospheric aerosols.

4. Multivariate Analysis for Source Apportionment of Water-Soluble OA

Regardless of the sophisticated and high-resolution multidimensional analytical techniques employed in the water-soluble OA analysis, the quality of the results is always dependent on the capacity to extract useful information from the acquired data. Yet, the processing and treatment of the data acquired by many of the multidimensional techniques presented in this review is not a trivial task. Some of these techniques can provide information regarding hundreds or even thousands of different compounds, which may cause a severe overlap of the different analytical signals obtained from a complex sample. In
addition, the data produced by these techniques, especially when combined with chromatographic techniques, may easily exceed more than three or four analytical dimensions.

Indeed, nowadays, one of the biggest limitations to a wide application of these advanced instrumental approaches is the lack of knowledge, and analytical expertise for interpreting the huge and complex data sets produced [43]. This lack of knowledge associated with the chemical complexity of the aerosol WSOM has limited the study of this matrix to the identification of some target components, the identification of functional chemical groups, and the assignment of sources and temporal characteristic patterns. In this sense, almost all applications of chemometric tools to the study of aerosol WSOM have been focused on mining the acquired data for highlighting different patterns that may be associated with source profiles and source contributions—the so-called source apportionment methodologies. There are several source apportionment methodologies already in use, namely the chemical mass balance (CMB) and chemical transport model (CTM), as well as some multivariate factor analysis methodologies, such as principal component analysis (PCA), PMF, and multivariate curve resolution-alternating least squares (MCR-ALS) (e.g., references [87–90] and references therein). The CMB model uses compounds associated with specific sources as references (e.g., levoglucosan as a tracer of biomass burning) to identify the contribution of that particular source. Therefore, the CMB model is mainly limited to the study of well-known emission source profiles [87,89]. The CTM requires descriptions of the main chemical and physical processes driving air concentration of primary and secondary pollutants, such as sulfur and nitrogen oxides, ozone, and atmospheric PM, being used for investigating and assessing ambient air quality at various spatial and temporal scales (reference [90] and references therein). On the other hand, multivariate factor analysis methodologies allow identifying profiles from experimental data based on numerical factors. Although not corresponding to a particular spectrum of a pure chemical compound, these factors may be related to emission sources and/or functional chemical groups. As pointed out by Alier et al. [87], the MCR-ALS and PMF provide similar results, whereas the PCA methodology produces profiles with the weakest physical meaning. An example of the application of these methods to the study of water-soluble OA is the recent work presented by Paglione et al. [35], in which MCR-ALS was applied to $^1$H NMR data of WSOM from atmospheric aerosols. In this work, the authors were able to identify a total of five factors: two factors were associated with chemical tracers of marine SOA and biogenic sources (e.g., methane-sulphonic acids and low-molecular-weight amines); other two factors were associated with “aged” components (one, of which was also associated with aged biomass-burning particles); and the fifth factor was associated with primary wood-burning particles and showed a chemical composition dominated by anhydrosugars, other polyols, and phenolic compounds [35]. This study also showed an agreement between these factors and the results derived from PMF analysis of the AMS data [35]. Undoubtedly, the combination of such powerful mathematical algorithms with chemical fingerprinting data provided by the diverse high-resolution offline and online multidimensional analytical techniques has an enormous potential to disentangle components in the unresolved aerosol WSOM matrices, as well as determine their atmospheric content and apportion of their sources. Yet, its implementation still is at an embryonic stage.

5. Conclusions

In recent years, the study of water-soluble organic compounds in atmospheric aerosols has become one of the areas of intense focus and interest in atmospheric chemistry research. The water-soluble fraction of OA plays an important role in climate, environment, and public health. However, due to the limited knowledge on the composition, sources, formation mechanisms, and transformation processes, their climatic and health impact are still far from being completely understood. Due to the inherent heterogeneity and presence in relatively low atmospheric concentrations, an important fraction of atmospheric WSOM still resides outside of the current analytical window. Nevertheless, during the past two decades, the rapidly evolving field of analytical instrumentation has produced sophisti-
In recent years, the study of water-soluble organic compounds in atmospheric aerosol WSOM still resides outside of the current analytical window. Nevertheless, and presence in relatively low atmospheric concentrations, an important fraction of atmospheric WSOM should provide detailed molecular and structural speciation with high-time resolution. However, these online techniques fail to provide detailed molecular speciation and consequently can hardly replace multidimensional offline strategies.

Ideally, multidimensional analytical strategies employed in the study of aerosol WSOM should provide detailed molecular and structural speciation with high-time resolution. Although the existent analytical approaches are not able to achieve these two requirements simultaneously, the synergistic information derived from both online and offline techniques should be able to fulfill this goal. Online AMS is probably the technique of choice for attaining high-time resolution in terms of bulk chemical information, with concomitant insight into brief events and daily variations of OA. However, these online techniques fail to provide detailed molecular speciation and consequently can hardly replace multidimensional offline strategies.

One of the most important steps for the implementation of offline multidimensional analytical strategies is the implementation of robust sampling, extraction and isolation procedures of field atmospheric WSOM samples. These requirements are of utmost importance, for example, for HR-MS studies of aerosol WSOM, which require the isolation of the organic analytes from the water-soluble inorganic constituents. The lack of standard procedures for sample collection and preparation is nowadays one of the biggest drawbacks for an extensive comparison of WSOM samples from different locations with different degrees of pollution (urban vs. rural vs. remote areas in different regions of the world). Therefore, future refinements may be directed toward analytical isolation procedures that allow the recovery of the majority of the WSOM without the artifacts associated with solid sorbent methods.

The use of offline MS methodologies, in particular with the advent of HR-MS detectors and their hyphenation with 2D chromatographic systems, or combination with EEM-PARAFAC methods, has allowed a huge improvement in the selectivity and molecular discrimination of the WSOM constituents, as well as the identification of specific chemical markers and tracers for OA source apportionment. Significant progress has also been achieved with the use of 1D NMR spectroscopy, particularly with liquid-state 1H NMR, for structural characterization and source apportionment of aerosol WSOM. In this field, future
work should explore the potential of using multidimensional liquid-state (2D or higher) NMR (e.g., $^1$H-$^1$H, $^1$H-$^13$C, and/or $^1$H-$^15$N correlation experiments) approaches. Alternatively, the use of solid-state advanced spectral editing $^{13}$C NMR techniques and the analysis of nuclei other than $^{13}$C (e.g., characterization of organic nitrogen) should improve the knowledge on the structural composition of water-soluble OA. Nevertheless, the lack of analytical expertise in the use of these NMR spectral editing techniques, as well as the difficulties in handling and decode highly overlapped 2D NMR data, are currently the major impediments for the application of these techniques to WSOM characterization. Furthermore, one of the biggest challenges for the future is the development of chemometric methods to deal with the huge amount of data sets generated by these multidimensional strategies. In addition, it is also necessary to develop software capable of processing and combine information withdrawn from different 1D and 2D spectra of the same WSOM sample, in an automatic or at least semiautomatic way, in order to unambiguously identify the organic structures present in the sample.

In the near future, with the development and further sophistication of the analytical and chemometric tools, the research in this scientific field should continue to evolve on the quest for attaining sound conclusions about the structure-origin relationship of OA fractions present in air particles and simultaneously build up scientific knowledge in the field of advanced structural and molecular analysis of complex OA.

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