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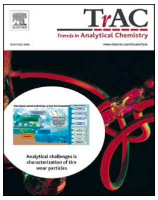

NMR spectroscopic applications to atmospheric organic aerosol analysis – Part 2: A review of existing methodologies and perspectives

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ABSTRACT

In the last decade, the considerable technological progress achieved in parallel science disciplines provides a potential for a breakthrough in NMR atmospheric applications. Improvements in probe sensitivity and in the NMR devices, like innovative tubes with a better filling factor, enable to improve the signal-to-noise ratios of an order of magnitude with respect to the earlier techniques. This already allows for the development of NMR techniques in experimental setups, like laboratory simulation chambers, where the limited sampling volumes and aerosol loads have hampered full application in the past. In parallel, the development of techniques for high-performance data analysis, including metabolomics, and multivariate statistical techniques applied to complex spectral datasets greatly expand the potential of NMR-based aerosol characterization techniques for individual compound identification and source apportionment. To achieve this goal, a more systematic NMR characterization of atmospheric tracers and relevant sources is required.

1. Introduction

NMR spectroscopy is, by far, the most powerful tool to investigate the structure of molecules and molecular aggregates in solution. Also, in the solid state it can provide essential information, although for crystalline substances X-Ray diffraction is still the leading technique. For such reason, NMR has assumed a huge and growing importance in a variety of fields in chemical, biochemical and related sciences [1]. The reasons of the outstanding role of NMR lies: i) in the low energy of the spectroscopic transition and the long life time of the resulting excited states, that ensures an extremely high spectral resolution; ii) in the strong dependence of the qualitative parameter, the chemical shift, from the chemical environment experienced by the resonating nuclei; iii) in a straightforward relationships between the intensity of the signal, the peak integral, and the absolute amount of a specific kind of atoms in the sample; iv) in the outstanding importance and diffusion of a few resonating nuclei species, chiefly ^{1}H and ^{13}C, in a wider context of not-active nuclei; v) in a peerless possibility to exploit tiny, but predictable, spectroscopic effects, such as the scalar spin-spin coupling or the dipolar spin coupling (NOE effect), to gain additional key structural information; vi) in the availability to implement 2D and 3D correlation experiments, either homo- or heteronuclear, that allows to selectively derive suitable information about the chemical structure of analytes. Last, an extremely important feature of NMR spectroscopy is its totally non-destructive nature: at the end of the experiment, the sample can be completely recovered by solvent removal (distillation or sublimation).

On the other hand, some important drawbacks affect the NMR techniques. First and most important, the small energy separation between the resonating energy states gives rise to a low sensitivity, that is particularly critical when low-mass samples are available. This drawback can be partially overcome by the possibility of averaging the signal in FT-pulsed NMR spectroscopy, at the cost of long experimental times, but remains a handicap in environmental studies. Moreover, the combination of low sensitivity, long acquisition times and solution-state experiments with strong limitation about solvent’s suitability, prevents the possibility of obtaining real-time continuous analyses (on-line experimental setting) in favour of discontinuous laboratory analyses (off-line experimental setting). Further critical issues are:

- The need of performing analysis of the samples in solution implies the choice of a specific solvent that could be suitable for a category of substances (e.g. water for highly polar ones, like salts or sugars) while totally ineffective for others. Moreover, solvents are traditionally deuterated, therefore more expensive and less available than their protonated counterpart. Magic Angle Spinning (MAS) solid

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Abbreviations

AMS (Q-AMS, HR-ToF-AMS):	Aerosol Mass Spectrometry (Quadrupole-AMS, HighResolution-Time-of-Flight-AMS)
ATOFMS	single-particle aerosol time-of-flight mass spectrometry
FTICR-MS	Fourier-transform ion cyclotron resonance-mass spectrometry
GC-MS	Gas-chromatography mass spectrometry
HULIS	Humic-Like Substances
LC-MS	Liquid Chromatography-mass spectrometry
MCR	Multivariate Curve Resolution
MSA	Methansulphonate
MSOM	Methanol Soluble Organic Matter
NMR (^{1}H NMR, ^{13}C NMR)	Nuclear Magnetic Resonance spectroscopy (proton-NMR, carbon-13-NMR)
OA	Organic Aerosol
PSOM	Pyridine Soluble Organic Matter
POA	Primary organic Aerosol
PCA	Principapl Component Analysis
PMF	Positive Matrix Factorization
SOA	Secondary Organic Aerosol
SPE	Solid-phase extraction
TFA	trifluoroacetic acid
WIOM	Water Insoluble Organic Matter
WSOC	Water Soluble Organic Carbon
WSOM	Water Soluble Organic matter

state NMR is a less common alternative, suffering from lower sensitivity and requiring even specialized hardware. Nevertheless, MAS has been successfully, but not routinely, applied in many fields, including environmental studies.

- Hyphenated techniques to simplify complex mixtures are available for NMR, specifically LC-NMR, but they have met much less success than the universally spread hyphenated MS techniques (GC-MS, LC-MS), mostly because of the complexity of coupling LC to high-field NMR, to the loss of sensitivity due to the stop-flow probe design, to the difficulties to efficiently suppress very strong solvent signals (usually acetonitrile and water).
- Last but not least, high-dispersion, high sensitivity NMR instruments are expensive instruments, also one order of magnitude more than many other analytical instruments, and are not suitable for mobilization, generally ruling out on-field implementation. Within this framework, the development of microcoils coupled with low-field benchtop NMRs could become an option in the future. For the moment only low-field spectrometers with poor resolution and sensitivity are available in benchtop version [2].

In this paper, Part 2 of a review about NMR applications to atmospheric aerosol analysis, we provide a survey of the existing methodologies developed for such applications, focusing on recent innovations and potentialities from emerging technologies. A review of analytical protocols for sample workup and NMR data analysis is also provided. Part 1 [3] provides instead a summary of the existing NMR studies on ambient aerosols and their sources.

2. NMR instrumentation

The characteristics of the NMR spectrometer are key to guarantee resolution and sensitivity to the analysis of environmental samples as well as to assure quantitative determination of the targeted analytes. Nevertheless, detailed information about spectrometers, probes and NMR tubes is rarely available in the literature of atmospheric samples analysis. Only the NMR field strength is systematically documented, because this is just the most important parameter governing dispersion and sensitivity in the analysis. Apart from the available sample amount, from a physical point of view, the signal-to-noise ratio (SNR) of an NMR spectrum is determined by: 1) the gyromagnetic constant (γ) of the observed nuclei; 2) the strength of the applied magnetic field (B_0); 3) the total acquisition time (TAT), that is closely related to the number of spectra acquired in the FT-mode. Considering these three parameters, SNR is the result of eq (1), where the constant K derives from a wide number of factors that will be considered and discussed later on (amount of sample, probe head design, etc.)

$$\mathrm{SNR} = K\,\gamma\,B_0^{3/2}\,\mathrm{TAT}^{1/2} \tag{1}$$

It is thus clear that the strength of the applied B_0 field can greatly improve the SNR, since doubling the field implies a gain of ~300% in the SNR when using the same TAT. Due to the square root relationship of SNR vs TAT, doubling the magnetic field reduces the TAT by about nine times. Unfortunately, while a 14.4 T magnet (corresponding to 600 MHz resonance frequency for ^{1}H) is nowadays the standard field for a "middle range" NMR spectrometer, the switch to 23.5 T or higher field causes a 10–20 fold multiplication of the cost of the spectrometer. At the present time, only about twenty NMR spectrometers exceed 23.5 T, being the 28.2 T (1.2 GHz for ^{1}H) spectrometer the actual cutting-edge limit for high-resolution NMR spectra (about 10 systems installed and running). The current literature on atmospheric aerosol sample analysis is most commonly based on middle range NMR spectrometers (500–600 MHz), although 700–750 MHz instruments have been sometimes used [4,5].

Being the cost and development of very high field magnets a serious drawback, orthogonal approaches to enhance sensitivity (keeping constant TAT and B_0) have been therefore pursued in the last decades towards two routes: 1) development of more sensitive probe heads and specialized NMR-tubes; 2) perturbation of the relative population of the involved energy levels by hyperpolarization techniques. To our best knowledge, hyperpolarization techniques have never been applied to the analysis of atmospheric samples, therefore we will focus here on the opportunities provided by probes and tubes.

2.1. Types of probes

The probe design has been the subject of many different approaches to enhance the absolute sensitivity of the spectrometer. One of the most important parameters for any NMR probe is the filling factor (FF). The filling factor of the probe is a measure of the fraction of the sample that contributes to the detected NMR signal within the sensitive region of the probe's coil. The filling factor can be calculated using the following equation:

Filling Factor (FF)=V_sample / V_coil

Where V_sample is the volume of the sample that is actively contributing to the NMR signal and V_coil is the volume of the sensitive region of the coil or resonator. The filling factor is typically expressed as a decimal or percentage. For example, if the calculated filling factor is 0.8, it means that 80% of the sample volume is effectively contributing to the detected NMR signal.

In NMR probe heads, two different coils architectures have been considered to fulfil the different requirements in different circumstances. Due to the small sample size (even in the case of standard 5 mm tubes) and to avoid reciprocal interferences, only one coil can be set at the minimum distance from the tube. This coil has the highest FF, thus the maximum sensitivity. When the inner coil is tuned to the X-channel, ("direct" design), the probe has the maximum sensitivity for the low-frequency X nuclei, and less sensitivity for ^{1}H. This is the probe of choice for direct ^{13}C and ^{15}N acquisition. On the other hand, the "inverse" design has the inner coil tuned to ^{1}H frequency (or to ^{1}H/^{19}F), thus achieving the maximum sensitivity for proton spectra and ^{1}H-detected nD spectra. Inverse probes are the standard choice for biomolecular NMR and metabolomics studies, including the first applications of "aerosolomics" [6]. Inverse probes have been used quite often by several research groups engaged in the characterization of atmospheric aerosols by means of 1D and 2D NMR techniques (e.g., Refs. [4,7,8]), although direct probes are still common (e.g., Ref. [9]).

A second approach to enhance mass sensitivity is to reduce the active volume of the sample by reducing its diameter. Within this framework, 3 mm NMR probes were developed, then 1.7 and 1 mm probe have been used from some years to record spectra of small molecules down to the nanomole range [10]. The main issue with such small diameters is the limited space where all the coils must be fit (at least 3 coils for deuterium lock, ^{1}H and X channel are required, but 4 coils are often needed for triple-resonance spectra of biomolecules), thus the theoretical gain due to higher concentration of the sample (when using the same amount of compound) is partially wiped out by a smaller FF and worst SNR of these probes. A 1.7 mm sample has a volume of about 60–65 μL, compared to the standard 500–700 μL of a 5 mm one. Albeit the gain in mass sensitivity should be about 8–10 times, the lower absolute SNR provided by the 1.7 mm probe reduces the gain in mass sensitivity to a factor of about 3. In spite of such potential, almost all current "atmospheric" NMR applications are based on standard 5 mm tubes. We could spot only one study targeting rainwater composition [11] and a second one dealing with secondary organic aerosol formation in laboratory conditions [12], in which 1.7 mm NMR tubes were adopted.

Attempts were also made by using 3 mm tubes within a 5 mm probe, with an enhancement up to 200% in mass sensitivity (compared to the theoretical 310% derived from the smaller volume of the sample). As a reward, the use of narrow NMR tube cushions the detrimental contribution of salts that are usually present in biological and environmental samples [13]. For the same task, shaped tubes are available to reduce the effects of salts on the pulse length and SNR [14]. In our best knowledge, such approach was never been adopted for atmospheric aerosol analysis.

The last source of noise within a probe head is due to the thermal noise along the signal pathway from the probe coils to the ADC converter. The thermal noise can be greatly reduced by reducing the operating temperature of all the electronic parts involved in the signal pathway, namely the probe coils and the pre-amplifiers. While the first spectra obtained with supercooled ("cold") probes were recorded in the middle of the 80's ([15,16]), the commercial implementation started in the last decade of the 20th century and the first installations started at the end of the century (for a technical review about cooled probes see Ref. [17]). Two designs of cold probes are actually available: the coils and the preamplifiers can be cooled to 77 K by the use of cheap liquid nitrogen, or they can be cooled down to ~20–25 K by the use of cooled helium. In the latter case the coils are usually made by films of high-temperature superconductors (HTS), and the performance are obviously better than the liquid N_2-cooled probe, but with substantially higher purchasing and management costs. At 600 MHz, the gain in SNR of a helium-cooled 5 mm inverse probe is about 5.5:1 with respect to a RT probe (in apolar solvents), while it is reduced to 2.5:1 when considering a N_2-cooled probe (data courtesy of Bruker Biospin). The gain in SNR is again reduced with highly polar solvents or salty samples, but this flaw can be greatly cushioned when using shaped NMR tubes [18] or by reducing the diameter of the sample to 3 mm or 1.7 mm. At present, only a few atmospheric aerosol NMR studies have reported analyses in cryoprobes ([8,19,20]).

2.2. Types of NMR tubes

When using standard NMR tubes, a non-negligible part of the solution is kept outside the active coil of the probe. This is required to avoid strong and localized changes in the magnetic susceptibility of the sample that would produce bad field homogeneity, thus unsymmetrical and broad spectral lines. A smart solution to this drawback has been solved some years ago by the Symmetrical NMR tubes developed by Shigemi (Shigemi Co, Ltd., Tokyo) where the bottom of the "outer" tube is filled by a glass plug whose susceptibility is matched with the solvent used for the experiment, and the "inner" tube has a tap that is again susceptibility matched (Fig. 1). The whole assembly allows to get a very good lineshape, with a reduction of the solvent volume to about 50% of the standard tube. Shigemi tubes are available also in 2.5 and 3 mm diameter, so the combination of reduced height and diameter of the active region can provide a gain in compound concentration by a factor of 4–5, hence a strong gain in SNR at the same TAT. Fig. 2 shows the spectra of two samples of biomass burning aerosols collected in a 10 m^3 reaction chamber, the FORTH-ACS (Patras, Greece [21]). While in field conditions, the sampling time can be prolonged virtually indefinitely until a sufficient amount of sample is collected, in simulation chambers the volume is fixed. In the past, this has limited the application of NMR techniques in chamber experiments to the very high concentration experiments. In more realistic concentration ranges (10–50 μg m^{-3}), the amount of sample available for collection on filters (~10–30 μg) is challenging even for the simple 1D ^{1}H NMR experiments. However, the use of Shigemi tubes, allowing for a better filling factor, can substantially improve SNR (upper panel in Fig. 2) with respect to traditional tubes (lower panel). These results demonstrate that substantial improvements can be achieved in the sensitivity of NMR analysis of atmospheric aerosol, enabling implementation in application fields, like in simulation chambers, in which sample load is a strong limiting factor.

As a conclusion, the combined use of cold probes with smaller sample diameters and susceptibility-compensated NMR tubes can greatly improve nowadays the mass sensitivity of NMR without the need for a substantially higher magnetic field. When considering a standard 5 mm direct probe at 600 MHz with room-temperature coils as reference for sensitivity, the gain is raised by a factor ot ~ 40 when using a 3 mm Shigemi tubes and an inverse detection 3 mm probe, with He-cooled coils. The switch to the actual cutting-edge field available (1.2 GHz) raises the gain to ≈100 times.

3. Methodologies for atmospheric sample preparation

Following the discussion in Part 1 of this review, the characterization of atmospheric aerosol samples by NMR spectroscopy requires the particulate matter collected on a substrate (fiber filters, filtering membranes or impactor plates) to be transferred into an NMR tube for analysis. For liquid NMR experiments, this implies a first extraction step with a suitable solvent, followed by the evaporation of the solvent by freeze-drying or in a rotary evaporator, redissolve the dried extract in a small amount of deuterated solvent [3]. The sample workup can include a derivatization step or, more frequently, a fractionation step aimed to reduce salt content or isolate a sub-sample in a medium suitable for other kinds of determinations (e.g. mass spectrometric analyses). In this section we deal with specific, crucial aspects of the NMR analytical protocols for atmospheric samples.

3.1. Solid-phase extraction and fractionation

The extraction of organic compounds from diluted water samples represents a common challenge in environmental analytical chemistry. This is particularly critical for sea water for which sample concentration under dryness is of no use for its high salt content. Solid-phase extraction (SPE) is therefore used for de-salting or for extracting organic compounds from a diluted solution into a smaller, concentrated liquid eluate avoiding the concentration step. SPE techniques are also common in several atmospheric applications, and especially in the workup of aerosol water extracts prior to organic analyses which can suffer from the interference of inorganic ions like FT-ICR mass spectrometry. The same eluates (often in methanol solution) can be employed to record NMR spectra and provide complementary chemical information with

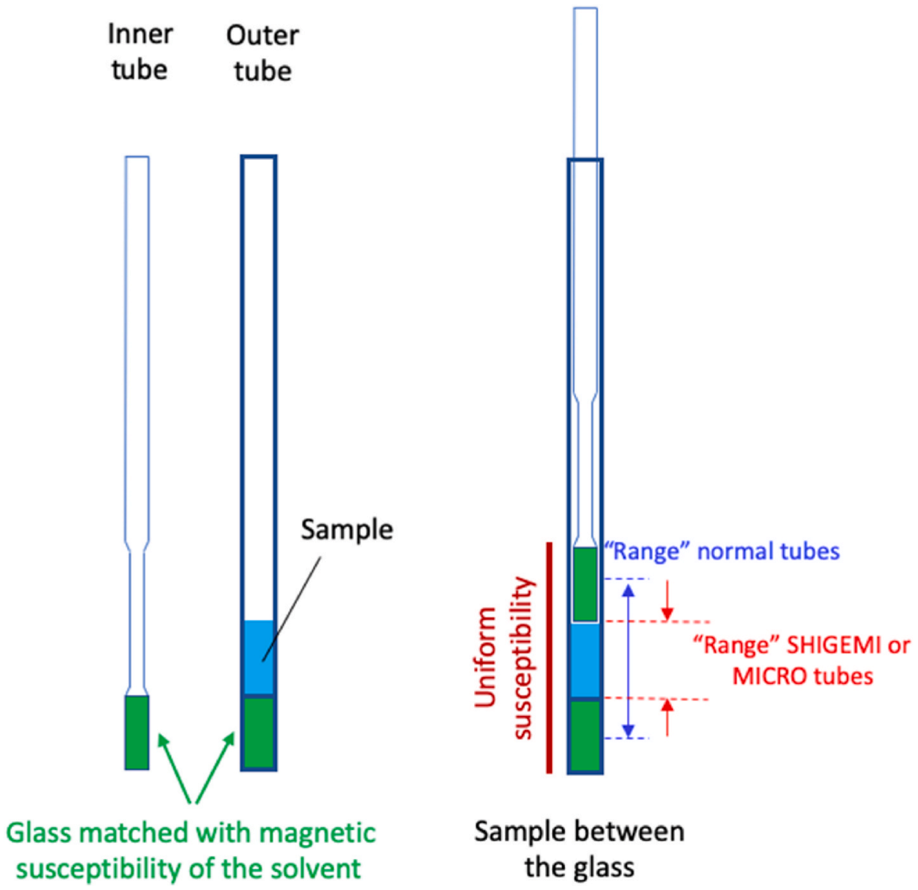

Fig. 1. Geometry of Shigemi tube (JEOL website, https://www.jeol.com/solutions/applications/details/NM200002E.php).

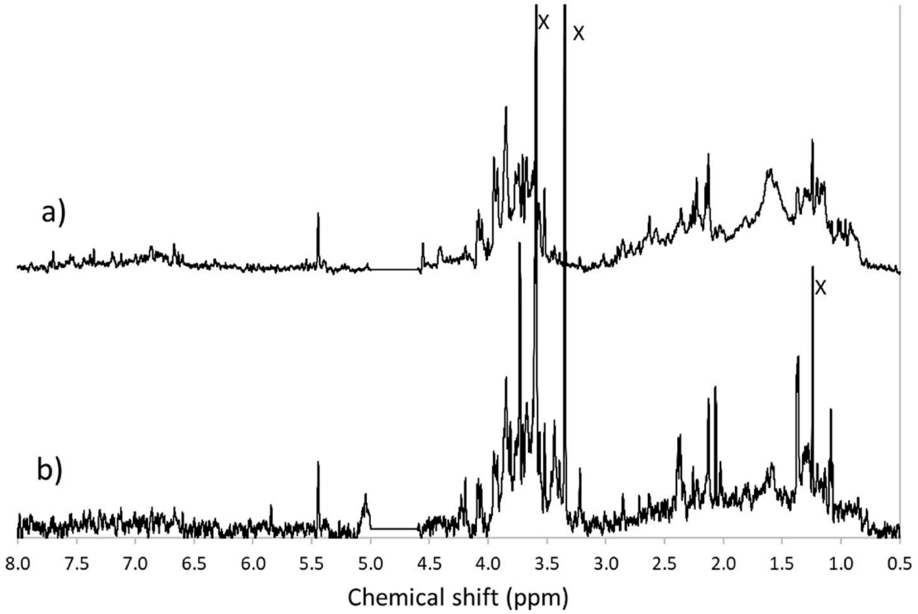

Fig. 2. Spectra of biomass burning aerosol produced in a 10 m^3 Teflon reaction chamber (FORTH-ASC, Patras, Greece) [21]. The aerosol concentrations in the chamber were about 20 μg m^{-3} and the sampling volume was ca. 0.5 m^3. The spectra were recorded at 600 MHz with a Varian Unity INOVA spectrometer a) in a Shigemi NMR tubes and b) in a traditional 5 mm tube. The peaks marked with "X" indicate contaminants. The aerosol samples were obtained during two distinct experiments performed in analogous conditions and producing similar amounts of aerosols, although the composition is not fully identical.

respect to high-resolution mass spectrometry [22]. The mixture of organic compounds recovered by the SPE cartridges is often referred to as "isolated organic matter", a definition which ignores the recovery of the SPE method toward specific classes of organic compounds. SPE recovery varies between adsorbing solid phases, but for the most commonly used types for atmospheric applications (OASIS HLB, C18, PPL, XAD-8, [23–27]) it is typically higher for relatively hydrophobic compounds and, when the pH is adjusted to ~2, for carboxylic acids. Since such techniques are adopted from protocols for isolation of humic substances, the isolated WSOC compounds are often termed "HULIS" (humic-like substances) [23] (Table 1). In contrast, strong organic acids, polyols and saccharides and amines typically exhibit low retention and are lost in the effluent [25]. In one study from Guangzhou (China) [28], 1D NMR spectroscopic analysis was carried out on unfractionated WSOC extracts of PM2.5 samples as well as on the eluates of SPE-isolates of the same extracts, showing that the SPE efficiency can be high (>60%) for OASIS HLB cartridges when elution is carried out using 2%-ammonia/methanol. The same study clearly shows that the SPE extraction can be selective, because the resonances in the range 3–5 ppm of the spectra of the unprocessed WSOC mixtures are only partially retained in the spectra of the SPE eluates of XAD-8 and OASIS HLB (when using 2%-ammonia/methanol as eluent) cartridges, indicating the complex mixtures of polyols and sugars present in the ambient WSOC samples are not quantitatively retained by the standard SPE methods. Follow-up studies ([27,29]) have confirmed a limited recovery of H–C–O -bearing organic compounds during SPE separation. Such effect, was already observed for XAD-8 resins by Ref. [30] employing ^{13}C NMR spectroscopy. Other examples of SPE isolation of WSOC fractions ("HULIS") using OASIS HLB or XAD-8 resins followed by NMR characterization are provided by Refs. [31–33]. To improve the recovery of strong acids, DEAE cartridges can be used. The main drawback of the employ of ionic exchangers such as DEAE for isolation is that they are unsuitable for desalting. A multi-step separation technique using a DEAE-based method was developed by Ref. [34], allowing the fractionation of aerosol WSOC into neutral/basic compounds (effluent), mono- and di-acids (retained on DEAE cartridges and eluted with a low ionic strength buffer) and poly-acids (or "HULIS", retained on DEAE cartridges and eluted with a high ionic strength buffer). The NMR characterization of the eluted fractions showed that the composition of the effluent is dominated by sugars and polyols, whereas the composition of the acidic fractions accounts for acyl and alkyl C–H groups and, in the case of poly-acids, aromatic C–H ([34,35]). In conclusion, only a few studies have attempted to characterize WSOC isolates by NMR spectroscopy, but these few ones agree on concluding that a large pool of compounds showing resonances in the chemical shift range of the polyols (H–C–O, 3–5 ppm) exist as a distinct chemical class with respect to aliphatic carboxylic acids showing bands in the ranges of alkylic (H–C, 0.5–2 ppm) and acylic (H–C–C=O, 2–3 ppm) groups. A further separation of mono- and di-acids from polyacids can be performed using weak-anion exchangers. Finer fractionation using preparative or semi-preparative liquid chromatography has never been attempted. The amount of the sample eluted out of the chromatographic column can be much less than the amount injected, and the identified components could be only a fraction of those eluted which becomes critical considering the inherent low sensitivity of NMR spectroscopy. To date, the only study employing semi-preparative HPLC separation with identification of aerosol organic compounds at the molecular level using NMR spectroscopy was carried out on extracts of secondary organic aerosol (SOA) samples produced in laboratory conditions [36].

Table 1

SPE stationary phase	Mobile phase	Recovery (% WSOC)	Chemical characterization	reference
DEAE	0.08–0.4 M Ammonium bicarbonate	52–73%	1D ^{1}H NMR spectroscopy	[34,35]
XAD-8	0.1 M NaOH	23%	1D^{13}C NMR spectroscopy	[30]
XAD-4, XAD-8	Methanol/ water (2:3; v/ v)	12–18% (XAD-4); 64–78% (XAD-8)	Elemental analysis, FT-IR, 1D^{13}C NMR spectroscopy	[26]
C18	methanol	60%	Several 1D & 2D NMR techniques, FT-ICR MS spectroscopy	[22]
ENVI-18, HLB, XAD-8, DEAE	40% methanol/ water (for XAD-8); methanol (for ENVI-18 and HLB), 2% (v/ v) ammonia/ methanol (for HLB), 1 M NaCl (for DEAE)	55% (ENVI-18), 51% (HLB-methanol), 68% (HLB – ammonia 2%), 53% (XAD-8), 32% (DEAE)	Elemental analysis, FT-IR, 1D ^{1}H NMR spectroscopy, thermochemolysis	[28]
HLB	methanol	45–66%	UV-VIS, FT-IR, 1D ^{1}H NMR, EEM fluorescence spectroscopy	[33]
HLB	methanol/ acetonitrile (1:1; v/v)	37–45%	UV-VIS, FT-IR, 1D ^{1}H NMR, XPS spectroscopy	[31]
HLB, PPL	methanol (for HLB and PPL), 2% (v/v) ammonia/ methanol (for HLB)	35–50% (HLB-methanol); 39–57% (HLB – ammonia 2%); 39–56% (PPL)	UV-VIS, 1D ^{1}H NMR spectroscopy, FT-ICR MS spectroscopy	[27]
HLB	methanol	32–65%	UV-VIS, 1D ^{1}H NMR, EEM fluorescence spectroscopy	[29]
HLB	methanol	43–60%	UV-VIS, FT-IR, 1D ^{1}H NMR, EEM fluorescence spectroscopy	[32]

NMR studies on isolated aerosol WSOC fractions ("HULIS") employing SPE (solid-phase extraction) techniques. HLB: OASIS hydrophilic/lipophilic balanced-resins; PPL: Bond Elut PPL (Priority PolLutant); DEAE: diethyl-aminoethyl- functionalized resins; XAD: Amberlite polymeric resins; ENVI-18 (Supelco): C18 polymerically bonded.

3.2. *Solvent extraction and liquid-state NMR spectroscopy*

The vast majority of NMR applications for atmospheric aerosol characterization relies on the use of solvents for sample extraction, laboratory workup (e.g., SPE fractionation) and analysis in an NMR tube. Although extraction in ultrapure water followed by vacuum-drying (or freeze-drying) of the extract and its re-dissolution in deuterated water represents the most common and straightforward protocol, other approaches have been explored. The extraction efficiency of hydrophobic acids can be improved by means of mild alkaline ([37,38]) or strong alkaline [39] aqueous solutions. Sample extraction with organic solvents is more challenging because their impurities become concentrated following vacuum-drying of the extract resulting in strong interference in the ^{1}H NMR analysis. Nevertheless [9], was able to record ^{1}H NMR spectra of pyridine-soluble aerosol organic matter (PSOM) in pyridine-D5 solution, while methanol-soluble organic matter (MSOM) was characterized in CD_3OD and $CDCl_3$ solutions by Refs. [29,40], respectively. The use of $CHCl_3$ for extraction coupled to NMR analysis in $CDCl_3$ was sometimes adopted in laboratory experiments such as in Ref. [41]. The characterization of PSOM and MSOM highlight chemical properties of aerosol compounds that are not accessible by means of water extraction. However, several overlaps must be expected with the composition of WSOM especially in the case of MSOM. To separate distinct solubility classes of aerosol organic compounds, more advanced protocols employing different solvent extractions applied in series must be adopted, the most complex of which was discussed in Ref. [42]. Simpler implementations consist in a first extraction in ultrapure water (for WSOM) followed by an extraction or the residues with organic solvents or alkaline solutions. Examples of this approach to characterize the water-insoluble organic matter (WIOM) are provided by the studies of [7,43] in which water-insoluble organic compounds are extracted in 0.1 NaOH and trifluoroacetic acid (TFA), respectively.

Beside the different solubilization properties, and the presence of impurities, the choice of an appropriate deuterated solvent for liquid NMR analysis must consider also the chemical shift range of the interfering peaks from residual protonated solvent molecules. Although the employ of solvent-suppression NMR sequences can successfully reduce the intensity of solvent peaks, it is often impractical to detect the resonances of the sample in the chemical shift window targeted by solvent suppression. The use of DMSO-D6 and acetonitrile-D3 causes residual protonated solvent peaks occurring at 2.6 and 2.1 ppm, respectively, in a range which is critical for the detection of the resonances of aerosol aliphatic carboxylic acids. Solvent peaks in deuterated water and methanol-D4 are found at 4.8 and 3.3 ppm, respectively, falling in chemical shift ranges less crowded of sample resonances. Methanol-D4 is therefore widely used for the analysis of WSOC extracts and isolates (“HULIS”) (e.g., Refs. [22,28]) also in virtue of its lower viscosity with respect to D_2O resulting in enhanced spectral resolution. Pyridine-D5 and $CDCl_3$ are example of NMR solvents with solvent peaks at high chemical shifts, interfering with the aromatic sample signals and not with the aliphatic ones. Finally, deuterated TFA exhibits a solvent peak in the very left end of the spectral window (>10 ppm), providing a wide unperturbed range for the sample resonances. However, TFA is an aggressive solvent which can cause hydrolysis reactions and other sample modifications. The hydrolysis of labile acetals and hemiacetals is often inevitable during sample extraction with water or other protic solvents. The use of methanol can also lead to transesterification reactions in the sample.

3.3. Use of buffered solutions

Liquid ^1H NMR analysis of environmental samples is affected by variations in solution ionic strength or pH. Even small variations in pH of the solution, indeed, can result in significant changes in the chemical shifts of weak acids and bases (e.g., carboxylic acids or amines). As the relative population of the protonated and deprotonated species vary with pH, the resulting shifts in the resonances signals pose a challenge to unequivocal molecular identification as well as to comparability between samples. This effect can be compensated for by the employ of a buffering agent in the NMR tube. This is a standard practice in many applications protein analysis [44] and particularly useful in biomedical metabolomics studies, in which a deuterated phosphate buffer is commonly used to maintain a physiological pH of 7.0–7.4 ([45,46]). Currently, the use of buffers in atmospheric aerosol NMR analysis is still very limited, in spite of the fact that the pH effect on the variability in the NMR resonances of atmospheric organic aerosols has been known for more than twenty years [47]. As atmospheric aerosols normally exhibit an acidic pH, phosphate buffers are not recommended. However, most buffers for acidic pH ranges are organic and interfere with the NMR analysis. Recent studies have employed a potassium deuterated-formate/formic-acid buffer to stabilize the pH to ~3.8 ([48, 49]). The residual protonated forms of formate and formic acid are responsible for a peak at 8.45 ppm of chemical shift, with only limited interference with the resonances of the sample. The use of pH buffers is particularly recommended in the elaboration of sets of ^1H NMR spectra by factor analysis and other multivariate statistical techniques for which a good alignment of the resonances between spectra is important.

Another approach to deal with the pH variability of the samples was used by Hornik et al. [[6,50]] and consisted in determining the pH of the individual samples according to the chemical shift of the peaks of formic and acetic acid and then processing them in Chenomx 8.0 (NMR Suite program, Edmonton, Alberta, Canada). Chemomx can compensate for the pH-dependent shifts. Since both formic acid and acetic acid are common constituents of atmospheric aerosol, they can be used as universal pH indicators in NMR aerosol analyses.

3.4. Chemical derivatization

Detection of ^1H resonances is, by far, the most important and widely exploited NMR spectroscopy in aerosol studies, as well as in most of other fields. Besides higher sensitivity, the constant presence of hydrogen in organic molecules makes this nucleus extremely useful in organic aerosol investigations, as previously discussed. Nevertheless, the final goal in organic chemistry is the to assess the presence and amount of different types of carbon atoms and their structural relations. Respect to this goal, scientists face the issue that some carbons are not directly bound to a hydrogen atom, and detection through ^1H NMR becomes indirect. This is of outstanding importance for some crucial functional groups, like the carbonyls of carboxylic acids, esters, ketones, that are the products of secondary oxidative processes in the atmosphere; and also substituted arylic, alkenylic and alkynylic carbons are missed in ^1H NMR. As we saw, direct carbon NMR resonance detection is challenging, although CP-MAS solid state investigation has experienced significant success. Indirect 2D spectroscopy, such as ^1H–^{13}C HMBC, is nowadays the most powerful technique and it has been successfully employed [22].

A different approach is based on the chemical derivatization [35]. Indeed, functional group transformation is a quite common technique in the analytical methodologies for organic compounds. For example, alcohols and carboxylic acids are usually transformed into their, respectively, trimethylsilyl ethers and esters to improve their gas-chromatographic behaviour; introduction of fluorescent moieties by derivatization is very useful to exploit very sensitive fluorescence detection in LC chromatography. In the derivatization technique developed by Ref. [35]the reaction with diazomethane $H_2C{=}N_2$ (CAUTION! Diazomethane is carcinogenic) is used to quantitatively and selectively convert carboxylic acids into methyl esters. In parallel, ketones can be transformed into the corresponding O-methyloximes by reaction with O-methylhydroxylamine. O–CH_3 singlets resulting from both treatments can be easily detected in the 3.5–4.5 ppm spectral region, and quantified respect to an internal standard, providing a direct overall assessment of the two functional groups, that cannot be obtained with different methods [51].

4. Methodologies for NMR data analysis

Atmospheric aerosol NMR spectra are most complex, involving large overlaps of resolved resonances with broad, unresolved bands arising from a myriad of organic compounds. There is not a standard methodology for the interpretation and analysis of such dataset. On the contrary, specific approaches have been transferred from NMR studies in parallel disciplines such as biogeochemistry and biology. In this section, we summarize the main approaches developed with a focus on 1D ^1H NMR datasets. The various methodologies are illustrated conceptually in Fig. 3.

4.1. Molecular speciation

NMR spectroscopy can be employed for identification and quantification of individual compounds in a mixture. Being NMR spectroscopy quasi-universal for organic compound analysis and its selectivity depending only on the compound molecular formula and largely not on instrumental and acquisition parameters, the technique can be ideal for non-targeted analysis of organic compounds in ambient matrices. However, NMR applications to atmospheric molecular speciation are much more limited with respect to more selective and sensitive MS-based methods. The slow pace at which NMR applications have progressed in the field is partly due to the challenges of resolving complex resonances with the 1D techniques. Further challenges are posed by molecular identification in absence of relevant atmospheric standards. For both aspects, significant progress was achieved in the recent years. Most importantly, just as in metabolomics, modern software for spectral

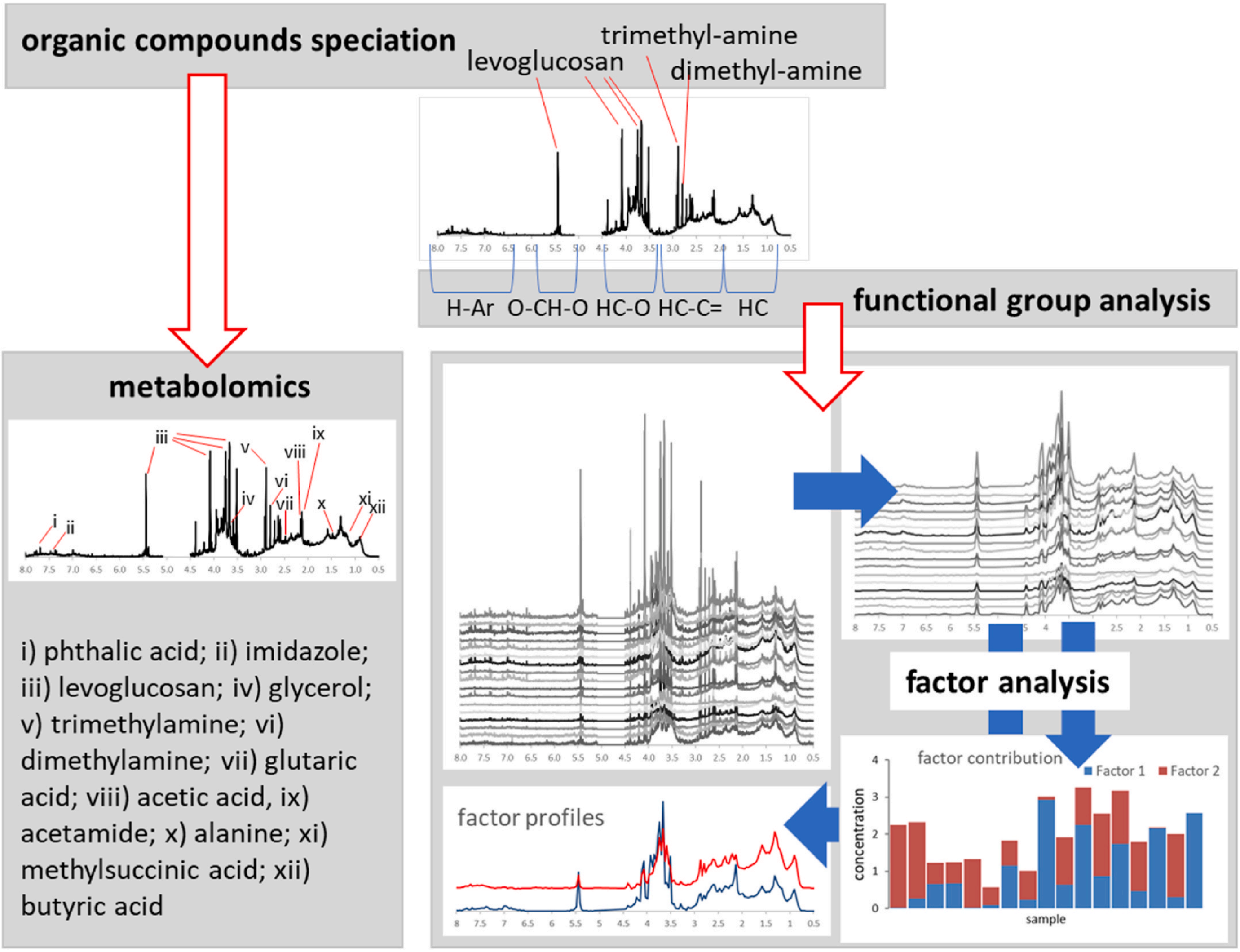

Fig. 3. Approaches to the elaboration of atmospheric aerosol NMR datasets. From the left: elaboration of individual spectra can involve the attribution of resolved resonances to specific organic compounds (normally through the comparison with the spectra of standard compounds) or the determination of major functionalities by integrating the main broad prominent unresolved NMR bands. The availability of large chemical databases together with the application of modern software for metabolomics enables the elucidation of complex overlapping resonances and the identification of a greater number of individual compounds. This approach is more powerful when applied to a set of samples. The entire set can be elaborated by multivariate statistical analysis like factor analysis (normally performed on binned spectra of reduced resolution) leading to spectral deconvolution into a few spectral profiles with varying contributions across samples.

deconvolution and peak fitting can turn particularly useful in extracting valuable chemometric information from 1D ^{1}H NMR spectra of ambient organic aerosols containing hundreds of overlapping resonances. The first application of such “aerosolomics” was shown by Ref. [6]: the authors were able to identify and quantify about 60 distinct organic species, with a relevant gain with respect to the traditional approaches (12 species in Ref. [47]). The potential of metabolomic approaches is conditioned by two factors: 1) the need of good SNR that allows to detect small multiplet signals in a potentially noisy spectrum; 2) the representativeness of available libraries with respect to the great diversity of atmospheric organic compounds. It is a matter of fact that current applications of aerosolomics have targeted low-molecular weight compounds like C1–C3 alkyl-amines, C3–C8 alkanoic acids and dicarboxylic acids, phthalic acid, formic and acetic acids, or classes of compounds which are common classes of biomarkers like saccharides, sugar-alcohols, amino acids and phenols [50]. The metabolomic software used in these studies, Chenomx 8.0, employs libraries which essentially miss all the specific tracers of atmospheric secondary organic sources, such as methyl-tetrols and all the products of monoterpenes oxidation in the atmosphere. In recent years, an increasing amount of new NMR data on such specific atmospheric organic tracers has been reported in the literature (see Part 1 of this review). Sharing the NMR spectra of secondary aerosol organic tracers and including them in metabolomics NMR libraries would greatly expand the application of NMR spectroscopy in organic aerosol molecular speciation studies.

4.2. *Functional group analysis*

The prominence of the broad, unresolved resonances in 1D NMR spectra of the extracts of ambient particulate matter poses different challenges in data analysis and interpretation with respect to the case of molecular speciation techniques. The problem of environmental samples showing broad, unresolved spectral features in virtue of their molecular complexity is not new, and it is in fact common in biogeochemistry where high-field spectroscopic techniques were successfully employed to gain structural information about complex mixtures of organic compounds eluding molecular speciation [52]. While high-resolution MS techniques like Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry can be used to explore the distribution of the stoichiometric rations between C, H and heteroatoms across thousands of individual molecular formulas, NMR spectroscopy provides information on the chemical environment of C, H (and more rarely N and P) atoms and especially on their short-range chemical order. When complexity is extreme, however, the overlap between the NMR resonances is typically very large. In the classical studies about NMR characterization of terrestrial and aquatic humic substances, for instance, 1D H NMR spectroscopy is mainly used to identify and quantify about four major organic functionalities bearing non-exchangeable protons: aromatic and alkenilyc (Ar–H or = CH) at 6.0–8.5 ppm, alkoxyl (HC–O) at 3.3–5.0 ppm, acyl and benzyl (HC(=O)- or Ar-CH) at 1.9–3.3 ppm and alkyl (-CH) at 0.7–1.9 ppm, regardless of the fine structure of the individual compounds underlying each region. The higher the overall molecular complexity, the larger the overlaps between the chemical shift intervals of such groups, but in general all four functionalities show a distinct maximum in spectral intensity and so it is often observed in atmospheric samples spectra, as well [34]. Aerosol samples show additional contributions besides the four CHxOy functionalities above, like amines and sulfonates (sometimes collectively called HC-X, where X is a heteroatom different from oxygen) in the δ_H range 2.2–3.5 ppm. The resonances of amines and MSA can sometimes be integrated separately and subtracted from the bands of alkoxyls and acyls.

Functional group analysis represents the most widespread approach used in NMR atmospheric aerosol studies. As discussed in Part 1 of this review, this approach turned particularly informative for source attribution of aerosol WSOC. A part of the possible inconsistencies between such studies in the protocols for deriving carbon concentrations for the specific functional groups, the main limitation of this approach stands in the limited ability of ^{1}H NMR spectroscopy to derive concentrations of carbonyls, carboxylic acids and esters. Even if an estimate of aliphatic (C=O) bearing functionalities can be derived from the H–C groups in alpha position ($\underline{H}$C–C=O), the stoichiometry of such structures is highly uncertain, and it is possible that increasing functionalization can eventually reduce the measured concentrations of $\underline{H}$C-C=O groups. To achieve a direct determination of carbonyls and carboxylic groups by ^{1}H NMR spectroscopy, derivatization techniques must be employed (see section 3.4) but at the cost of a considerable additional sample workup and sophistication in the analysis.

4.3. PCA and FACTOR ANALYSIS

NMR functional group analysis is based on the analogy between atmospheric aerosol organic matter and humic substances. Nevertheless, differences between atmospheric humic-like substances (HULIS) and aquatic and terrestrial humic and fulvic acids have been documented [53]. In respect to the spectroscopic properties, the 1D H NMR spectra of atmospheric WSOC are often much richer of features that can be resolved by high magnetic field instruments although not necessarily at the molecular level. Reducing such complexity down to four main functionalities means ignoring a large fraction of the information available in the spectra. At the same time, most of this information is carried by complex resonances with uncertain attribution to specific chemical structure, impeding the use of the functional group analysis method. The problem of managing high-resolution spectroscopic information without any clear molecular-level identification is common to methodologies other than NMR like many mass spectrometric techniques, including the most successful technique developed for aerosol observations, the AMS. Factor analysis for reducing the number of variables and for spectral deconvolution has played a role of primary importance in the AMS spectral analysis, leading to innovative methods for source apportionment of organic compounds in the aerosol ([54, 55]). Factor analysis in 1D H NMR spectroscopy can follow the same approach, in analogy with the protocols developed for offline AMS applications when the number of samples is much smaller with respect to the (standard) online methodologies [56]. While AMS-based factor analysis methodologies have become a standard in organic aerosol source apportionment, the NMR-based methods are still under development and they are better suited to support the AMS approach and characterize source types for which the AMS provide an ambiguous identification. In fact, NMR factor analysis was often performed in conjunction with parallel source apportionment by quadruple AMS (Q-AMS) or high-resolution time-of-flight AMS (HR-ToF-AMS), and at least in one case also in parallel with organic characterization by single-particle aerosol time-of-flight mass spectrometry (ATOFMS) or thermal desorption aerosol (TAG) GC/MS. The most used factor analysis techniques for NMR datasets are Principal Component Analysis (PCA), Positive Matrix Factorization (PMF) [57] and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) [58]. PCA is especially suitable for dimensionality reduction and more in general for exploratory data analysis and for identifying the number of factors explaining the largest fraction of variance in a dataset. PMF and MCR-ALS include the most common NMR spectral unmixing techniques in several chemometric applications [58]. They are free from the orthogonality and normality constraints of PCA and are non-negative factor analysis methods, making them suitable for spectral deconvolution and source apportionment applications. Since PMF explicitly accounts for the uncertainty in the data, it often yields more accurate results than traditional factor analysis methods. MCR-ALS can be a suitable alternative to PMF in absence of the uncertainty matrix and the results from the two techniques were shown to compare well in some NMR aerosol source apportionment studies [59]. In all these techniques, prior to factor analysis and in order to remove spurious sources of variability, the original NMR spectra are subjected to several pre-processing steps. A polynomial fit is usually applied to correct the baselines. Careful horizontal alignment of the spectra is performed using the reference standard singlet (Tsp-d4 in D_2O solution) and other known signals (e.g, MSA or MeOH singlets at 2.8 and 3.35 ppm, respectively) as reference positions. Blank signals, corresponding to impurities of filters or solvent or ambient contaminations are removed. The spectral regions containing only sparse signals (chemical shift $<$0.5 ppm or $>$ 8.5 ppm) or under the disturbance of the solvent peak (HDO; 4.7 $<$ chemical shift $<$5.2 ppm) are omitted from the data set. Binning over a range between 0.02 and 0.03 ppm of chemical shift intervals is then needed to remove the effects of peak position variability caused by matrix effects. Low-resolution (from 200 up to 400 points) spectra are finally obtained and processed by factor analysis.

In the recent years the application of PMF using the ME-2 solver [57], controlled within the Source Finder Igor-based software package (SoFi, Datalystica inc [60], used in Igor Pro, WaveMetrics) enhances the possible control of the users over the factor solutions, allowing the possibility to impose “constrains” such as specific factor profiles corresponding to expected sources in a known environment. This approach, that applied on AMS datasets demonstrated a very good ability to separate specific sources in environments characterized by very mixed emissions and processes [61], is promising also for NMR-based organic source apportionment whenever source-specific reference spectra are let available. This calls for more experimental research in simulation chambers and other laboratory setups for improving the NMR libraries of reference spectra.

5. Conclusions

In more than two decades of atmospheric research with NMR spectroscopy, several techniques have been explored but a few (specifically 1D ^{1}H NMR methods) have gained relatively extended application. It should be noted that the best-developed techniques are based on offline analysis in solution state, with no suitable methodology for characterizing the total organic aerosol: organic fractions are rather obtained for NMR analysis following extraction in a suitable solvent and possible further steps of sample preparation, desalting and fractionation. 2D heteronuclear techniques are already in place in many NMR studies and are expected to improve their performance, opening a clearer window to the detection of ^{13}C, today, and of ^{15}N in the next future.

It is high time to exploit the enormous progress achieved in the last decade in the fields of metabolomics and protein analysis. First examples of metabolomics applied to aerosol characterization ("aerosolomics") have appeared in the literature, highlighting the potential of modern software tools for NMR spectral data analysis for extracting structural information from the great complexity of resonances which are typically encountered in atmospheric aerosol NMR spectra. Aerosolomics can represent, therefore, an emerging analytical methodology for organic aerosol molecular speciation, provided that good sensitivity can be achieved.

Although NMR spectroscopy has an inherent limited sensitivity with respect to mass spectroscopy, new probes equipment, including cryoprobes, can greatly improve the signal-to-noise ratio in NMR analysis of H, C and heteroatoms. Filling factor is another key parameter in the analysis of aerosol samples, in which the small sample load often represents a bottleneck. In this regard, innovative NMR tubes allows for a better filling factor and enhanced signal-to-noise ratios. Such technical improvements have already allowed to extend NMR characterization to laboratory secondary organic aerosol studies at realistic atmospheric concentrations in which the available sampling loads (<20 µg) were excessively small for the traditional approaches. The combination of inverse probes, helium-cooled coils and NMR tubes with a high filling factor can enhance NMR sensitivity of a factor of 40. To achieve higher gains in sensitivity, cutting-edge field spectrometers (1.2 GHz) must be employed.

Several innovative NMR methodologies remain unexplored in atmospheric aerosol research. For instance, spin hyperpolarization techniques involve manipulating the nuclear spin polarization of a sample to achieve a higher degree of alignment of the nuclear spins, resulting in stronger NMR signals with respect to the Bolzmann distribution achieved in the magnetic field [62]. at the best of our knowledge there are no data obtained on environmental sample taking advantage by spin hyperpolarization.

As the NMR research field is rapidly reaching very high levels of sophistication, an efficient cooperation between NMR experts and atmospheric scientists will be of paramount importance, as well as the creation of an NMR research community devoted to compare protocols for spectra acquisition and data analysis, constructing new libraries of reference spectra and explore new methodologies for NMR aerosol characterization.

CRediT authorship contribution statement

Emilio Tagliavini: Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing. **Stefano Decesari:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Marco Paglione:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Andrea Mazzanti:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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