

Monitoring anthropogenic particles in the environment: recent developments and remaining challenges at the forefront of analytical methods

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Abbreviations

3D – 3 dimensional

AF4 – asymmetric flow field flow fractionation

AFM – atomic-force microscopy

BSE – back scattered electron

CCD – charged couple device

CFFF – centrifugal or sedimentation field flow fractionation

CryoTEM – cryogenic transmission electron microscopy

DLS – dynamic light scattering

DLSI – dispersive light scattering image

EDS/EDX – energy dispersive x-ray spectroscopy

ENM - engineered nanomaterials

FAST – fast acquisition speed technique

FFF - field flow fractionation

FOQELS – fiber optic quasi-elastic light scattering

FPA – focal plane array

FTIR – fourier transformed infrared

HF-FFF – hollow fiber field flow fractionation

m-PTA – multispectral particle tracking analysis

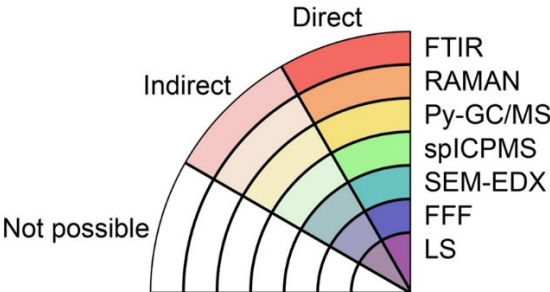
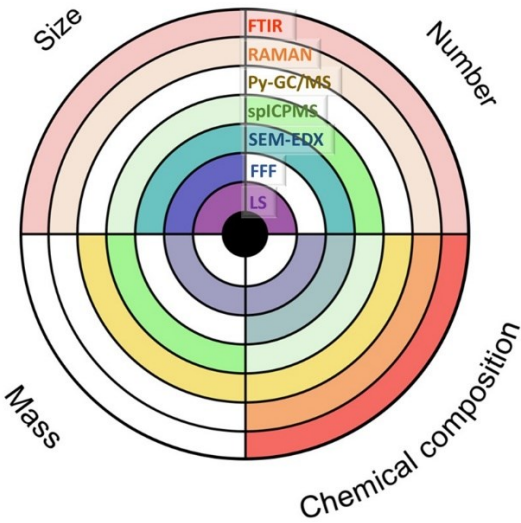
MALS – multi-angle or static light scattering

MAPNTA – maximum a posteriori nanoparticles tracking analysis

NIST – National Institute of Standards and Technology
NP – nanoparticles
NTA – nanoparticle tracking analysis
PE – polyethylene
ppb – part per billion
PS – polystyrene
PTA – particle tracking analysis
Py-GCMS – pyrolysis gas chromatography mass spectroscopy
QCL - quantum cascade laser systems
S-SNOM - scattering-type near-field optical microscope
SE – secondary electrons
SEM – scanning electron microscopy
SERS – surface enhanced Raman scattering
siMPLE – systematic identification of microplastic in the environment
SNOM – scanning near-field optical microscope
SP-DLS – single particle dynamic light scattering
spICPMS single particles inductively coupled mass spectroscopy
SRS – stimulated Raman scattering microscopy
STEM – scanning transmission electron microscopy
TEM – transmission electron microscopy
TRWP – tyre and road wear particles
UV-vis – ultraviolet visible

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30 Graphical abstract
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Abstract

Anthropogenic particles at the micro- and nano-scale are posing risks to human health and the ecosystem. Engineered nanomaterials, micro- and nano-plastics, soot, road and tire wear are a few prominent examples of particles that are either intentionally manufactured or incidentally produced and released into the environment. Analytical developments in the past few decades have made possible to study particles in the micro- and nanoscale, however there is still no universal protocol of analysis and caveats exist in the use of the most prominent techniques. The task is challenging due to the large variety of particle properties and the complexity of environmental media. This review discusses a selected group of techniques most likely to play a key role in future monitoring activities and discuss recent developments and inherent shortcomings.

49 1. Introduction

50 Anthropogenic particles are a broad category of particles manufactured (engineered) or
51 incidentally produced by human activities. These particles can be released either intentionally
52 or unintentionally into the environment, potentially causing adverse health effects and
53 ecosystem impairment. This category of particles includes engineered nanoparticles, plastic
54 particles (micro and nano), soot, paint flakes, tire and road wear particles (TRWP), as well as
55 other combustion and friction particulate byproducts. Many of them are known to cause health
56 effects and/or contain toxic compounds.

57 Anthropogenic particles (organic and inorganic) can be found in a wide range of sizes and those
58 on the micro and nano scales are currently receiving attention from society and governments
59 around the world due to the increased awareness of their existence and potential harmful
60 consequences. Microlitter generally refers to particles debris ranging from 1 μm up to 5 mm in
61 length, whereas environmental nanoplastic is defined as plastic particles between 1 nm and
62 1000 nm [1]. However, engineered nanomaterials are typically defined as solids with at least
63 one dimension smaller than 100 nm, while it has been proposed that a threshold of 30 nm ought
64 to be used instead, since novel functionalities become prevalent at this size range [2]. Here, the
65 term “nanoparticles” is used to describe anthropogenic particles with size $< 1\mu\text{m}$, including
66 both nanoplastic and engineered nanomaterials. Regardless of size categorization, both
67 anthropogenic micro- and nanoparticles have been detected in several environmental
68 compartments, such as air [3], freshwater and marine water bodies [4,5], soils [6], marine
69 sediments [7], aquatic organisms [8], as well as food and food packaging [9,10] and in the
70 human body [11]. Overall, the number of particles found in the environment increases
71 exponentially as particle size decreases [12] and it is the smaller size fractions that are more
72 likely to induce adverse effects, owing to their reactivity, ability to pass through biological
73 membranes (e.g. organ tissue) [13], transport behavior, and potential to be mistaken for food
74 by organisms. Towards the smaller particle size, the particle analysis is arguably more complex
75 with several analytical challenges ranging from sampling to particles characterization.

76 Since anthropogenic particles vary greatly in terms of composition, size and morphology, there
77 is no single analytical method that covers these features entirely, and different analytical
78 techniques are applied to assess these pollutants in the environment. The scientific community
79 has been developing analytical methods aiming mainly at specific categories of particles and
80 applying different analytical techniques to address the multiple scientific questions regarding
81 the anthropogenic particles and their effects. In general, these methods apply frontier techniques
82 to overcome several challenges posed by the particle’s features, mainly size limitation. To
83 address the multiple scientific questions related to the occurrence, environmental fate and
84 toxicity of anthropogenic particles, there have been advancements in analytical methods and
85 techniques, particularly with respect to throughput analysis and lower limits of detection for
86 particle size and mass or number concentration.

87 This paper provides a review of the most used analytical techniques for separation,
88 characterization, and quantitation of anthropogenic micro- and nanoparticles. These techniques
89 fulfill one or more of the following criteria and are considered fitting for a broad range of
90 applications: (i) parameters measured are relevant to characterize and/or determine particle
91 abundance (number of particles, size distribution, composition, total mass and mass per
92 particle); (ii) versatility in application to environmental compartments and particle
93 characteristics (e.g. organic or inorganic); (iii) limits of detection that are relevant for
94 environmental studies. (iv) technology maturity and instrument availability; (v) ease of use and
95 established algorithms for data interpretation. The techniques included in this review are

Fourier-transformed infrared spectroscopy, Raman spectroscopy, pyrolysis gas chromatography mass spectrometry, single particle inductively coupled mass spectrometry, electron microscopy, and field flow fractionation with light scattering. In the following sections, an overview of each technique is presented, followed by a discussion of specific analytical challenges and limitations, recent advances (~5 years), and new directions. We address the qualitative and quantitative aspects of each technique along with challenges related to sample preparation and treatment, interferences and artefacts, and data analysis and interpretation. It is intended that this review serve as a basis for addressing challenges in producing quality data for monitoring activities and investigations on the abundance, fate, and toxicity of anthropogenic particles, which are urgently needed in risk assessment and regulatory efforts.

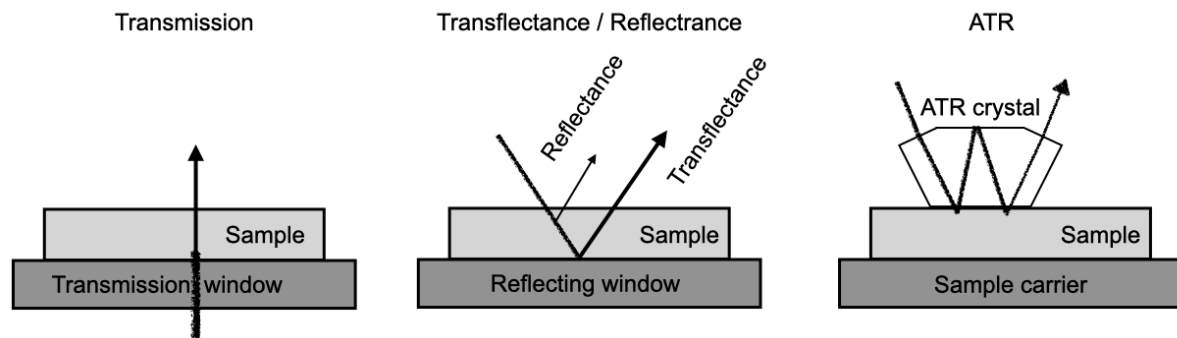
2. Vibrational spectroscopy

2.1 Fourier-transformed infrared spectroscopy

Fourier-transformed infrared (FTIR) spectroscopy is one of the most common techniques used for the characterization of microplastics in the environment. This technique measures the transition of molecular vibration states by the absorption of infrared radiation, in which the photon energy is transferred to the molecules, changing their vibrational state. For a molecule to absorb infrared radiation there must be a variation in the dipole moment due to its vibrational or rotational movements, such as the polymeric materials [14]. As a result, characteristic spectra profiles of the analyzed material are obtained with fast and nondestructive measurements, requiring little or no sample preparation [15,16]. Infrared instruments can be combined with optical microscopes (μ -FTIR) for hyperspectral image acquisition, simultaneously collecting chemical and spatial information of several particles at the same time by automated sample mapping [14]. Hyperspectral images bring several advantages for microplastic analysis, where a sample after purification can be filtered directly on a membrane (transparent at infrared range) for mapping, allowing the characterization and quantification of microplastic particles and the evaluation of their morphological features (size distribution, area, etc.) without manual sorting.

Similarly to conventional infrared instruments, infrared microscopes are flexible and different measurement modes (Figure 1) can be applied for spectra acquisition, e.g. transmittance, reflectance and attenuated total reflectance (μ -ATR) [16]. Each measurement mode should be applied according to the particles' features and size, as summarized in Table 1. ATR measurements provide the best spatial resolution and the reflective crystal must be in full contact with the particle to produce desirable spectra [17]. Therefore, particles smaller than the reflective crystal should be avoided in μ -ATR mode. Transmittance is well suited for sample mapping and it is the most common approach used for microplastic analysis. In this mode, the source light is transmitted through the sample and particles thicker than 300 μ m provide weak or no analytical signal due to total light absorption [16]. On the other hand, this limitation can be overcome when reflectance mode is used. However, this measurement mode depends on the particle morphology; there can be light reflection errors due to the light scattering and samples with a wide range of particle sizes can face challenges in defining the camera focus for quality spectra measurements [15].

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139 **Figure 1.** Overview of the measurement modes applied to μ -infrared spectroscopy. Figure
 140 adapted from Ref. [16].

141 Hyperspectral image measurements can be time consuming, mostly depending on the detector
 142 applied in the instrument, such as single point or focal plane array (FPA). Unlike single-element
 143 detectors, FPA-based imaging uses several detectors placed in a grid pattern for area mapping,
 144 improving the measurement time and analysis throughput [18]. Even faster analysis can be
 145 obtained with recently developed Quantum Cascade Laser systems (QCL), where an FPA-
 146 based instrument is combined with high brilliance infrared sources based on broadly tunable
 147 external cavity quantum cascade lasers. Although this system also analyzes larger areas with
 148 14 times more pixels than a high-end μ -FTIR instrument, measurements are often limited to the
 149 fingerprint region, in which important peaks for polymer identification are missing, such as the
 150 CH and OH signals in the range from 3600 to 2750 cm^{-1} [19].

151 Most of the μ -FTIR instruments can reach small pixel sizes ($\sim 1 \mu\text{m}$) and detailed information
 152 about small particles can be obtained. However, it should be noted that the smallest detectable
 153 particle size in infrared microscopy is determined by the microscope diffraction limit (e.g. 10
 154 μm at 1000 cm^{-1}), regardless of the pixels size applied [17]. For infrared analysis of particles
 155 below the diffraction limit, more advanced techniques must be applied such as nano-FTIR,
 156 which is a combination of a scattering-type near-field optical microscope (s-SNOM) with a
 157 broadband infrared source [20,21]. This technique is based on atomic-force microscopy (AFM),
 158 where a probe tip is approached to the sample, creating a nano-focus for surface measurement
 159 with a spatial resolution that depends on the radius of the cantilever tip, which is up to 20 nm
 160 [22]. Nano-FTIR has been demonstrated for nanoplastic analysis, and further advancement and
 161 investigation of this developing technology needs to be explored [20].

162 Infrared microscopy has been successfully applied for microplastic analysis with several
 163 publications worldwide applying this technique for analysis of this anthropogenic litter in
 164 sediment [23–25], water [26,27] and biota [9,28] samples. Although the feasibility of this
 165 technique has been demonstrated, the analysis protocol needs to be harmonized for more
 166 accurate data comparability, such as the filters applied to reduce interferences and sample
 167 pretreatment to avoid organic matter and biofilm that can hide the polymer signal.

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169 2.2. Raman Spectroscopy

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Raman spectroscopy is a technique based on the inelastic scattering from monochromatic light, such as laser light. As light interacts with the sample, photons are absorbed and re-emitted with a shifted frequency. The shift provides information about vibrational, rotational, and low-frequency transitions in molecules, resulting from changes in their dipole moment while absorbing radiation. Micro-Raman spectroscopy is the combination of Raman spectroscopy with optical microscopes, making it possible to focus the excitation light on a small spot [17]. However, nowadays, most Raman microscopes are confocal, which gives a weaker Raman signal but provides a higher depth resolution and image contrast. Raman is a single particle method, which simultaneously provides chemical information of each measured particle, as well as its size and morphology. The analysis can be done in two ways, imaging, or particle measurement. For imaging measurements, the entire substrate or a section is scanned, and a spectrum is received from each image pixel. For particle measurements, the analysis is done in two steps, first an optical image is acquired with a light microscope and then the spectra are acquired where particles are detected. Both methods are three-dimensional with two spatial dimensions and one spectral dimension.

The main advantage of Raman microscopy is the detection limit, around 1 μm , in addition to not being sensitive to water signal as infrared spectroscopy [19]. A limitation with Raman spectroscopy is the weak signal it provides, where less than one in a million excitation photons give rise to a single Raman photon [29]. This problem can be overcome applying longer integration time. However, longer exposure can degrade the sample, and this should be done carefully. Raman spectra may have problems with cosmic ray events generated when high-energy particles pass through the detector and generate many electrons that the detector interprets as a signal. However, by recording more than one spectrum, the problem will be neglected. Additionally, Raman spectra can suffer from a fluorescence background, which can be more than six orders of magnitude higher than the efficiency of the Raman interactions. To reduce the fluorescence signal, it is possible to change the excitation wavelength or reduce the detection volume. There is less fluorescence using a confocal Raman since it only collects photons emitted from the focal plane [30]. Impurities and organic matter can also cause fluorescence and can be reduced by cleaning the particles before the analysis. The cleaning procedure might degrade the plastics and must be done carefully.

The intensity of the Raman scattering is proportional to ν^4 , where ν is the frequency of the laser. Shorter wavelengths lead to higher Raman signals, but many samples show strong fluorescence when excited with shorter wavelengths such as 400 nm [30]. Shorter excitation wavelengths give higher lateral resolution ($0.61 \cdot \lambda/\text{NA}$), where λ is the laser wavelength, and NA is the numerical aperture, showing that both factors are equally important. The Raman signal is proportional to excitation power; however, the excitation power can cause thermal decomposition because of absorption. Other common problems with Raman spectroscopy are interfering signals from the underlying substrate, additives, fillers, dyes, or coloring agents that can affect the spectra (Figure 2).

Recently, Raman microscopy has been used to identify microplastic particles in environmental samples such as in wastewater, seawater [31], sediment [32,33], mineral water [34], drinking water [35] and air [3]. In general, two workflows have been used: (i) visual identification where a subset of suspected microplastic particles has been moved and identified with Raman and (ii) direct particle identification on filters of different substrates such as polycarbonate or silicon filter [31]. The first workflow is not optimal since smaller particles are hard to visually identify and it is not possible to move them with tweezers. It also relies on visual identification and there can be many false negatives, i.e., particles that are not visually identified as plastics but are

plastics. The second workflow works for smaller particles and it is possible to identify all particles, however, often only a subset of the filters is analyzed to reduce running time.

Raman spectroscopy can also be used for analysis of single molecules on solid surfaces by applying surface-enhanced Raman scattering (SERS). Molecules are adsorbed onto a metal surface, which enhances their Raman scattering by factors of up to 10^8 [36]. SERS has therefore been applied to detect metallic nanoparticles, however challenges pertaining to high variability, interferences from sample matrix and impurities, and the presence of “SERS hotspots” dominating the signal [37]. A few studies have used SERS to identify plastic particles in the nanometer size range in environment matrices such as seawater [38] and river water [39], as well as atmospheric particles [40]. Another Raman approach is stimulated Raman scattering microscopy (SRS), which is a technique where two laser beams are used, one that has a higher photon energy and one with a lower photon energy. It is faster than confocal Raman microscopy since it only measures signals at single wavenumber and it is less sensitive for fluorescence. However, since it only measures one wavelength, identification of different polymers is difficult. For instance, Zada *et al.* [41] used six wavenumbers to be able to identify five polymer types.

Confocal Raman microscopy has been gained more information about the samples such as Raman-AFM [42], Raman-SNOM [43] and Raman-SEM [44]. Raman tweezers, a combination of optical tweezers with Raman spectroscopy, has been used to identify micro- and nano-plastics in solution [45], and can further be combined with field flow fractionation (FFF) to identify size-fractionated polymers and inorganic particles between 200 nm and 5 μm in diameter [46]. Nevertheless, Raman imaging has been used to identify plastic particles down to 100 nm [47–49].

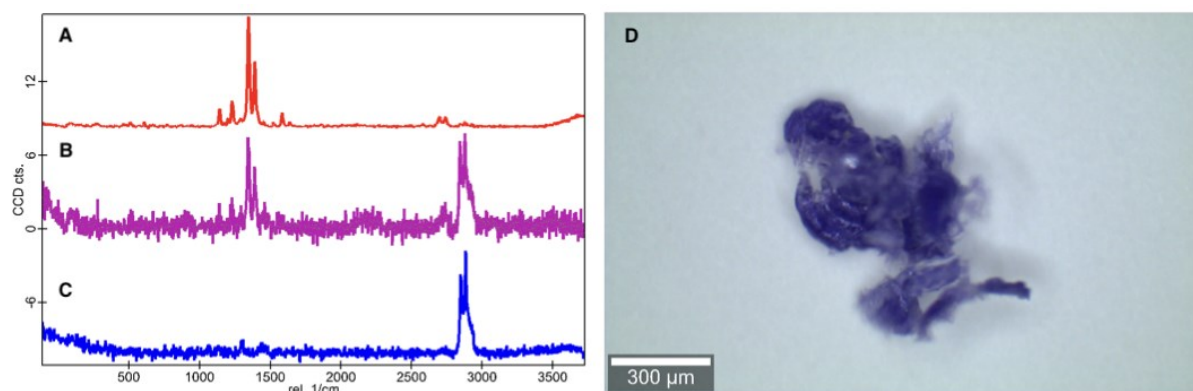


Figure 2: Confocal Raman microscopy spectra (A-C) and image (D). Spectra showing (A) mainly the dye, (B) both dye and polymer (C) only polymer.

2.3 Raman and FTIR data analysis

Raman and FTIR are complementary vibrational spectroscopies that have great similarity in the way their data are processed. In general, the data set for both techniques is complex and quite large when hyperspectral images are obtained. For microplastic analysis, where these techniques are massively applied, the most common data processing pipeline is the use of library search and multivariate data analysis for particle characterization based on the spectra information. Library search is currently the most common strategy applied for both Raman and

μ -FTIR. This approach compares the sample spectra with a reference library for matching and assigns the spectra when the similarity surpasses a given threshold [18,24,50]. Pearson's Correlation or Euclidian distance commonly determines the spectral similarity and the results often only rely on this index, which is a potential source of error due to its dependence on the spectral library and the threshold applied. Despite the fact that the spectral match depends heavily on the reference library comprehensiveness, this straightforward approach does not require an expert operator to acquire the information [15]. This analysis may last from a few seconds to several hours and depends on the size of the data set, since all the spectra must be compared with all the reference spectra in the matching routine. These reference spectra libraries can be purchased or customized, with specific spectra libraries already published for microplastic analysis using Raman [51] and μ -FTIR spectroscopies [52], as well as dedicated software for this purpose, such as siMPLe software [53].

Alternatively, multivariate data analysis (chemometrics) is well suited to retrieve the microplastic information for these complex data, with their feasibility already demonstrated by several publications in the literature. The typical multivariate analysis procedure consists of: (1) data processing to eliminate spectral interferences [16]; (2) exploratory analysis to identify patterns in the dataset [54] and; (3) modeling to develop statistic models to retrieve the plastic information based in the representative sample spectra [15,55–57]. Chemometric approaches are validated, and a statistical evaluation of the models are performed to reduce bias and increase confidence in the particles/spectra assignment. This analysis strategy is faster than the traditional library search with results obtained in a few minutes once the classification models are developed, and morphological information about the particles is possibly obtained. The use of chemometrics for image analysis is well established; however, it needs to be further explored for microplastic analysis, to better understand and extract as much information as possible from these complex data and provide more accurate results and robust statistical models for high throughput analysis.

3. Pyrolysis gas chromatography mass spectrometry

In pyrolysis - gas chromatography mass spectrometry (Py-GCMS), the samples are thermally decomposed in a pyrolysis step, followed by separation of the degradation products using gas chromatography, ionization, and further fragmentation, and finally detection of the ionized fragments using mass spectrometry (Figure 3). Py-GCMS is commonly used to identify and determine the mass concentrations of anthropogenic particles such as plastics and TRWP. Identification of the thermal degradation products provides structural and compositional information about the particles in the sample and allows the determination of mass concentrations of specific compounds per unit of mass or volume of the sample.

Multiple Py-GCMS approaches have been used in the analysis of plastic particles and TRWP. These involve various thermal programs or configurations of the pyrolysis step, such as single-shot Py-GC/MS (single temperature, > 500°C) [58–60], sequential Py-GCMS (variable temperature, from low to high) [61], thermal desorption pyrolysis (concentration of degradation products onto adsorbent media, followed by release through heating of adsorbent) [62] and thermochemolytic Py-GCMS (allows chemical reaction to occur which makes the detection and identification of degradation products easier) [63].

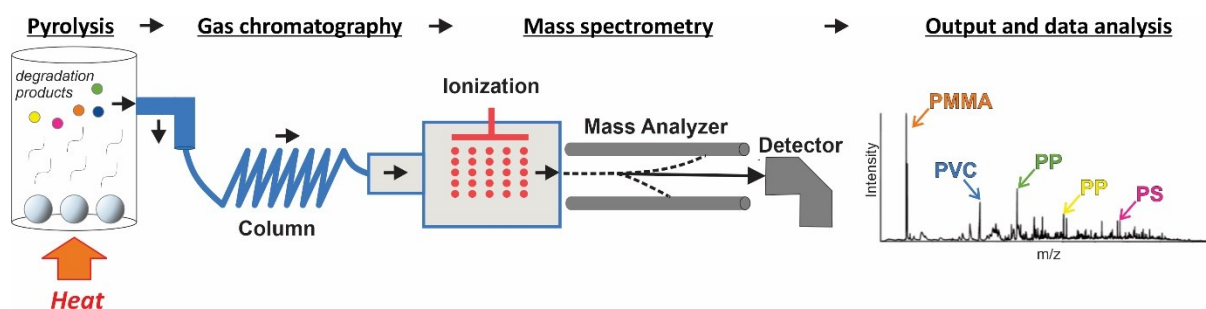


Figure 3. Schematic of pyrolysis-GC/MS for analysis of plastic particles

Py-GCMS is a complementary technique to vibrational spectroscopies, which in addition to providing information on sample composition, delivers quantitative information on the mass of specific polymers, from which the polymer mass concentration per unit mass (e.g., per g sediment) or volume (e.g., per liter water) can be calculated. This is in contrast to FTIR and Raman, which can provide particle number concentration only. The ability to provide mass data is a strength of Py-GCMS. Mass data are critical for investigations of fate and transport as well as exposure and toxicity, such as in mass balance approaches for studying transformations and bioaccumulation and the development of reference doses which are given in units of g per kg body weight. In addition, Py-GCMS analyzes all particles at both nano- and micro-scale, providing a more accurate understanding of the particle composition and structural complexity, including differentiation between plastic polymers and organic additives. However, unlike vibrational spectroscopies, Py-GCMS is a destructive technique, and the sample cannot be re-analyzed in case of non-successful data collection.

Py-GCMS has successfully been used for the analysis of micro- and nanoplastics and TRWP in a variety of environmental and biological media, such as sediments, soils, air, surface runoff, suspended particulate matter, sewage sludge and aquatic organisms [60,64–66]. Py-GCMS has been used for the analysis of single and bulk plastic particles which first have been isolated from the sample media (e.g. [61]), as well as for the direct quantification of plastics without particle isolation, such as through the use of accelerated solvent extraction to extract polymers from the sample matrix (e.g. [67],). With respect to particle size and mass limits, polymer mass detection limits below 1 μg have been achieved, with the mass detection limit as a function of the polymer type [58]. The minimum size in which a single particle can be analyzed is limited by the feasibility of isolating and transferring it to the sampler system. The smallest single particle which has been analyzed using Py-GCMS had a diameter of 500 μm [68,69]. The smallest bulk particles which have been analyzed thus far are nanoplastic particles with diameter < 1 μm [70].

A major challenge in Py-GCMS is the identification of polymers in complex environmental and biological matrices. The matrix complexity can result in high background signals and interferences from natural organic matter (e.g., naturally occurring proteins, fats, polysaccharides) with structures similar to the polymer degradation products and their ionization fragments. Sample processing methods for the concentration of polymers and removal of potential matrix interferences and background signals have been employed to improve the signal to noise ratio and lower mass detection limits. This includes: sample digestion followed by polymer preconcentration [63], pressurized liquid extraction onto silica gel [60], and pre-cleaning steps to remove natural organic matter from the particle surface, for example, by means of methanol extraction, enzymatic and/or oxidative decomposition.

However, sample pre-treatment procedures that are too aggressive can result in alteration of the particle prior to analysis [63,71–75].

Additionally, methods for improved data analysis and interpretation of pyrograms which increase the certainty of polymer and/or additive identification are currently being explored, such as the development of optimized Py-GCMS methods for specific polymers/additives; the identification of common interferences from various sample matrices; principal components analysis to identify specific polymer indicators; selected/single ion monitoring to compensate for background effects; and methods of reducing artefact formation, such as the use of deuterated internal standards [76]. A comprehensive, freely accessible shared database for thermal degradation and ionization products and polymer indicator ions (e.g., Tsuge et al. (2011) [77], NIST and Wiley mass spectra libraries) would be tremendously helpful for Py-GCMS users. To date, only a limited number of plastic polymers and additives have been identified, suggesting that this technique needs to be further explored. Future development of this method can benefit from advances in miniaturization of mass spectrometers, with portable mass spectrometry becoming relatively common in air quality monitoring [78]. A custom portable Py-GCMS has recently been developed by Zhang et al. and holds promise for providing rapid, in-field identification and mass quantification of microplastics [79], e.g. for marine sampling and monitoring.

4. Single particle inductively coupled plasma mass spectroscopy

Single particle inductively coupled mass spectroscopy (spICPMS) has evolved as a premium method for quantifying particles in complex aquatic matrices and one of the very few methods that may provide both number concentration and size distribution of particles in suspension. ICPMS instruments are used for the quantification of the majority of elements in the periodic table of elements, based on the mass-to-charge ratio. Prior to its application in particle analysis, ICPMS has been used for decades in the determination of metals at trace concentrations in acid-dissolved samples, due to its high sensitivity and elemental selectivity. Because of its wide use in trace metal analysis, the technology is well established and is readily available for spICPMS analysis, although some software and hardware upgrades may be necessary for some models. Briefly, the liquid sample is mixed with argon gas at the tip of a nebulizer to produce a spray of droplets. Larger droplets are removed in a spray chamber while finer droplets are directed to a plasma torch (6000 – 8000 Kelvin), where the sample is desolvated, vaporized, atomized, and ionized. The charged atoms are then separated under an electric field, based on their mass to charge ratio, and detected. When a particle enters the plasma torch, a plume of ionized atoms is generated, which is detected as a spike (particle event) over the background signal (Figure 4). The number of spikes detected is used to derive the number concentration of particles in the suspension, while the area under the particle event is used to derive the amount of analyte in single particles, which is then translated to particle size. Two critical conditions ought to be met: (i) the particle number concentration needs to be low enough to reduce the chance of more than one particles entering the plasma at the same time and (ii) the data reading frequency needs to be high enough to allow identification of the particle event over the background signal. For a detailed review of the method and its applications prior to 2016, the reader is directed to an exhaustive review by Montano et al. in 2016 [80].

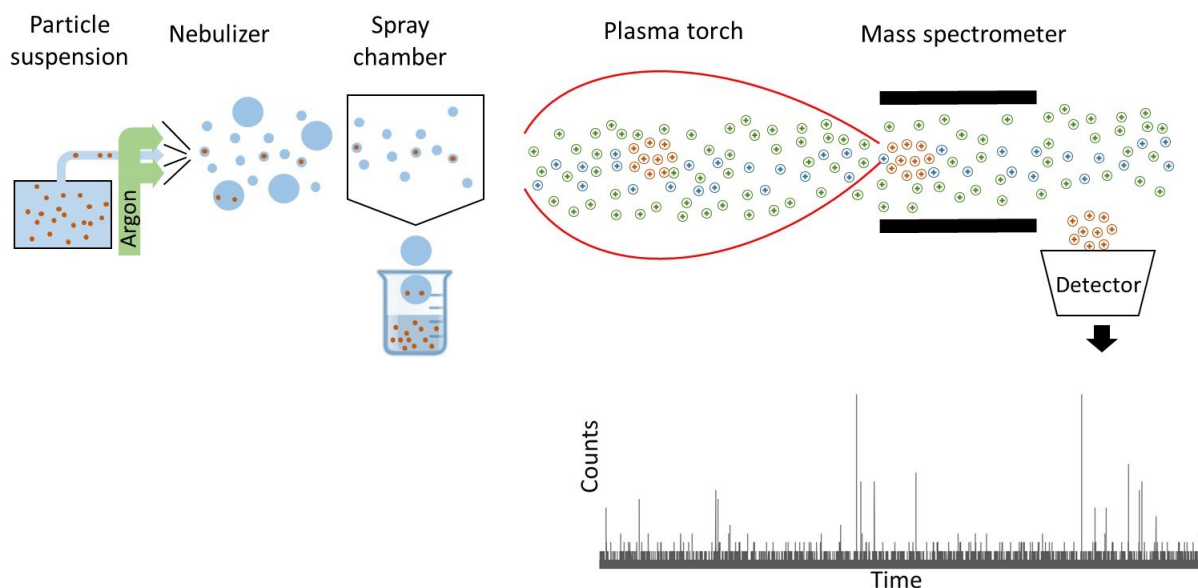


Figure 4. Schematic representation of particle analysis with spICPMS

The technique has gained wide acceptance due to several key advantages: (i) high sensitivity that allows particles as small as 5 nm to be measured [81]; (ii) high selectivity, meaning that particles containing elements of interest are readily quantified; (iii) high through-put allows the analysis of a substantial number of samples within a reasonable timeframe and; (iv) readily available equipment, owing to the use of ICPMS for trace metal analysis. In addition, the parameters measured, i.e. particle number concentration and size distribution are key for the appropriate characterization of a particle suspension. However, keeping in mind that what is measured is the amount of one element during a particle event, the quantification of these parameters is challenged by analytical limitations and a complex data processing routine. An obvious challenge is the assumptions necessary to convert the analyte mass to size of a particle that is likely to contain other analytes, some of which may be impossible to measure with this technique (e.g. light elements such as oxygen and hydrogen). For this conversion, particle composition, density, and shape must be known. When these parameters have not been determined using complementary techniques, arbitrary assumptions are made, typically using the composition of the most common mineral of the target element, the density of the bulk mineral, and spherical shape.

Recent studies have improved the accuracy of particle size calculation from analyte mass, by improving the sample introduction system or using advanced instrumentation. Hyphenated methods are emerging as powerful tools with applications in complex matrices. Among them, asymmetric flow field flow fractionation (AF4) with its high-resolution size fractionation is used to force particles to enter the spICPMS in an orderly fashion, based on their size and shape. The method also removes dissolved components and concentrates particles of the same size, thus enabling the detectability of dilute samples with high dissolved background [82,83]. Hydrodynamic chromatography has also been applied for the determination of gold nanoparticles and their agglomeration state in gastrointestinal fluids [84] and the method has been validated for nanoscale liposomes [85]. Capillary electrophoresis also has been applied as a high through-put hyphenation technique with spICPMS for the detection of 10 nm gold nanoparticles [86]. Another approach to reduce uncertainties in size calculation is to utilize advanced instrumentation to quantify more than one element in single particles, thus acquiring

more complete information about their composition. A time-of-flight mass spectrometer enhanced with machine learning algorithms was used to distinguish naturally occurring from engineered cerium oxide nanoparticles, taking into account that natural particles are likely to contain other elements as “impurities” compared to their pristine engineered counterparts [87]. When hyphenated with AF4, a holistic set of data is acquired on the particle composition and structure and is probably the most advanced technique for the characterization of a nanoparticle suspension to date [88].

Another challenge for spICPMS is the detection of nanoparticles in samples with elevated background noise, which may be a result of dissolved components of the target analyte or interferences on the measured isotope. When the background noise is elevated, the intensity of spikes generated by small particles is challenging to distinguish from the fluctuating background signal, thus resulting in increased limits of detection. As already mentioned, hyphenation with AF4 will remove the dissolved background to a large extent. Due to the wide use of ICPMS in trace analysis, technical solutions developed to address polyatomic or isotopic interferences on the target analyte are also available for spICPMS. Sector field instruments utilize a magnetic field in addition to the electrostatic field, thus achieving high resolution between isotopes; in tandem ICPMS instruments, a collision and/or reaction gas mixture can be used to eliminate interferences [89,90]. A third approach to address the challenges imposed by elevated background is data treatment, where a threshold of 3 up to 7 times the standard deviation of the background is applied to separate background (below the threshold) and particle spikes (above). An improvement of the traditional threshold method is the deconvolution of the background from particle signals using a mixed Polygaussian probability mass function [91]. Furthermore, by increasing the data acquisition frequency (FAST analysis), i.e. reducing the duration of signal acquisition window (dwell time), the background signal is reduced (by division in more dwell times) making particle events more prominent. Recently, a deconvolution method has been applied in combination with short dwell times to detect 20 to 100 nm silver nanoparticles in the presence of ppb levels of dissolved silver ions [92].

Until recently, spICPMS was not applied to measure carbon-rich particles, such as micro- and nano-plastics, due to the high ionization potential of carbon, which results in low ionization efficiency at the argon plasma. In addition, the background of ^{12}C (the most abundant isotope of carbon) is very high in aqueous samples. However, when the ^{13}C isotope is measured and the particle is large enough, carbon from particles can be detected above the background, as demonstrated for 1 – 5 μm spherical polystyrene microbeads dispersed in ultrapure water, i.e. without background from dissolved organic compounds [93,94]. Another approach for indirectly measuring plastic particles in the nanoscale has been demonstrated, using functionalized gold-containing nanoparticles, whose coating selectively adsorbs on carboxyl groups on the surface of nanoplastics; the metal from the conjugated nanoparticles is then detected with spICPMS [95].

In addition to the particle characteristics (composition, density, shape) and the background level, analysis with spICPMS is often hampered by the low concentration of nanoparticles in suspension and the multitude of organic and inorganic materials present, especially in environmental samples. To address this challenge, particle concentration and extraction techniques and novel sample introduction systems have been recently applied. A colloid extraction technique using surfactants [96] was able to extract all particles, irrespective of size or composition, and concentrate them in a smaller volume while maintaining particle integrity. A flow injection system was applied to dilute seawater samples, while minimizing the risk of altering particle stability [97]. Furthermore, enzymatic and chemical treatment has been applied

to decompose organic and inorganic material in complex matrices, thus simplifying the analytical task for spICPMS [98,99].

The spray chamber is a critical part of the sample introduction system and is used to protect the plasma torch from large droplets that would otherwise absorb heat and cause fluctuations in the plasma temperature, even causing the flame to extinguish. As a result of the droplet size discrimination, approximately 10% of the sample reaches the torch (the term transport efficiency is used to correct this effect), while the rest is discarded in the waste. Larger particles are less likely to survive the spray chamber, thus adding bias to the determination of the particle size distribution and number concentration. To address this issue, novel nebulization systems have been developed with direct injection of the sample, assisted by heating or gas displacement, achieving close to 100% transport efficiencies [100–104]. To accurately determine the transport efficiency, standard nanoparticle suspensions are necessary. However, these standards are not available for most elements, they are often not adequately characterized, and are prone to changes with time (losses on sample vials and pipette tips, gradual dissolution and/or aggregation, etc.). A microdroplet generator used to produce droplets of precisely defined size has been proposed to bypass the need for standard suspensions. Using solutions that contain pre-defined concentrations of dissolved components, it is possible to matrix-match a sample and conduct a calibration for the instrument response to the mass of analyte [105]. An alternative sample introduction system with application on solid samples is with laser ablation, with promising preliminary results on the achieved transport efficiency [106].

Overall, the analytical advantage of spICPMS is demonstrated by the wide range of its applicability in complex matrices, such as biological fluids [107], food [108], drinking water [109], surface waters [110], waste leachates [111], soil extracts [112], and plant extracts [113], among others. Identifying the most suitable components in terms of sample handling, sample introduction system, instrumentation, and data analysis algorithm depends on the target nanoparticles' physicochemical characteristics and the matrix [114]. It should be kept in mind, however, that the amount of analyte in each particle event may in fact originate from a particle comprised of various mineral forms (e.g. mix of iron oxides, crystalline and amorphous), a pure element (metallic silver or copper) or ions adsorbed on a larger particle (e.g. arsenic ions adsorbed on iron oxide particles). Distinguishing between these sources requires advanced instrumentation and possibly complementary techniques. Furthermore, data analysis is a complex process due to handling of large data files and the challenging separation of particles and background signals. Further hardware and software advancements are needed in these directions. Although the development of the micro-droplet generator offers an alternative, standard materials are a simple and cost-effective solution for calculating transport efficiency and calibrating the method, so further development in standard materials is also necessary.

5. Electron microscopy

Electron microscopy is a powerful tool for acquiring morphological information, such as size, shape, surface topography, and aggregate structure, in addition to qualitative and semi-quantitative elemental composition and crystal structure. The technique resembles the traditional light microscope instruments, but with substantially higher resolution, owing to the use of electrons instead of light. Similar to light microscopy, a sample is placed on a flat surface and areas of interest are magnified and examined. The electron beam is directed onto the sample and, through its interactions with the sample material, the particles morphology, composition, and crystal structure may be acquired. In transmission electron microscopy (TEM), a parallel electron beam is used, which penetrates the sample. High resolution morphological information can be obtained on particles up to 1 μm in thickness, while composition and crystal structure

may be obtained on particles up to 0.1 μm [115]. Although the accuracy and specificity of TEM are advantageous, the method is rarely used to characterize a population of particles in complex samples with an abundance of particles of diverse compositions and dimensions. The reason is that locating particles of interest is a time and effort consuming process, making it impossible to examine more than a few hundreds of particles per sample within a reasonable timeframe. Manually searching for particles of interest introduces operator bias, therefore TEM is often used as a complementary technique to verify the presence of target particles. In combination with bulk elemental analysis of filtered fractions, it was used to visualize inorganic nanoparticles in waste leachates [116]. A scanning unit can be installed to a TEM instrument (STEM), which resembles the operation of a scanning electron microscope (SEM). In SEM, a convergent electron beam is used for scanning areas in a raster motion. Several detectors are installed to capture electrons and x-rays following the interaction of the beam with the sample material. Secondary electron (SE) detectors may be used for high resolution topographical information, backscattered electron (BSE) detectors for elemental contrast, and energy dispersive x-ray spectroscopy (EDS or EDX) detectors for elemental composition. TEM detectors for higher resolution images and quantitative EDX analysis (STEM) may also be installed, but only on very thin samples (up to 1 μm). SEM is more appealing for application with environmental samples owing to the possibility of semi-automatic procedures supported by image analysis tools, which may be used to acquire morphological characteristics and qualitative particle composition, but not for quantitative composition and crystallography [115,117].

Analysis of inorganic particles with SEM-EDX is possible when particles are deposited on a surface, which is a challenging task to accomplish without introducing artefacts. Alternatively, CryoTEM may be used, but this is an expensive and time-consuming solution that is typically avoided. A particle suspension is deposited on a surface, e.g. TEM grid, tape or membrane, and is allowed to dry. The water removed during drying reduces the distance between particles, thus enhancing the formation of agglomerates which were not present in suspension. An artificial corona on particles may also appear during sample preparation, due to changes in ion concentrations and pH during drying [118]. One solution is to remove the suspension, by means of a lint-free wipe, before the liquid volume is significantly reduced by drying. However, only the particles that attach on the surface during this time are analyzed further. An alternative is to add a suitable stabilizer in the suspension that prevents particle agglomeration [119]. Recently, the application of cloud point extraction treatment of environmental water samples prior to TEM-EDX analysis has shown promising results for metal-containing nanoparticles [120].

High vacuum is applied in the sample chamber to maintain stability of the electron beam, however under these conditions non-conductive samples will cause charging effects, thus distorting the acquired image. A conductive layer by means of sputtering is therefore applied on non-conductive materials. Because this layer is often not uniformly applied and will contribute to the EDX compositional analysis, an alternative is to conduct the analysis under variable pressure conditions, which is a promising tool for environmental and biological research [121]. The compositional information acquired with EDX is qualitative since the intensity of the detected characteristic x-rays depends on the interaction volume of the electron beam with the particle material. In turn, the interaction volume depends on the energy of the electron beam and the dimensions of the object examined. On smaller particles, the electrons will penetrate the particle and the support underneath, thus producing mixed signals from both the particle and support. Using STEM, it is possible to acquire semi-quantitative information on particles with thickness (dimension parallel to the electron beam) less than 1 μm .

Plastic particles may be analyzed with SEM-EDX, producing morphological and compositional information. The concentration of carbon is qualitatively determined; however, it is not possible to identify the type of polymer. SEM has been used to determine changes on the surface of plastic particles caused by weathering under environmentally relevant conditions [122]. Concentrations of human exposure to microplastic particles from bottled mineral water have been calculated using analysis with SEM-EDX [123]. The particles containing carbon were assumed to be plastics, however, since mineral water contains inorganic and organic particles as well, which also contain carbon (e.g. carbonate salts, microorganisms and their residues), their identification is questionable [124]. Moreover, studies that have used SEM to verify plastic particle concentrations based on topographical contrast and elemental composition derived number concentrations of questionable validity, since no determination of polymer structure was made to verify these results [123,125].

6. Particle tracking analysis and dynamic light scattering

Particle tracking analysis (PTA) and dynamic light scattering (DLS) are two often complementary quantitative techniques which are used to determine the hydrodynamic diameter of micron- and nanometer-sized particles (≈ 1 to 10^3 nm) in synthetic and filtered surface water samples [70,126–128]. PTA and DLS theory are based on the movement of particles suspended in a fluid as a result of the Brownian motion of the surrounding fluid molecules. In PTA, particles in the sample are inundated with light at a single wavelength and the movement of particles is visualized and tracked using a charged coupled device (CCD) camera. The hydrodynamic particle diameter is then calculated based on a relationship between particle size and the diffusion coefficient, typically using the Stokes-Einstein equation for spherical particles. DLS, on the other hand, measures the intensity of the light scattered by the suspended particles over time (10s to 100s of seconds). An autocorrelation function representing the rate of fluctuation of the intensity of the scattered light (due to particle motion) as a function of time is processed to obtain the diffusion coefficient (Brownian motion) of the particles and thus the particle diameter. With respect to additional particle characterization, DLS analyzers are often coupled with either an electrophoretic light scattering mode to measure particle electrophoretic mobility and zeta potential or a static light scattering mode to measure the radius of gyration, while PTA can quantify the particle number concentration, along with qualitative surface properties resulting from the interaction of the particle surface with light.

A major limitation of DLS is that smaller size fractions often remain undetected due to relatively higher light scattering by larger particles. As a bulk or ensemble measurement, conventional DLS is more sensitive to larger particles that can obscure the scattering signal of smaller particles in the population (Figure 5a), and hence intensity-weighted size distributions are most immediately derived from the autocorrelation function. On the other hand, PTA tracks single particles and is comparatively more sensitive to particles at the lower end of the size range, producing number-weighted size distributions (Figure 5b); however, there is room for improvement in PTA in terms of the detection limit (currently ~ 10 nm) and susceptibility to visualization artefacts [126,129]. Hence, DLS is better suited to monodisperse rather than polydisperse particle size distributions, while PTA performs well with monodisperse particle size distributions and better than DLS with polydisperse particle size distributions, though it still suffers from scattering errors in polydisperse suspensions [129]. A prefiltration step is often necessary to remove background signal from colloids in environmental samples. Another limitation is that there is an optimum range for the intensity of the scattered light that restricts the usable particle concentration range; the sample scattering must be sufficiently high to provide good signal-to-noise ratio [130] (e.g., for a background scattering of 10 kcps, sample

scattering of > 20 kilo counts per second (kcps) or higher would be required). Neither technique performs well with highly concentrated particle suspensions, where particle interactions can influence the particle motion, and so sample dilution is often required.

To address the challenge of accurate particle size measurements in polydisperse samples, multiple approaches have been developed. For example, algorithms which deconvolute autocorrelation functions produced by polydisperse samples (e.g., CONTIN, CORENN) [131,132] and statistical models (e.g., MApNTA) [133] can be used to identify multiple particle size distributions in the same particle suspension. Data can also be averaged over shortened time intervals to identify, remove, and/or differentially analyze “unwanted” size fractions such as large dust impurities or aggregated particles [134]. Finally, multispectral PTA (m-PTA) utilizes several incident light wavelengths and allows for improved sensitivity of polydisperse particle distributions [129]. To address the challenge of size measurements for highly concentrated samples, approaches such as modulated 3D cross-correlation DLS [135,136], fiber optic quasi-elastic light scattering (FOQELS) [137,138], and ultrasonic attenuation spectroscopy [139–141] have been developed or are under development. Differences in the intensity of scattered light of aggregated particles and natural components using PTA have been utilized to study aggregation kinetics of nanoparticles in landfill leachates and natural seawater [142,143].

New directions in the particle sizing field include the use of single-particle DLS (SP-DLS) to identify the shapes of individual particles [144], though this technique is in its infancy. Image-based DLS (DLSI) [145], in which the intensity of the scattered light is imaged over time and in multiple space-dimensions, reduces the time required to obtain data compared to conventional DLS and improves the resolution of conventional DLS. DLSI has the potential to be applied to real-time flow through systems, possibly even *in situ* size characterization. The hyphenation of DLS with separation techniques such as field flow fractionation (FFF) has also been performed and provides a wider particle size range of detection.

7. Field flow fractionation

Field - flow fractionation (FFF) is a chromatographic method that is capable of separating macromolecular and particulate species, typically by size (Figure 5c) [146]. In FFF, the sample is injected into the FFF channel, and an applied force pushes the particles toward an “accumulation wall”. Particles equilibrate at different distances from the accumulation wall depending on their properties or interaction with the applied force. The particles are then eluted under laminar flow pattern, with particles further from the accumulation wall experiencing higher flowrates and hence eluting earlier. The applied force can be established by a variety of means. Most typically, fluid cross flow (termed flow FFF) is applied using an ultrafiltration membrane as the accumulation wall. Flow FFF can further be categorized by the channel and cross flow configuration, with asymmetric flow FFF (AF4) performed with the membrane at the bottom of a flat channel, and hollow fiber FFF (HF-FFF) using a tubular membrane with radial cross flow. In flow FFF, particles are separated by their diffusion rate toward the center of the channel, with smaller particles residing further from the accumulation wall and eluting faster. Centrifugal FFF (termed centrifugal or sedimentation FFF, or CFFF) can also be used to separate particles by their buoyant force (size and density). Potential advantages of FFF over other size separation methods include the gentle nature of the separation and capability for separation over a continuous size distribution (as opposed to discrete size cutoffs). Coupling FFF with online detectors then produces size-resolved characterization of the nanoparticles.

Recent advances in FFF for nanoparticle analysis in environmental samples address challenges in both the *separation/sizing* and *compositional analysis*.

Environmental samples covering a wide size distribution pose challenges in FFF separation. For example, in AF4, the cross flow is typically held fixed, allowing optimal separation across only a relatively narrow size range, e.g. 1-100 nm. Recent studies have achieved separation across a broader size range by employing a variable cross flow profile, as demonstrated on carbon-based aggregates and nanoplastics in AF4 (separation across 1 to 800 nm) [147] and PS nanoparticles in frit inlet AF4 (separation across 50 nm to $\sim 1 \mu\text{m}$) [148]. Challenges remain in extending the size separation range beyond $1 \mu\text{m}$. Above this size range, a transition from normal to “steric” elution mode occurs, where the large size of the particle prevents approach to the accumulation wall, such that larger particles experience faster flow rates and elute more quickly rather than more slowly. Hence, reliable size separation will not be achieved in polydisperse samples with sizes spanning the normal and steric elution modes, and pre-separation, for example by centrifugation, would be required before AF4 analysis [149–151]. As the elution time can also be affected by the shape of the particle [152–154] and interactions with the accumulation wall, coupling FFF with online DLS or collecting fractions for offline characterization (e.g., by AFM [153], TEM [155], or SEM [156]) is important to directly measure sizes and shapes. Furthermore, the use of both online DLS and multi-angle or static light scattering (MALS or SLS) to compare the hydrodynamic radius and radius of gyration yields a shape factor that can be used to distinguish solid spheres from other structures (e.g., rods, core-shell structures, etc.) [157–161]. However, care must be taken to avoid errors in online DLS analysis if the particle concentrations are low [162] or the AF4 flow velocity is significant relative to the diffusion rate of the particles [163]. Recent advances in online sizing include the coupling of FFF with nanoparticle tracking analysis (NTA), as demonstrated on mixtures of different sizes of PS nanoplastics [164], which is advantageous for small particles that do not scatter as efficiently in DLS analysis.

Characterization of the composition of the fractionated particles also poses challenges, especially in natural samples. First, sensitivity can be a limiting factor, as the sample is diluted after injection in the FFF mobile phase and may show poor recovery from the channel [149,151]. Introducing surfactant in the mobile phase can minimize losses but may disrupt the natural aggregation state of the samples. Alternatively, higher sample masses can be injected using semi-preparative FFF [161,165]. Dilution can also be minimized by using HF-FFF instead of AF4 [166], or by splitting particle-free flow from the AF4 effluent, such that a more concentrated sample stream is sent to the detectors. The capability to quantify mass concentrations in the AF4 eluent, as well as the sensitivity and selectivity, will also depend on the choice and optimization of online detectors. In the most common online detectors (UV-Vis and light scattering), anthropogenic nanomaterials often do not show any distinguishing features from other natural colloids. UV detection is also prone to interferences from particle light scattering, which is disproportionally weighted to large particles. If heteroaggregates of anthropogenic and natural colloids are present, distinguishing the presence or size of the anthropogenic material will be even more challenging. To help overcome these issues, FFF has frequently been coupled with ICP-MS for selective and quantitative detection of anthropogenic nanoparticles in environmental samples containing natural colloids. For example, FFF-ICP-MS has been applied to detect TiO_2 and Ag nanoparticles in wastewater [167]. It has further been applied to identify individual nanoparticles and those heteroaggregated with soil or sediment colloids, for example for CdSe/ZnS quantum dots [168], Au NPs [169], and phosphorus (P) in black carbon [165]. Recent advances in FFF-ICP-MS include the use of TOF-ICP-MS to rapidly monitor multiple isotopes, enabling more comprehensive analysis of highly complex

mixtures of engineered and natural nanoparticles and colloids [88]. For nanoplastics, AF4 has been applied to collect size fractions for offline ICP-MS analysis of nanoplastics doped with Pd as a tracer [170]. AF4-ICP-MS and CFFF-ICP-MS have also been applied to characterize composite particles of metal nanoparticles within plastics [171]. These two modes produced complementary information: AF4 separated the composites by their overall hydrodynamic size, whereas the plastic matrix could be rendered “invisible” in CFFF by density-matching the mobile phase, resulting in separation by the number of metal nanoparticles per plastic. Another selective detection mode recently coupled to AF4 is magnetic particle spectroscopy for magnetic nanoparticles, such as iron-based biomedical imaging agents [172].

Online detection and identification of purely plastic or carbon-based nanoparticles is currently a major challenge in AF4 analysis, as AF4-ICP-MS does not provide information on the molecular composition of organic materials such as plastics. Light scattering has been explored as an AF4 detector to detect PS and PE nanoplastics in fish after enzymatic digestion; while the spiked PS could be detected, the PE nanoplastics were not distinguishable from the light scattering background of the fish digest [150]. Hence, more selective detectors are required. Herrero et al. coupled AF4 with atmospheric pressure photoionization (APPI) - Orbitrap mass spectrometry for identification of fullerene nanoparticles and aggregates [173]; however, this direct interfacing would not be well suited for larger nanoplastics. Schwaferts et al. have made a major recent advancement in hyphenating AF4 and centrifugal FFF with optical tweezers to concentrate the eluting nanoplastics followed by Raman detection to identify the plastics [46]; this method can hold great promise for nanoplastics analysis if the availability of the instrumentation is expanded. However, owing to the multiple conditions that can be adjusted, the method development of FFF techniques is a time-consuming process [174], which remains a major barrier for its broad utilization. Further improvement in detection capabilities and commercialization of accessories to directly couple them with AF4 will also be a critical area to expand the applicability of AF4.

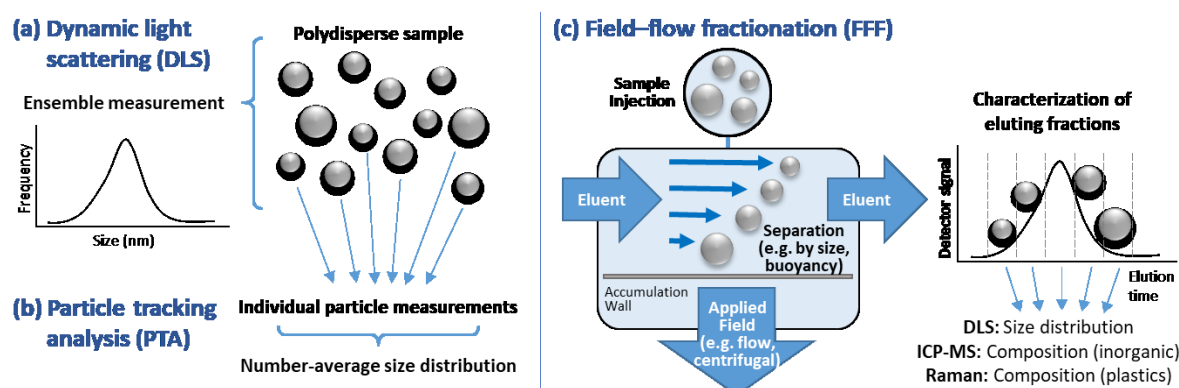


Figure 5. Particle sizing methods – dynamic light scattering (A) and particle tracking analysis (B), and field-flow fractionation (C) for particle separation with optional “hyphenation” to online detectors for size-resolved analysis.

8. Future perspectives

The release of anthropogenic particles to the environment is arguably one of the biggest concerns of societies and governments today worldwide due to the potential risks that these particles pose to human health and ecosystems. The complexity and abundance of anthropogenic particles increases toward the smallest size ranges (e.g., nanoscale), where

particles have lower mass but larger surface area available for mass transfer and surface reactions and are more likely to be ingested by animals, especially invertebrates that are in the lower trophic levels of the food chain. To adequately address the questions of human health and ecosystem risks, analytical methods must be used which provide particle number, size, mass and composition data at increasingly small scales and levels of complexity. However, sample preparation and analytical methods for particle identification are more established for microparticles, while to some extent there is still a methodological gap for nanoparticles. This gap hinders the progress to universally understand the source, fate, and effects of nanoparticles in the environment; hence, one of the future directions in this field is to fulfill this research gap by developing and improving methods and techniques for nanoparticles analysis, as demonstrated in Figure 6.

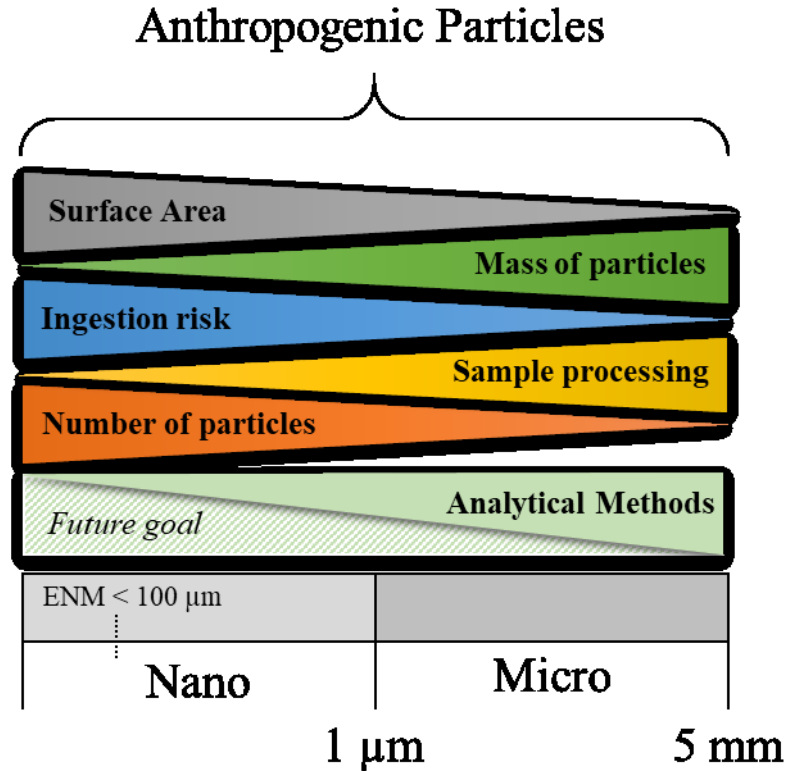


Figure 6. Schematic representation of the relative importance of anthropogenic particle features in nano and micro scale (top four bars), including the current state of development and anticipated future direction of analytical methods (figure adapted from [175])

In this review, advances in several analytical methods with these capabilities are discussed. However, there are still challenges such as sampling harmonization, analytical identification, and data processing which, if addressed, can significantly improve data quality and reproducibility, resolution, and representativeness (Table 1). Harmonization efforts such as standardization of reference materials, methods and sample processing protocols and the development of shared databases for data analysis and interpretation are sorely needed in order to provide reliable and comparable results across laboratories. For instance, vibrational spectroscopy is one of the most advanced and commonly used technique for microplastic identification, but development is still needed in the direction of data processing and validation. For inorganic particles on the other hand, methods such as spICPMS and SEM-EDX call for further development in data management and processing, in addition to validation and

standardization of sample handling protocols. Moreover, for all methods discussed, there is a need for development to increase throughput with standardized and automated systems and to expand the limit of detection or separation to broader size ranges. Miniaturization is also expected to play a critical role in enabling field applications and thus facilitating monitoring efforts.

Other foreseeable future analytical directions include the maturation of semi-quantitative aspects of the various techniques and further development of hyphenated techniques, particularly combining new techniques targeting nanoparticles of emerging concern, including plastics, paint flakes, TRWP, engineered nanomaterials. Some of the techniques discussed produce semi-quantitative data, e.g., from a two-dimensional image it is possible to estimate the particle mass if the thickness of the particle is also assumed and its chemical composition and density are known. Similarly, spICPMS results can be used to derive particle size distribution by making assumptions on the density, composition, and shape of the particles. Hyphenation of techniques has provided significant leaps forward for particle analysis. For example, the current state-of-the-art for microplastic analysis is μ -FTIR and μ -Raman, i.e. the combination of microscopy with a spectrometer, where chemical composition can be obtained in combination with two-dimensional size distribution and number of imaged particles. For example, Mattsson et al. used a combination of techniques including FTIR, Raman, SEM, and NTA to study environmental weathering of expanded polystyrene particles covering a size range from 0.01 to 1,000 μ m [176]. Similarly, Py-GCMS is a combination of techniques providing chemical composition and masses of particles in both the micro- and nanoscale. New combinations such as FFF coupled to optical tweezers and Raman spectroscopy are only recently being developed for analysis of emerging anthropogenic particles such as plastics, paint, tire wear and road particles. All these hyphenated methods produce multi-dimensional data sets (e.g., size- or spatially resolved spectra), and software tools need to be developed for automated data integration and visualization.

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Declaration of interests

X 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.

Author contribution

KM and AG conceived the idea and drafted the outline. All authors wrote the manuscript. All authors discussed, reviewed, edited, and approved the manuscript.

816 **Table 1.** Overview of the most common analytical techniques for anthropogenic analysis of nano- and microparticles.

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Technique	Analytical information	Size or mass concentration limit of detection	Advantages	Limitation	Analytical information	General remarks	Applicable materials
μ-FTIR <i>Transmittance</i> <i>Reflectance</i> <i>ATR</i>	<i>Chemical fingerprint.</i>	<i>< 10 μm</i> <i>< 10 μm</i> <i>< Reflective Crystal Size</i>	<i>Sample Mapping.</i> <i>Sample Mapping.</i> <i>Lower detection limit.</i>	<i>Particle thick. >300μm.</i> <i>Reflective error.</i> <i>Not suitable for mapping.</i>	<i>Chemical fingerprint.</i>	<i>Particle morphology can display spectral interferences mostly in reflectance and ATR measurements.</i> <i>Reflective crystal must be in full contact with the particle for ATR measurements.</i> <i>High interference from water signal.</i>	<i>Microplastics</i>
Confocal Raman	<i>Chemical fingerprint.</i>	<i>< 1 μm</i>	<i>Low interference from water signal.</i> <i>Less sensitive for degradation.</i> <i>Aqueous samples.</i>	<i>Fluorescence signal.</i> <i>Weak signal.</i> <i>Might burn the sample.</i>	<i>Chemical fingerprint.</i>	<i>Sample mapping or particle measurements is still time consuming.</i>	<i>Microplastics</i>
Py-CG/MS	<i>Mass concentration, polymer identification and particle composition.</i>	<i>< 1 μg</i>	<i>Whole particle analysis.</i> <i>Quantitative analysis (mass).</i> <i>Differentiation between plastic polymers and organic additives.</i> <i>Low mass detection limit.</i>	<i>Destructive technique.</i> <i>Only information about organic compounds.</i>	<i>Mass concentration.</i> <i>Polymer identification.</i> <i>Particle composition.</i>	<i>Complementary to Raman and FTIR spectroscopies.</i> <i>Appropriate sample pre-treatment may be needed for removal of potential environmental or biological matrix interferences.</i>	<i>Micro-nanoplastics, TRWP</i>

FFF	Size fractionation	< 1 nm	Relatively gentle (aqueous) sample separation across continuous size distribution Can be coupled with various detectors for sizing and compositional analysis	Low sample injection volume, high sample dilution, or low recovery in analytical FFF mode may result in low sensitivity.	Size fractionation, size distribution, and composition (with online detection, or fraction collection with offline analysis)	Sample pretreatment may be needed to separate < 1 µm and > 1 µm particles that elute in normal and steric elution mode, respectively. Surfactants may need to be added to achieve higher sample recovery	Nanoplastics, ENMs, Microparticles
PTA	Particle concentration and size distribution.	< 10 nm	High throughput and easy to use. Low limit of detection for particle size. Performs better with polydisperse samples compared to DLS.	Visualization artefacts. Poor performance with highly concentrated samples. No chemical composition data	Particle concentration and size distribution.	Provides particle number concentration. Complementary to DLS.	Nanoplastics, ENMs, soot, TRWP
DLS	Particle size distribution and particle properties (zeta potential, electrophoretic mobility)	< 10 nm	User friendly. High throughput and short analysis time. Low limit of detection for particle size.	Skewed towards larger particle sizes. Not useful as a standalone technique in polydisperse and highly concentrated samples. No chemical composition data	Particle size distribution and particle properties (zeta potential, electrophoretic mobility).	Provides data on electrophoretic mobility and zeta potential. Complementary to PTA.	Nanoplastics, ENMs, soot, TRWP
SEM-EDX	Size, shape, number and composition of particles	< 100 nm	Holistic analysis includes number, size, shape, and composition, applicable on a wide range of particle sizes.	Sample preparation artefacts, qualitative or semi-quantitative composition.	Size, shape, number and composition of particles	Careful sample preparation in contamination-free environment is necessary. The analytical process is time consuming (low throughput)	ENMs, soot
spICPMS	Particle concentration and estimation of size distribution	< 10 nm	High throughput analysis, low limits of detection, mature technology with a variety of solutions available for most issues.	Scarcity of properly characterized and stable reference materials, complex and developing data analysis, destructive technique	Particle concentration and estimation of size distribution	Composition, shape, and density assumptions to derive size distribution necessitate use of complementary techniques.	ENMs, microplastics

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