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Analytical strategies for micro- and nanoplastics in aqueous matrices: Progress and the separation bottleneck

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ABSTRACT

Microplastics (MPs) and nanoplastics (NPs) are widespread contaminants, yet analytical workflows fail to generate consistent and comparable measurements, particularly at the nanoscale. Aggregation, together with interactions with dissolved and particulate matter, hinders MP and NP isolation and compromises analytical resolution. This review examines advances from 2019 to 2025 in sample preparation, separation and identification/quantification, with emphasis on aqueous matrices. Digestion methods have improved matrix removal while limiting polymer damage, although Quality Assurance/Quality Control (QA/QC) remains essential. Separation remains the main bottleneck, since conventional methods show low recovery for particles smaller than 100 nm and limited robustness in complex matrices. Emerging strategies improve size resolution but face challenges in scalability, reproducibility and consistency. Key gaps include lack of harmonised analytical protocols, limited certified reference materials and insufficient nanoscale sensitivity. By integrating recent methodological evidence, this review identifies key analytical developments required for reproducible and environmentally relevant monitoring of MPs and NPs.

1. Introduction

Microplastics (MPs) and nanoplastics (NPs) are now recognised as ubiquitous contaminants, occurring in marine and freshwater systems, soils, the atmosphere, and even drinking water. Global estimates indicate that more than 20 million tonnes of plastic waste enter the environment each year, and MPs/NPs have already been detected in diverse biota as well as in human tissues and biological fluids, underscoring the scale and urgency of their environmental and health implications [1,2]. Their widespread occurrence has intensified concerns regarding ecological and toxicological effects and has placed these particles high on scientific and policy agendas [3–5]. Despite this increased attention, analytical approaches still face major challenges in producing reliable, comparable and scalable data on their occurrence, fate and behaviour [6,7].

MPs are typically defined as plastic particles between 1 and 5000 μm, whereas the definition of NPs remains debated. A cut-off of <0.1 μm is adopted here as a stricter criterion, although other studies extend the upper boundary to 1 μm [8–10]. MPs and NPs arise from diverse sources, including cosmetics, abrasive cleaning agents, pre-production pellets, packaging, construction materials, agricultural plastics and tyre-wear particles (TWPs) [3]. Once released, they persist in the environment and interact with organic and inorganic matter, undergoing ageing processes such as eco-corona formation and surface oxidation [11]. These transformations modify stability, aggregation behaviour and apparent density, complicating detection and risk assessment [12,13].

Analytical limitations are particularly severe for NPs. Their behaviour in environmental matrices is governed by colloidal instability and surface ageing, which strongly influence isolation, preconcentration and quantification. These constraints contribute to the large variability reported across studies and highlight the need for multi-technique workflows tailored to polymer type, size range and matrix complexity [14–16].

Among the workflow stages, separation from the surrounding matrix remains one of the most critical bottlenecks. MPs and NPs typically occur at trace levels and coexist with mineral grains, organic debris and

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heterogeneous particulates. Inadequate pretreatment, such as incomplete digestion or insufficient matrix removal, propagates into the separation stage and compromises downstream identification and quantification [17]. These risks are further exacerbated by laboratory contamination, underscoring the importance of rigorous quality assurance and quality control (QA/QC). Quantification is additionally complicated by methodological variability and the lack of consensus on reporting units. Fig. 1 illustrates a typical workflow for MPs and NPs analysis in environmental samples.

Several reviews published before 2019 provided valuable overviews of MP analysis but focused largely on larger size fractions or specific environmental matrices, leaving nanoscale methodologies underexplored, particularly for particles below 100 nm [13,18–23]. In this review, we evaluate analytical methodologies reported between 2019 and 2025 for the preparation, separation and identification/quantification of MPs and NPs, with emphasis on aqueous matrices. Unlike earlier reviews, we examine not only methodological principles and performance, but also scalability, limitations, and comparability. We identify key gaps, including the scarcity of robust nanoscale workflows, the lack of certified reference materials (CRMs) and the limited applicability of many laboratory techniques to environmental monitoring. We also highlight recent advances, including solvent-based liquid–liquid extraction approaches, the maturation of field-flow fractionation (FFF) and the integration of artificial intelligence (AI) with hyperspectral imaging.

2. Sample preparation

Sample preparation is a critical stage in the analytical workflow for MPs and NPs, as it directly affects recovery and reliability. Methods must remove matrix interferences while preserving particle integrity [24]. As particle size decreases, the formation of heteroaggregates between nanoplastics and natural organic matter (NOM) increases, meaning that larger quantities of non-plastic material must be removed prior to analysis [22]. Incomplete organic-matter removal or unintended polymer degradation can therefore reduce recovery and compromise representativeness [25].

These constraints require effective preconcentration and enrichment strategies for plastic particles in complex matrices, particularly in environmental samples where organic and inorganic components interfere with spectroscopic and thermal analyses [26]. As a result, protocols originally developed for MPs often lack the sensitivity required for NPs, reinforcing the need for adapted or novel approaches capable of accurately capturing their presence and degradation behaviour [27–29].

Another challenge associated with nanometre-sized particles is the higher risk of contamination compared with micrometre-sized samples, making contamination prevention strategies and the systematic analysis of procedural and laboratory blanks essential [22]. In practice, sample preparation comprises three main components: (i) pretreatments to remove matrix interferences, (ii) effective preconcentration to compensate for the typically low abundance of MPs and NPs, and (iii) rigorous contamination control [25].

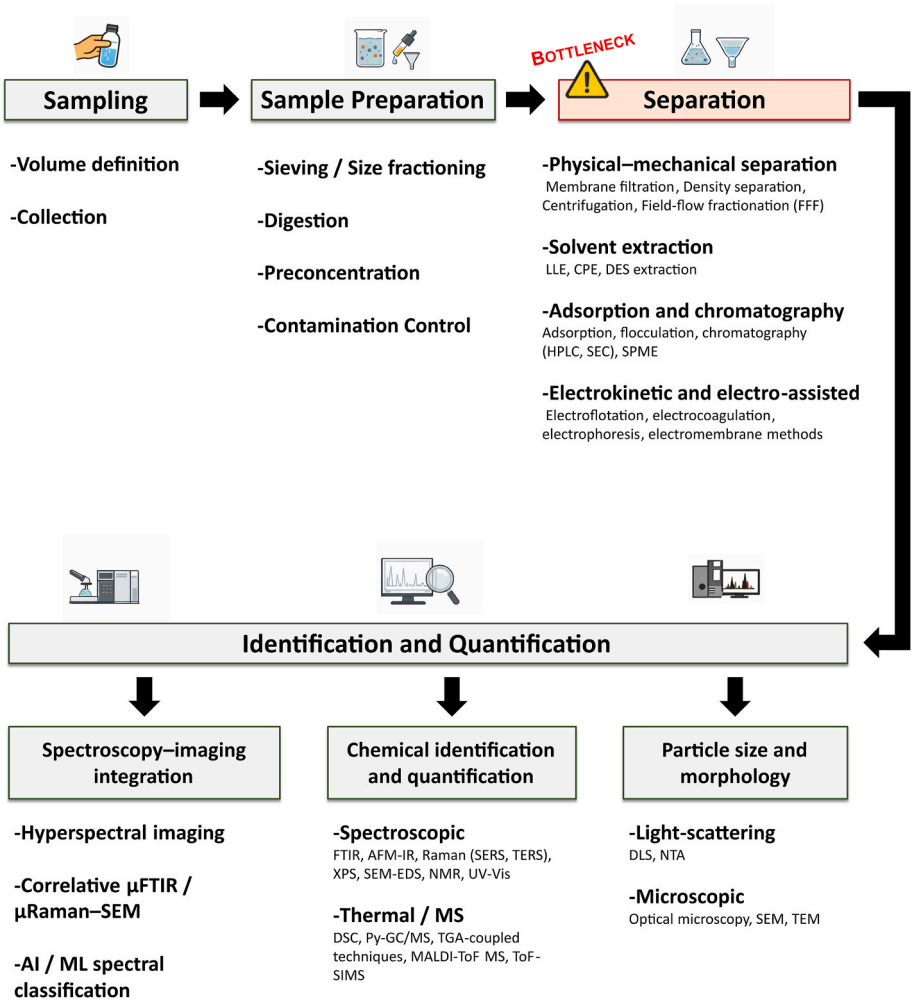

Fig. 1. General workflow for the analysis of MPs/NPs in aqueous matrices.

Sampling and pretreatment of NPs are inherently more complex than the dead-end filtration commonly applied for MPs [30]. Because NP detection and characterisation require more sensitive analytical methods, even trace levels of matrix interference must be removed to obtain reliable analytical signals [31]. In addition, the small size of NPs makes them more susceptible to damage during processing, which may lead to mass loss and reduced analytical signal [32]. Consequently, nanoplastics often need to be isolated from large sample volumes and separated from coexisting components prior to analysis, requiring effective preconcentration and fractionation strategies.

Among the available strategies, pretreatments have received considerable attention, as they directly influence particle recovery and integrity. An analysis of 102 articles published between 2019 and 2025 (Table S1) indicates that digestion-based protocols dominate current practices, accounting for approximately two-thirds of the reported approaches (Fig. 2). Oxidative digestion is the most frequently applied strategy, followed by alkaline and acid treatments, whereas enzymatic digestion remains comparatively underutilized despite its milder operating conditions.

Other pretreatment approaches, including solvent washing, filtration, polymer dissolution, sonication and heating, are generally used as complementary rather than standalone strategies [33–37]. This reliance on digestion reflects the analytical priority given to efficient matrix removal, but also introduces trade-offs between pretreatment efficiency (reducing analytical interferences) and polymer preservation, particularly for nanoplastics [38].

2.1. Sieving and size fractionation

After sampling, sieving is typically the first step used to remove particles larger than 5 mm, such as leaves, shells, organisms or other synthetic materials present in the sample matrix. This enables the isolation of MPs within the required size range and the fractionation of retained particles using different mesh sizes [39]. In general, fractionation can be achieved by stacking sieves with mesh sizes ranging from 5 mm down to 20 μm [40,41]. This step does not separate NPs but remains an essential initial processing step for environmental samples. Sieves are typically made of stainless steel due to its chemical stability and mechanical robustness [42].

In MP analysis, dry sieving is commonly used for larger particles. However, this method may cause electrostatic charging and agglomeration of fine particles [43]. For this reason, wet sieving is more appropriate for the separation of smaller particles in environmental samples [43]. Smaller mesh sizes increase processing time, clogging and susceptibility to contamination [43]. To ensure robust and comparable results, analysts should use standardized and multistage sieving procedures [43]. After sieving, the retained fractions still contain a mixture of inorganic particles, natural organic matter and plastic-associated material, requiring further treatment before downstream analysis [42].

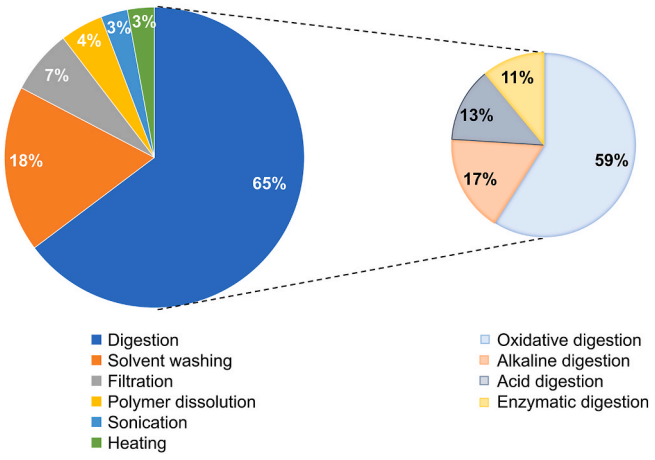

Fig. 2. Distribution of sample pretreatment methods reported for MPs and NPs analysis in spiked and environmental samples (N = 102 articles, 191 methods in total).

2.2. Digestion methods

Digestion is central to removing biological and organic matter while preserving MPs and NPs for downstream analysis [25]. However, this step must be carefully controlled, as inappropriate conditions may damage polymers [29,44,45]. Digestion efficiency depends on reagent type, incubation time, temperature and polymer-specific properties. Acid digestion employs concentrated acids such as nitric or sulphuric acid across a range of temperatures and reaction times [46]. Although effective for biological samples rich in proteins, carbohydrates or lipids, it carries a significant risk of polymer degradation under prolonged exposure [47]. For example, treatment with 65% HNO3 at 70 °C removed all organic matter yet severely damaged polyamide (PA), polystyrene (PS) and poly (ethylene terephthalate) (PET) after 24 h [48]. In another study, no damage or aggregation of PS NPs was reported during further analytical procedures of aqueous samples after the following digestion protocol: 10 mL of water sample, 50 μL of HNO_3 (1 M), and 400 μL of HF (1 M) added in sequence. The pH of the digestive solution was 2.44 and samples were shaken for 40 min [49].

Alkaline digestion uses strong bases such as 10% KOH to hydrolyse proteins and lipids in biological samples [50]. It is less effective for hard tissues at low temperatures, while rapid or excessive heating can also damage polymers [50]. Accurate temperature control is therefore essential to prevent degradation [51,52]. Oxidative digestion, typically based on hydrogen peroxide (H_2O_2) or Fenton's reagent, is widely applied to complex organic matrices [53]. Although generally less aggressive than acids or bases, over-oxidation can lead to discolouration or deterioration of the mechanical properties of fibrous polymers [54,55]. Enzymatic digestion is the mildest approach, achieving high recoveries with minimal polymer damage. Enzymes such as proteinase K, alone or in combination with other enzymes or Fenton's reagent, are commonly used to degrade complex organic matter [56]. However, high costs and long incubation times still limit widespread application [29].

The choice of digestion protocol therefore depends strongly on matrix composition, polymer susceptibility and analytical objectives. In practice, matrix-specific preferences are observed in the literature, with alkaline digestion dominating food matrices [57], Fenton's reagent commonly applied to soils [58], and oxidative digestion with H_2O_2 (30% w/v) favoured for aqueous matrices. Whether conducted at 60 °C for 12-48 h [59–61], or at room temperature for 5–6 days [62,63], there is a clear preference for progressive oxidation under milder conditions. Oxidant-to-sample volume ratios also vary widely depending on the type of water analysed, ranging from 1:1 (v/v) for filtrate from surface seawater of the Venice lagoon [63] to 1:15 (v/v) for Australian potable water. Because the amount of organic matter to be removed is generally lower in aqueous matrices, greater preservation of polymer integrity can often be achieved under milder conditions with extended digestion times [59,63]. Furthermore, recovery tests with representative polymers are recommended whenever possible to validate digestion efficiency while ensuring minimal alteration of polymer morphology [64]. Depending on sample concentration and method sensitivity, a preconcentration step is often required after digestion prior to separation and analytical identification of MPs and NPs.

2.3. Preconcentration techniques

In environmental samples, preconcentration is often required to improve the limit of detection (LOD) and limit of quantification (LOQ) when analysing MPs and NPs, as particle concentrations are typically low [26]. No single technique is universally applicable, and selection depends on sample volume, matrix complexity and compatibility with

downstream analytical methods [32].

Membrane filtration remains the most widely applied approach for aqueous samples due to its operational simplicity and scalability [28]. However, decreasing pore size increases fouling and processing time, limiting feasibility in high-volume applications such as wastewater monitoring [26,28]. In the case of NPs in aqueous samples, filtration requires very small pores (<200 nm) or molecular-weight cut-off membranes to retain nanoplastic colloids while allowing dissolved substances to pass through [38].

Ultrafiltration extends this principle by enabling tighter molecular-weight cut-off control (typically 10–500 nm depending on membrane type) in stirred-cell or centrifugal configurations [25]. Despite improved selectivity for smaller particles, ultrafiltration faces similar constraints, including aggregation during processing and higher operational costs, which restrict their feasibility for large-scale monitoring campaigns [56,65].

Ultracentrifugation concentrates particles under high gravitational forces and may be applicable to multiple polymer types; however, low sample capacity and density overlaps in complex matrices can compromise selectivity [25,28]. The smaller the particles, the greater the centrifugal force and the longer the time required to effectively separate particles above a given size threshold. The combined use of density gradient ultracentrifugation (DGU) at very high G-forces and layered solutions of different densities (e.g., sucrose or silica gradients) can separate nanoplastics from natural colloids [38].

Liquid–liquid extraction (LLE) may function as both a preconcentration and separation strategy, with performance governed by solvent–polymer affinity and phase stability (see Section 3.2). Solvent evaporation is generally restricted to small sample volumes and is rarely suitable for large-scale environmental monitoring.

In practice, combined workflows often provide the most reliable outcomes. For example, bulk concentration via ultrafiltration or diafiltration followed by asymmetrical flow field-flow fractionation (AF4) can improve nanoscale fractionation while minimising artefacts propagated into identification and quantification. Nevertheless, sampling and laboratory artefacts may still affect representativeness, reinforcing the need for integrated contamination control throughout sample preparation.

2.4. Contamination control

Rigorous QA/QC is essential for reliable analysis of MPs and NPs due to the high risk of laboratory and airborne contamination, particularly for nanoscale particles. Best practices include using non-polymeric tools whenever possible, maintaining clean laboratory environments and applying field and procedural blanks to quantify external contamination [51,66]. Ideally, sample processing should occur under a laminar-flow hood or in an International Organization for Standardization (ISO) Class 5–7 cleanroom, with controlled access and documented cleaning routines [67]. Laboratory personnel should wear cotton lab coats and sterile gloves, and procedures should be conducted on ethanol-cleaned surfaces within filtered-air environments.

Glass fibre filters, stainless-steel tools and glassware require rigorous cleaning, either by muffling at 450 °C for 4 h or by acid washing (10% HCl or HNO_3 overnight) followed by thorough rinsing with Milli-Q water [68–71]. Other labware can be rinsed with ethanol or dichloromethane followed by ultrapure water [60]. Glass and metal tools should replace polymeric alternatives whenever possible, and unavoidable use of plastics must be explicitly reported. Samples should be stored in sealed containers, and all solutions used during sample preparation should be filtered through membranes with pore sizes equal to or smaller than those applied to the samples.

Method validation relies on replication and careful interpretation of blanks to ensure that background contamination is not statistically significant [27,72]. Inter-laboratory comparisons are particularly valuable, as identical protocols applied by different researchers allow reproducibility to be assessed using known or spiked reference materials [73]. Certified reference materials (CRMs), although limited, are increasingly recommended for validating recoveries and supporting standardisation [74]. Ideally, CRMs should reflect environmentally relevant polymer types, irregular particle shapes and broad size distributions to better represent environmental conditions [75]. Once such materials are employed, matrix spikes are recommended in all studies [74]. Another parameter commonly reported in analytical chemistry, but still absent from many microplastic studies, is the working range together with the corresponding limits of detection (LOD) and quantification (LOQ) [74].

Several laboratory sources of contamination warrant particular attention. Jones et al. [76] demonstrated that untreated glassware can release more MPs than certain single-use plastic items. Laboratory dust, conventional fume hoods and tap water have also been identified as major contamination sources. Aluminium foil, often used to protect samples, can accumulate dust and release particles due to electrostatic charging. Pre-washing with dichloromethane has been suggested to mitigate this issue [60].

Contamination control must also extend to the sampling stage. Field blanks, pre-cleaned containers and sealed transport conditions are essential to prevent artefacts prior to laboratory processing. This aspect is particularly important when analysing MPs and NPs and becomes even more critical for NPs due to their lower environmental concentrations and higher susceptibility to contamination. Transparent reporting of these measures underpins reliable results across all stages of sample preparation.

3. Separation techniques for micro- and nanoplastics

After preconcentration, separation is applied to isolate MPs and NPs with minimal losses and polymer alteration, which is a prerequisite for accurate detection and quantification [77]. However, variability in particle size, method-specific limitations and interactions with organic and inorganic matrix components pose major challenges to existing methodologies [51]. To provide an updated overview of current practices, a Scopus search was conducted using the keyword “nanoplastics” in combination with terms related to “separation” or “extraction” in “aqueous” or “water” matrices. From 234 articles initially screened, 112 studies were retained, encompassing 127 individual reports of separation techniques grouped into 21 methodological categories (Table S2 in the Supplementary Material). Publication activity has increased markedly over the past five years, reflecting rapid methodological diversification and growing recognition of the analytical challenges associated with nanoplastics (Fig. S1).

The retained studies were classified according to their separation or extraction mechanism. For clarity, the 21 methodological categories were consolidated into four overarching classes: physical-mechanical, sorption-based, solvent-assisted and electro-assisted (Fig. S2 in the Supplementary Material). Physical-mechanical techniques, including membrane-based operations, centrifugation and field-flow fractionation, clearly dominate current research, accounting for nearly half of the reported strategies. Sorption-based and solvent-assisted techniques represent a substantial but secondary share, whereas electrokinetic and electro-assisted methods remain comparatively underexplored.

Beyond this classification, the diversity of polymers analysed across these categories is summarised in Fig. 3. Polystyrene (PS) overwhelmingly dominates the literature, largely due to its commercial availability, spherical morphology and analytical convenience. In contrast, polymers more prevalent in environmental samples, such as polyethylene (PE), polypropylene (PP), PET and polyvinyl chloride (PVC), are far less frequently investigated. Although PS serves as a practical model material, its physicochemical behaviour does not fully represent that of more crystalline, hydrophobic or heterogeneous waste-derived polymers. This imbalance underscores the need for broader polymer coverage in future method validation to ensure environmental representativeness [78].

The choice of separation technique depends strongly on the

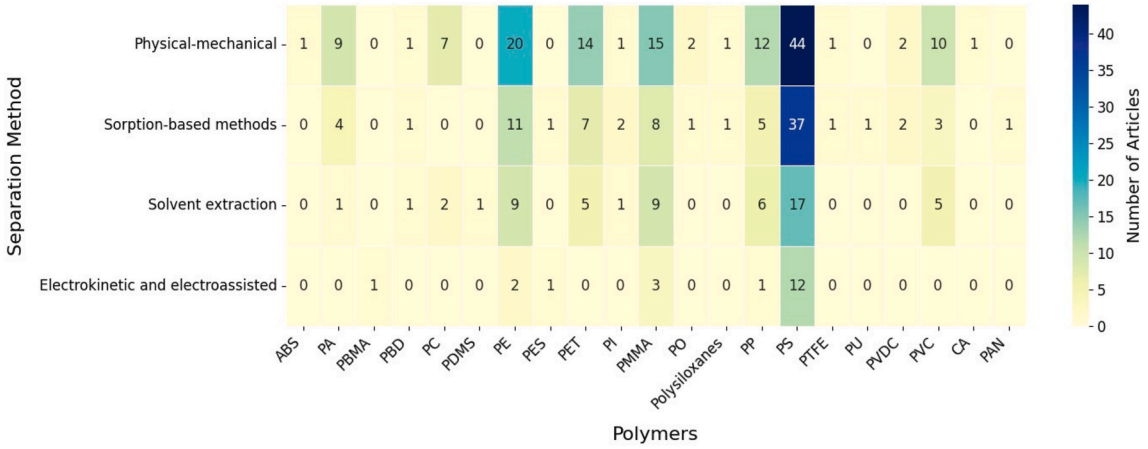

Fig. 3. Heatmap showing polymers analysed across four separation categories, based on 127 individual applications of separation techniques identified in 112 studies (2019–2025) involving micro- and nanoplastics in spiked and environmental aqueous samples.

physicochemical properties of MPs and NPs (size, shape, density, hydrophobicity and surface charge) as well as on matrix characteristics. Traditional methods, such as membrane filtration and density separation, remain widely used but often fail to efficiently recover particles smaller than 0.1 μm, particularly in saline or heterogeneous matrices [25]. Effective separation must therefore achieve two goals: (i) removal of matrix components and (ii) maximisation of nanoscale particle recovery. The following subsections critically evaluate the main classes of separation techniques. Extended comparative tables summarising all methods (physical-mechanical, solvent-assisted, adsorption-based and electro-assisted) are provided in the Supplementary Material (Table S3–S6).

3.1. *Physical-mechanical separation methods*

3.1.1. *Membrane filtration*

Membrane filtration is widely used to isolate or fractionate MPs and NPs by size and can serve both as a large-volume concentration step and as a final separation stage. Membranes are typically made of cellulose acetate, polycarbonate or polytetrafluoroethylene (PTFE) [79]. Filtration performs well for MPs larger than 1 μm but becomes less efficient for smaller MPs (~0.1–1 μm) and NPs (<0.1 μm) [28]. Capturing smaller particles requires very narrow pore sizes, which greatly increase filtration time and fouling, particularly in matrices with high particulate loads or complex compositions [79]. These factors reduce scalability and operational efficiency in environmental monitoring [26,28,80,81]. Small-pore membranes are also more expensive and more prone to physical damage, adding analytical complexity [25].

Membrane composition strongly affects recovery, particularly for hydrophobic polymers such as PS. Common materials include glass fibre [82], silicon and aluminium oxide [83], polyurethane [84], mixed cellulose ester [85], polycarbonate, polyvinylidene fluoride (PVDF), nylon, quartz [86], polytetrafluoroethylene (PTFE) [87] and cellulose [88]. Glass-fibre filters are inexpensive but may shed fibres that interfere with spectroscopic or mass-based identification [68]. Inorganic membranes such as aluminium oxide minimise spectroscopic interference and improve NP identification [83,89]. Organic membranes like PVDF and nylon often achieve high retention (>84% for PS) [78], but can adsorb particles, requiring correction for recovery losses [28]. Overall, membrane selection must balance retention efficiency, chemical neutrality and operational robustness [90].

Membrane charge and pore size both influence NP recovery. Positively charged membranes can enhance retention of aged or slightly charged NPs through electrostatic attraction; for example, Wang et al. [91] reported PS removal efficiencies of 99.4% (500 nm), 99.3% (100 nm) and 89.9% (50 nm), outperforming neutral membranes. Performance remains matrix-dependent and requires validation for each sample type [91]. Pore size also plays a critical role, as pores <0.1 μm improve retention but increase fouling and adhesion, reducing throughput [45]. Cascade filtration, where progressively finer filters are applied sequentially, helps reduce clogging and prolong membrane lifespan [92,93].

Another approach to improve filtration performance involves the development of advanced functional membrane materials designed to reduce fouling, enhance permeability and improve mechanical and thermal stability [94]. In this context, several nanomaterials, including zeolites, metals and metal oxides, carbon nanotubes (CNTs) and graphene derivatives, have been explored for membranes targeting the removal of MPs and NPs from water [95].

Among these materials, metal–organic frameworks (MOFs) and covalent organic frameworks (COFs) have recently attracted attention due to their highly porous structures and tunable surface chemistry [96]. COFs are crystalline porous frameworks with high surface area and tunable pore structures, although their application to MP and NP removal is still at an early stage [96–98]. MOFs consist of metal ions or clusters coordinated with organic ligands, forming highly porous structures with large pore volumes and high adsorption capacity [99]. These properties can enhance interactions between membranes and plastic particles, potentially improving separation efficiency [99]. However, their practical implementation remains limited by challenges related to aggregation, structural stability in aqueous environments, scalability and cost [100,101].

Other functional membrane materials include metal and metal oxide-based systems, such as silver, ZnO, TiO_2, and iron oxides, which can provide high reactivity, antibacterial properties and photocatalytic activity [102]. These materials may enhance MP and NP removal through electrostatic interactions and photocatalytic degradation under UV irradiation [103]. Nevertheless, potential metal leaching, production costs and fouling remain important limitations for large-scale applications [102,103].

Carbon-based materials, particularly CNTs, have also been investigated due to their high mechanical strength and chemical stability. However, their use is still constrained by high fabrication costs and the risk of nanoparticle aggregation or release into treated water [104]. Overall, although engineered nanomaterials offer promising routes to improve membrane-based separation, their large-scale application remains limited by issues related to material stability, economic feasibility and potential environmental risks associated with nanoparticle release [105]. Future studies should prioritise validation under realistic water matrices and operational conditions to assess their practical applicability [103].

3.1.2. Density separation

Density separation relies on differences between polymer and solution density to isolate MPs and NPs from sediments or solid matrices [106]. Sodium chloride (NaCl, 1.2 g/mL) is widely used because it is inexpensive and environmentally benign [107]. Denser polymers such as PET and PVC require higher-density solutions such as zinc chloride ($ZnCl_2$) or sodium iodide (NaI), which can reach ~1.6 g/mL [45,108]. Although effective for heavier polymers, these media introduce ecological risks, disposal challenges and higher costs.

The technique performs poorly for NPs because weak buoyancy and hydrophobic interactions promote aggregation and particle loss [109]. Thus, density separation is best suited for coarse MP fractionation rather than primary NP recovery, particularly in marine or high-salinity matrices [110].

Flotation can act as a complementary step. Hydrophobic MPs and NPs attach to rising gas bubbles and accumulate at the air–liquid interface, while hydrophilic particles remain dispersed [111]. However, bubble size is difficult to control, losses are common and oil-removal steps may damage particles and compromise downstream analysis [77].

3.1.3. Centrifugation and ultracentrifugation

Centrifugation separates particles based on sedimentation velocity and density contrast, with reported recoveries of 73–93% for mixed polymer suspensions (50–1200 nm) in freshwater, wastewater and seawater matrices [49]. Performance depends on rotor type, relative centrifugal force (RCF), dispersion quality, salinity and natural organic matter [112].

Ultracentrifugation reaches much higher RCF values, enabling concentration of smaller particles [113]. Density-gradient ultracentrifugation (DGU) improves density resolution by applying layered gradients of increasing medium concentration, yielding separation by buoyant density [114–116]. Gradient composition, stepwise versus continuous profiles and controlled deceleration are essential to maintain band integrity and minimise resuspension [114,117,118]. Isopycnic ultracentrifugation, widely used in molecular biology [119], has been successfully adapted for MPs, with recoveries of 86–99% for polyamide and PET particles (128–1636 μm) in soil matrices at 60,000 rpm for ≥48 h [117].

Despite their potential, centrifugation and ultracentrifugation require careful control of ionic strength, dispersion and sample transfers. Throughput remains modest, and polydisperse samples or high salinity can reduce selectivity, making these techniques more suitable as preconcentration or fractionation steps prior to spectroscopic or imaging-based identification.

3.1.4. Field-flow fractionation (FFF)

Field-flow fractionation (FFF) separates suspended components in a ribbon-like channel under laminar flow without a stationary phase [66]. An external field applied perpendicular to the flow induces separation based on particle size, mass or density. In asymmetric-flow FFF (AF4), a cross-flow pushes particles toward the accumulation wall; smaller particles diffuse back into faster-flow regions and elute earlier, while larger particles remain closer to the wall and elute later (Fig. 4) [66,120]. Centrifugal FFF (CF_3) uses channel rotation to generate a centrifugal field that separates particles according to buoyant mass over typical sizes of ~10 nm to 20 μm [66].

FFF is particularly suited to NPs because it covers the full nanometre range and can be coupled online with detectors for enhanced characterisation [66]. Ultraviolet-Visible (UV–Vis) detectors provide concentration profiles, while multi-angle light scattering (MALS) yields size distributions [120]. AF4 coupled with UV–Vis Diode Array Detector (DAD) and MALS have achieved recoveries up to 88.5 ± 0.3% for PS nanoparticles (50–100 nm; UV linearity $R^2 > 0.99$), though recoveries are lower for larger or agglomerated particles [121,122]. Sensitivity is polymer-dependent and requires validation for each material.

High equipment cost, specialised expertise and low throughput currently limit routine environmental use [123]. For complex matrices, AF4 performs best when integrated with MALS and UV/DAD detectors. Cross-validation with Nanoparticle Tracking Analysis (NTA) or electron microscopy and the use of internal standards improve quantitative accuracy. Reporting recoveries and associated uncertainties is essential for inter-study comparability. A comparative overview of physical-mechanical separation approaches is provided in the Supplementary Material (Table S3).

3.2. Solvent extraction

Liquid–liquid extraction (LLE) is widely used to separate hydrophobic analytes, including MPs and NPs, by transferring them into an immiscible organic phase (Fig. 5). Its effectiveness for polymers such as PS and PET depends primarily on solvent–polymer affinity and matrix composition [124].

Conventional organic solvents (e.g., hexane, dichloromethane, xylene; including pressurised fluid extraction) show significant limitations for MPs and NPs. Co-extraction of organic matter frequently interferes with downstream spectroscopic or mass spectrometry (MS)-based analysis [125,126], and the risk of polymer softening or dissolution can compromise morphological integrity [125]. Apolar solvents such as toluene have achieved recoveries of 55–82% in freshwater [127], although reproducibility decreases in complex matrices. Oil-based LLE systems such as olive oil offer lower toxicity but still suffer from particle loss during demulsification and interference from persistent hydrophobic residues [128].

Recent developments include cloud-point extraction (CPE), natural organic solvents and eutectic solvents (ES). CPE concentrates particles into a surfactant-rich phase. Natural oils provide simple and relatively benign extraction media, though emulsion stability remains problematic. ES exploit tunable hydrogen bonding, hydrophobic interactions

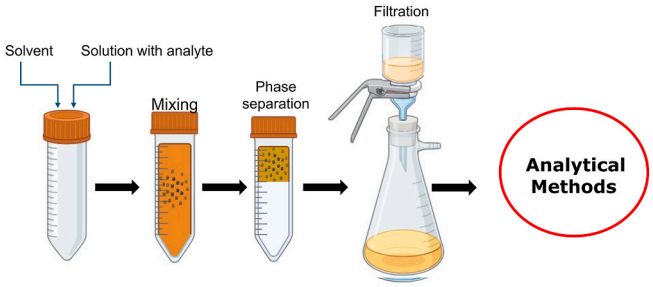

Fig. 5. Example of a liquid–liquid extraction workflow.

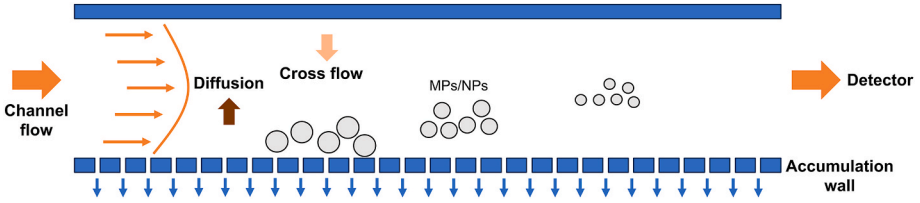

Fig. 4. Operating principle of an AF4 separation method.

and π–π stacking to extract non-polar polymers with high selectivity.

3.2.1. Surfactants (cloud-point extraction)

Cloud-point extraction isolates hydrophobic particles using surfactant-rich phases that encapsulate MPs and NPs (Fig. 6).

Surfactants such as Triton X-45 and cetyltrimethylammonium bromide (CTAB) have shown high recoveries. Li et al. [86] demonstrated that Triton X-45-based CPE can isolate both PS and poly (methyl methacrylate) (PMMA) NPs from environmental waters. In their workflow, MPs >1 μm were first removed by filtration, and NPs were enriched in the surfactant phase, achieving recoveries above 90%. Li et al. [129] further optimised CTAB-based CPE for PS NPs in biological matrices by combining gold-nanoparticle labelling with single-particle ICP-MS (SP-ICP-MS), enabling detection of low-abundance NPs. However, CTAB performance is highly concentration-dependent: excessive surfactant can leave persistent residues that interfere with spectroscopic or MS-based measurements, particularly in organic-rich matrices [129]. Similar issues occur with non-ionic surfactants such as Triton X-45, underscoring the need to optimise surfactant type and dosage to enhance compatibility with complex matrices and improve reproducibility [130].

3.2.2. Eutectic solvents (ES)

Eutectic solvents (ES) have emerged as promising and more sustainable alternatives for MP and NP extraction [131]. Composed of a hydrogen-bond donor (HBD) and acceptor (HBA), ES offer advantages over conventional organic solvents, including lower toxicity, potential biodegradability and tunable polarity [132]. Their adjustable hydrophobicity makes them suitable for non-polar polymers such as PS, PET and poly (lactic acid) (PLA) [109].

Hunter et al. [128] evaluated hydrophobic ES composed of decanoic acid:menthol mixtures (1:1 and 1:2), reporting recoveries of 50–93% for PS, PET and PLA in freshwater and saltwater. The study also noted challenges including particle aggregation and reduced extraction efficiency in saline matrices. Molecular-dynamics simulations confirmed that hydrophobic interactions and hydrogen-bond networks drive polymer–solvent affinity [128].

Zhang et al. [131] investigated lignin-derived ES (e.g., thymol:2, 6-dimethoxyphenol; menthol:2,6-dimethoxyphenol), reporting extraction efficiencies above 95% for PS and PET. These high recoveries were attributed to hydrophobic interactions, hydrogen bonding and π–π stacking. However, prolonged exposure of PS to thymol-based ES caused softening and partial dissolution, while menthol-based systems showed reduced stability. Ishtaweera et al. [133] explored tetra-alkylammonium-bromide-based ES (e.g., [N4444]Br:decanoic acid 1:2), obtaining >98% recovery for NPs from 100 to 1000 nm. These systems performed particularly well in saline matrices, where enhanced aggregation promoted transfer into the ES phase [133].

Overall, ES offer tunability, high recovery and improved sustainability, but challenges remain, including viscosity, emulsion stability and occasional polymer softening. Performance depends strongly on solvent composition, solvent-to-water ratios and mixing conditions, and is inconsistent in saline or organic-rich waters. Continued optimisation via interfacial engineering, controlled mixing and deeper mechanistic understanding will be essential to broaden applicability in environmental workflows. A comparative overview of solvent-based extraction approaches is provided in the Supplementary Material (Table S4).

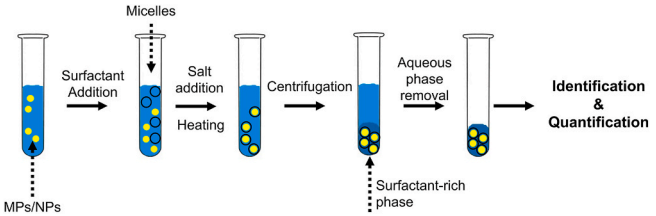

Fig. 6. Example of a Cloud-point extraction workflow.

3.3. Adsorption- and chromatography-based methods

Adsorption- and chromatography-based approaches offer alternative mechanisms for MP and NP separation, although performance is strongly dependent on material properties and matrix composition (Fig. 7).

In adsorption-based extraction, efficiency depends primarily on sorbent type, ranging from biomass and magnetic nanoparticles to engineered materials such as hydrogels, metal–organic frameworks (MOFs) and carbon nanostructures [134]. Adsorption is widely applied in water treatment due to its low energy requirements, modest operational cost and favourable environmental profile [135,136]. Removal efficiency is strongly influenced by sorbent physicochemical properties, including nanostructure, composition and surface chemistry [135]. Functional groups such as –OH, –COOH, $–NH_2$, $–N–CH_3$, Fe–OH and –Si–O enhance NP removal [135], and may be introduced through acid oxidation, metal-salt activation, ball milling or chemical modification of renewable materials and magnetic nanoparticles [137–139].

Biomass-derived sorbents such as biochar offer sustainable alternatives but may be difficult to recover at scale due to fine particle size. Magnetic functionalisation (e.g., Fe_3O_4-doped biochar) improves retrievability while preserving electrostatic and hydrophobic adsorption mechanisms [139]. CTAB-modified magnetic rice-straw biochar has achieved removal efficiencies of up to ~99.6% for sub-100 nm PS, although performance decreases in the presence of competing ions or natural organic matter (NOM) [139]. Hydrophobised iron-oxide nanoparticles (IONPs) also enable rapid magnetic retrieval, achieving ~89–93% recovery for 100–1000 nm PS in seawater and nearly 100% for millimetre-scale pellets [140].

Despite their advantages, adsorption systems face three recurrent constraints in environmental matrices: (i) limited adsorption rates and capacities; (ii) low selectivity and occasional sorbent instability; and (iii) strong sensitivity to pH, temperature and salinity [136,137]. Sorbent synthesis is often labour-intensive, and the risk of secondary contamination via leaching of functional groups or additives must be considered [141]. Furthermore, most studies employ monodisperse test plastics that do not reflect real wastewaters, where adsorption sites compete with NOM and co-contaminants [141,142]. For methods claiming reusability, at least five adsorption–desorption cycles under realistic ionic strength and NOM conditions should be demonstrated.

Polymer-assisted flocculation aggregates MPs and NPs through charge neutralisation and bridging by cationic or amphoteric reagents, supporting rapid settling or flotation and providing an effective preconcentration step (Fig. 8).

Recoveries of 65–90% have been reported for PS and PMMA NPs around 100 nm in wastewater, although efficiency drops significantly for micrometre-scale particles [143]. Bioflocculants such as jellyfish mucin offer improved sustainability but remain sensitive to matrix

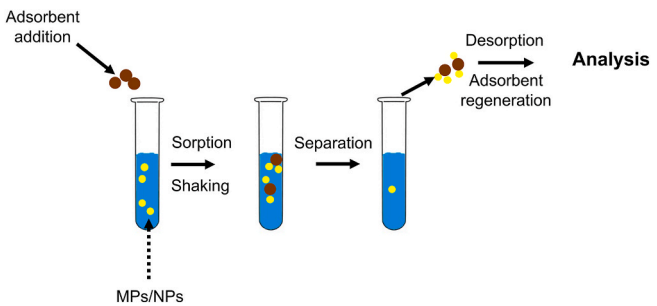

Fig. 7. Typical adsorption process for MP/NP removal.

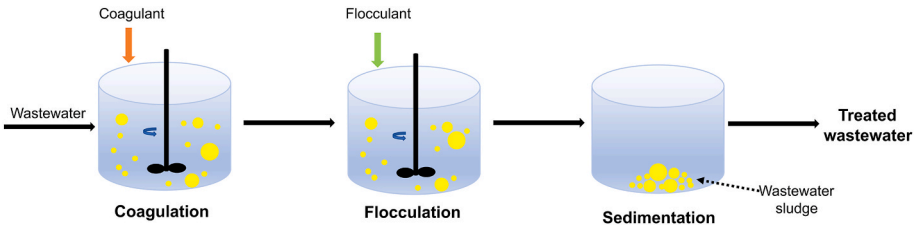

Fig. 8. Example of coagulation-flocculation process in wastewater treatment plants.

composition. Synthetic coagulants lack biodegradability and generate sludge that can interfere with downstream analysis [144].

Chromatographic approaches, including high-performance liquid chromatography (HPLC) and size-exclusion chromatography (SEC), can separate MPs based on interactions with stationary phases but often suffer from clogging and high maintenance in particulate-rich samples [145]. Nevertheless, when coupled with spectroscopic or MS-based detectors, chromatography provides valuable information for polymer identification [146]. Reported applications include SEC with PLgel MIXED-D columns, HPLC with advanced polymer chromatography (APC) columns [146,147], reversed-phase HPLC on C18 columns [148, 149], and normal-phase HPLC using aqueous columns [112].

Solid-phase microextraction (SPME) is a separation technique that can be applied prior to thermo-analytical methods to minimise contamination of thermo-analytical instruments and reduce interference from thermally decomposed compounds, which may otherwise reduce sensitivity and increase analytical uncertainty [150]. SPME devices are relatively simple to operate and consist of sorbent-coated supports that extract analytes directly from the sample or its headspace with minimal solvent consumption [151]. These devices are available in several configurations, most commonly fibres, arrows and thin films [151]. Xu et al. [150] demonstrated an approach combining thermal decomposition of PMMA particles (500 nm–3 μm) with extraction of indicator compounds prior to GC–MS analysis. Spike recoveries were determined by adding 5 and 20 μg/g of PMMA to water and soil samples. Recoveries ranged from 91.8 to 98.9% in water and 75.6–89.0% in soil. The reported limit of detection was 0.28 μg using pyrolysis at 350 °C with an incubation time of 13 min. These results indicate the potential applicability of the method for determining mass concentrations of MPs and NPs in environmental samples [150]. Despite these advantages, several limitations of SPME must be considered. Variability between batches and manufacturers of SPME fibres often requires optimisation prior to analysis to ensure reliable results [152]. In addition, the intrinsic fragility of SPME fibres necessitates careful handling and conditioning procedures to avoid physical damage or unintended loss of sorbent coatings [153]. The choice of sorbent material may also introduce matrix interferences or competitive binding effects, particularly in complex environmental matrices [154]. Furthermore, the efficiency of analyte desorption can vary depending on analyte properties and matrix composition [154]. Šunta et al. [155] reported that SPME can be applied for detection of common polymers such as PET, PS, PVC and PP. However, uncertainties were observed in the identification of PE MPs in environmental samples with high hydrocarbon loads, due to similarities between PE thermal degradation products and hydrocarbon-derived compounds. They also highlighted that method robustness depends partly on manual procedures and operator experience during sample preparation and analysis. In heterogeneous samples containing polymers with different melting temperatures, polymers with lower melting points may degrade prematurely. To address this limitation, multi-step melting procedures involving several controlled heating stages were proposed [155].

For adsorption-based separation strategies to be analytically useful, regeneration and desorption processes must be considered, as they determine sorbent reusability and polymer recovery. These processes are also critical for the sustainability and large-scale applicability of sorption methods [156]. However, many adsorbents cannot be reused efficiently because their performance decreases after successive adsorption–desorption cycles [157]. In addition, ecological risks are associated with the handling, regeneration and disposal of MP- and NP-loaded sorbents, for which well-established protocols remain limited [158]. Leakage of adsorbent components into water bodies may also occur, as discussed for engineered functional materials in membrane systems [159]. Furthermore, regeneration processes are often energy-intensive and may involve the use of strong acids, bases or inorganic solvents, generating potentially hazardous liquid effluents [160]. Despite the recognised importance of desorption and regeneration, many studies provide limited information on the operational conditions used for sorbent regeneration, which hinders reproducibility and critical assessment of sorbent reusability.

Thermal regeneration involves heating sorbents to desorb or decompose retained adsorbates [161]. This approach, commonly applied to biomass-based sorbents such as biochar, typically relies on progressive heating under inert gas flow [162]. Although thermal regeneration can effectively remove adsorbed MPs and NPs, degradation of functionalisation agents and aggregated pollutants may generate secondary by-products such as CO, SO_2, HCl, NOx, highlighting the need for careful control of regeneration conditions [162].

Solvent regeneration represents an alternative strategy in which organic solvents are used to desorb MPs or NPs from the sorbent surface. Ethanol is among the most frequently reported solvents for this purpose [163]. Compared with thermal regeneration, solvent-based methods can better preserve the mass and porous structure of the adsorbents, although they may increase operational costs and reduce overall process sustainability [162].

Several studies have evaluated the recyclability of sorbent materials. Han et al. [137] investigated the regeneration of poly (dimethyldiallylammonium chloride)-modified magnetic bentonite (PDMMB), reporting PS nanoplastic removal efficiencies decreasing from 95.95% in the first cycle to 86.41% after five cycles. Tang et al. [164] regenerated magnetic carbon nanotubes by thermal treatment (400–600 °C under N_2 for 2 h), maintaining removal efficiencies above 80% over multiple cycles. Modak et al. [165] described the regeneration of a chromium-based metal–organic framework (Cr-MOF/MIL-101) using ethanol combined with dilute NaOH, although adsorption efficiency decreased after repeated cycles.

Overall, a comprehensive assessment of adsorption- and chromatography-based methodologies should consider adsorption efficiency, desorption performance and sorbent regeneration, as these factors determine their analytical applicability and operational sustainability. Although these techniques can serve as complementary tools for the separation and identification of MPs and NPs, their broader implementation remains constrained by several limitations, including limited selectivity, complex synthesis routes, insufficient validation in real environmental samples, fouling susceptibility and challenges associated with sorbent regeneration [166]. A detailed comparison of adsorption- and chromatography-based separation approaches is provided in the Supplementary Material (Table S5).

3.4. *Electrokinetic and electro-assisted methods*

Electrokinetic and electro-assisted methods separate particles

according to their charge-related properties under an applied electric field [167]. These techniques have shown good performance at laboratory scale, but broader applicability remains limited by low throughput and demanding operational requirements [168,169].

In electroflotation, particles attach to electrolytically generated bubbles and rise to the surface. However, bubble size and rise dynamics are difficult to control, frequently resulting in particle losses, and additional collector or oil-removal steps may damage particles or interfere with downstream analysis [77,170]. The use of saturated salt solutions has been proposed to improve recoveries in flotation-based systems [44].

In electrocoagulation, sacrificial electrodes generate coagulant species in situ that neutralise particle charge and promote aggregation. Although this provides more controllable aggregation than chemical dosing alone, electrode-derived species and local pH shifts can alter NP physicochemical properties and complicate subsequent analysis [171,172].

In electrophoresis, charged particles migrate through solid or liquid media under an electric field. Migration velocity depends on field strength, medium porosity and particle properties such as charge, size and mass [173]. Capillary electrophoresis often requires surfactants to regulate surface charge, but these can interfere with downstream NP identification [28]. While preconcentration improves performance [25], UV absorption and overlapping peaks limit resolution in NP mixtures, and particles larger than ~300 nm may clog capillaries or cause peak tailing [174]. A voltage-driven solvent-drop microextraction variant of capillary zone electrophoresis achieved recoveries of 47–60% for ~200 nm PS in tea matrices, providing rapid cleanup for subsequent quantification [175].

Electromembrane configurations apply an electric field across ion-exchange or nanoporous membranes to induce selective transport, offering membrane-level selectivity under electrical control. However, these systems currently operate at low throughput [175].

Overall, electro-assisted techniques offer clear advantages for charged or surface-modified particles, but limitations in throughput, ecological footprint and particle specificity restrict their standalone applicability. Their most promising role is in hybrid workflows that integrate electrokinetic steps with membranes, adsorption or solvent-based extraction to improve robustness and resolution in complex matrices. A detailed comparison of electrokinetic and electro-assisted separation methods is provided in the Supplementary Material (Table S6).

3.5. Comparative evaluation and recommendations

Accurate analysis of MPs and NPs depends critically on separation efficiency, which directly influences downstream identification and quantification. Variability in particle size, shape, polymer chemistry and matrix complexity further complicates this stage.

Membrane filtration is accessible and effective for larger particles. Hydrophobic membranes, such as geopolymer foams modified with silane coupling agents, can reach recoveries of ≈85% for micrometre-sized PS, although fouling and particle adhesion remain major barriers for NPs <100 nm [79,176]. Ultrafiltration provides precise molecular-weight cut-offs but is prone to fouling and incurs higher operational costs [177]. Density separation performs well for MPs but is less effective for NPs, especially for denser polymers such as PET and PVC [25]. Sodium chloride (1.2 g/mL) is a low-cost, benign option, whereas sodium iodide (~1.6 g/mL) improves recovery but increases cost and disposal complexity [45,108]. Flotation can support density-based workflows, but matrix interferences and reagent carry-over limit reproducibility [44]. Centrifugation and ultracentrifugation are widely used, but aggregation, overlapping densities and transfer losses may reduce selectivity at scale [115]. FFF provides the highest size resolution and is compatible with online detectors, but high cost, specialised expertise and low throughput restrict routine use [123]. Hybrid workflows combining FFF with membrane filtration, adsorption or solvent-based extraction can balance precision with practicality [120].

Solvent-based routes offer alternatives for hydrophobic MPs and NPs. Conventional LLE is limited by co-extraction of matrix constituents and risks of polymer softening or dissolution, whereas hydrophobic ES can achieve >95% recovery via hydrogen bonding, π–π stacking and hydrophobic interactions. Challenges remain with emulsion stability and scale-up [125,131]. Cloud-point extraction provides a milder approach by concentrating particles into a surfactant-rich phase, though performance is dose-dependent and surfactant residues may interfere with downstream analysis [130].

Adsorption methods offer flexibility across matrices. Magnetic nanoparticles enable rapid recovery, although efficiency depends on polymer type and surface functionalisation. Engineered bio-based sorbents provide tunability but may face selectivity or stability limitations under realistic conditions [136,137]. Chromatographic approaches, including SEC and HPLC, are valuable for polymer identification, but clogging and maintenance requirements limit use in particulate-rich samples.

Electro-assisted techniques expand the available toolbox. Electroflotation reduces reliance on added collectors but requires precise bubble control [77,170]. Electrocoagulation generates coagulants in situ with fewer added chemicals, though electrode species and pH shifts require careful management [171,172]. Electrophoresis provides high resolution for charged particles at low volumes when charge control is feasible [173]. Electromembrane systems deliver electrically driven selectivity but currently suffer from low throughput.

In summary, the effectiveness and scalability of each method vary considerably, and no single technique is universally applicable across matrices and size ranges. Table 1 and Fig. 9 summarise the principal separation methods, their indicative working volumes and associated costs. Future progress will depend on materials innovation, greener extraction media and the development of standardised operating protocols with clearly defined conditions [66,133,166]. These advances will improve robustness under real-world conditions and support more reliable assessment of the ecological impacts of plastic pollution.

4. Identification and quantification methods

Accurate identification and quantification of MPs and NPs remain major analytical challenges due to limitations in sensitivity, matrix interference and the lack of standardized workflows [13]. Despite significant progress, no single analytical technique captures all relevant dimensions of characterisation, including size, morphology, chemical composition and abundance [22]. As a result, multi-technique workflows are increasingly employed. However, even combined approaches may fall short when confronted with sensitivity limits, matrix interferences and constraints on scalability and representativeness [220]. This section reviews the main analytical methods, their principles, advantages and limitations, and highlights the gaps that continue to hinder reliable, reproducible and standardised analysis.

4.1. Size and shape characterisation

4.1.1. Light scattering techniques

Dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) are widely used for nanoparticle size determination, but both exhibit inherent limitations [195]. DLS estimates hydrodynamic diameter from Brownian motion [221], yet its extreme sensitivity to aggregation and polydispersity makes it unreliable in heterogeneous samples. NTA, which tracks individual particle trajectories, performs better in polydisperse systems but still requires particle concentrations rarely achieved in environmental samples [222].

To illustrate these limitations, DLS was applied to polystyrene (PS) suspensions at increasing concentrations. Polydispersity indices ≥0.5

Table 1
Overview of key separation methods (with working volumes and estimated cost).

Method	Description	Advantages	Limitations	Working volumes	Estimated cost	References
Membrane filtration	Size-selective exclusion	Accessible; dual purpose (preconcentration, separation)	Fouling; limited for particles <100 nm	Moderate–High	Variable	[60,82,83,85,87,88,150,175,178–189]
Ultrafiltration	Molecular-weight cut-off membranes	Precise cut-offs for small particles	Aggregation; higher cost	Low	Medium–High	[84,116,185,187,190,191]
Density separation	Buoyancy in salt solutions	Benign reagents (e.g., NaCl)	Ineffective for NPs, contamination risks	High	Low–Medium	[85,116]
Flotation	Bubble attachment (collector-assisted)	Simple, high throughput	Low selectivity, reagent carry-over	High	Low	[111,192]
Centrifugation/ ultracentrifugation	Sedimentation in a centrifugal field	Simple operation, preconcentration	Agglomeration, overlapping densities, transfer losses	Moderate	Medium–High	[49,112,118,193–197]
Field-Flow Fractionation (FFF)	Size separation under an external field	High precision, on-line detection	High cost, specialised equipment, low throughput	Very low	High	[66,120,193,198,199]
LLE (Conventional organic solvents)	Phase transfer into organic phase	Compatible with Py-GC/MS	Toxicity, co-extraction, low selectivity	Low–Moderate	Low–Medium	[200–202]
CPE (Surfactants)	Micelle encapsulation (non-ionic/cationic)	Mild, effective for hydrophobic polymers	Emulsion stability, surfactant residues	Moderate	Low	[49,86,118,196,197,202,203]
LLE (Eutectic solvents, ES)	Phase transfer into immiscible hydrophobic ES	Greener, tuneable interactions	Viscosity, emulsion stability, scale-up feasibility	Moderate	Low	[128,131,133]
Adsorption (magnetic nanoparticles)	Functionalised magnetic nanoparticles	Fast, easy magnetic recovery	Requires functionalisation, polymer-specific	Moderate	Low–Medium	[137,139–141,204–211]
Adsorption (advanced materials, biomass)	Biomass/MOFs/ Graphene Oxide sorbents	Tuneable surfaces, potentially sustainable	Limited selectivity, stability varies	Moderate	Low–Medium	[135,136,138,205,212–217]
Electroflotation	Bubble attachment generated by electrolysis	Low energy, reduced sludge	Bubble control, potential particle damage	Moderate–High	Low–Medium	[218,219]
Electrocoagulation	In-situ coagulant generation at electrodes	Tuneable aggregation, fewer added chemicals	Electrode species, local pH shifts	High	Medium	[171]

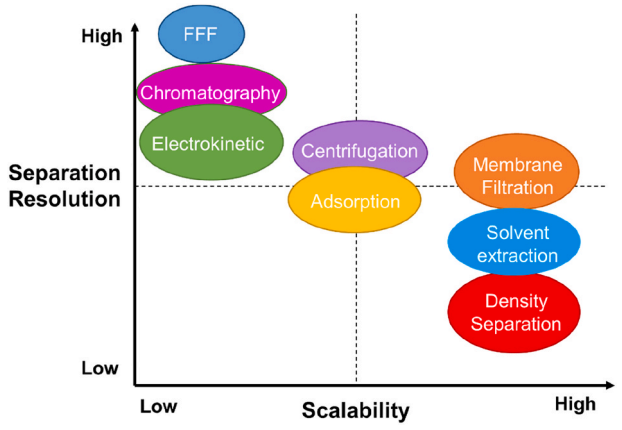

Fig. 9. Qualitative trade-off between scalability and separation resolution for major micro- and nanoplastics separation techniques. The positioning reflects the qualitative discussion presented in Section 3.5, where scalability relates to practical applicability and throughput, while resolution reflects the ability of each method to selectively separate particles across relevant size ranges.

indicated aggregation at all tested concentrations (Table S7). These observations reinforce the need for complementary methods to validate particle-size data under environmentally relevant conditions, where aggregation and matrix interferences are common [66,113]. Scattering techniques should therefore be treated as first approximations and validated with independent methods. Transparent reporting of polydispersity index (PDI) values, autocorrelation curves and NTA particle counts (within the optimal operating range of the instrument) improves reproducibility.

4.1.2. *Microscopic techniques*

Microscopy provides essential morphological information but is limited by low throughput and preparation artefacts [223]. Optical microscopy is inexpensive and accessible but cannot resolve particles below ~200 nm, thereby missing a substantial fraction of NPs. Scanning electron microscopy (SEM) offers high-resolution surface imaging but is susceptible to charging effects and preparation artefacts. Transmission electron microscopy (TEM) achieves even higher resolution and can reveal internal structures, yet it is labour-intensive, requires demanding preparation protocols and provides a narrow field of view, limiting its suitability for large datasets.

SEM analysis of PS particles (Fig. S3 and Table S8) revealed broad size distributions spanning the nano-to microscale, with pronounced aggregation and adhesion consistent with the heterogeneity observed by DLS. The limited number of particles typically analysed further compromises statistical robustness, meaning that microscopy alone cannot reliably represent whole populations [86]. Microscopy is therefore most effective when integrated with higher-throughput techniques that provide more representative particle statistics.

4.2. *Chemical identification and quantification*

4.2.1. *Spectroscopic methods*

Spectroscopic methods are indispensable for polymer identification, although their performance for NPs is constrained by weak signals, matrix interferences and limited representativeness. Fourier-transform infrared spectroscopy (FTIR) identifies polymers through characteristic vibrational fingerprints and performs reliably for MPs, but sensitivity declines sharply below ~100 nm due to low signal intensity and the need for concentrated deposits [22,224]. Nano-FTIR extends the detection range into the nanoscale but is strongly influenced by sample geometry and plasmonic artefacts, complicating spectral interpretation.

Atomic force microscopy infrared spectroscopy (AFM-IR) combines nanoscale topographical imaging with chemical specificity, enabling detection of functional groups in the 10–100 nm range. Developments such as quantum cascade lasers have improved sensitivity, yet the technique remains limited by small sampling areas, which restrict

representativeness in environmental assessments [25,28].

Raman spectroscopy provides higher spatial resolution than FTIR and can detect particles down to ~300 nm. Surface-enhanced Raman scattering (SERS) and tip-enhanced Raman spectroscopy (TERS) further extend detection limits but remain highly sensitive to substrate preparation, fluorescence artefacts and reproducibility challenges [225,226]. For particles <300 nm, careful preparation, cross-validation with polymer standards and fluorescence mitigation strategies are essential. Correlative Raman–SEM approaches have enabled identification close to the 100 nm scale [22].

X-ray techniques provide complementary information. X-ray photoelectron spectroscopy (XPS) resolves surface bonding states, while energy-dispersive X-ray spectroscopy (EDS), typically coupled with SEM, yields elemental composition. However, limited sensitivity to light elements constrains their applicability for most polymers [227,228].

Nuclear magnetic resonance (NMR) provides structural information at the bulk level, but conventional ^{1}H NMR requires high mass loadings often incompatible with NP yields. Sensitivity limitations and long acquisition times further restrict applicability, and pretreatment steps may exacerbate solvent interference or signal suppression [227,229].

UV–Vis spectroscopy offers a rapid, field-deployable approach for NP quantification. It is effective for stabilised colloidal dispersions, but performance is strongly limited by aggregation and polydispersity [230]. Reliable quantification is generally restricted to polymers with well-defined absorbance profiles (primarily PS). Weak absorbance and colloidal instability substantially restrict UV–Vis as a stand-alone method. In laboratory tests, UV–Vis applied to PS NPs extracted with hydrophobic ES revealed dissolution effects consistent with hydrogen bonding and π–π stacking [131]. High PDI values (>0.5) skew concentration estimates, although dispersants may improve stability. Cross-validation with complementary techniques is indispensable [113,231].

4.2.2. Thermal analysis and complementary MS approaches

Thermal methods remain central to the chemical fingerprinting of MPs and NPs. Differential scanning calorimetry (DSC) provides information on melting and crystallisation, although its applicability to NPs is limited by low concentrations and overlapping transitions among polymers with similar thermal behaviour [227,232].

Pyrolysis gas chromatography–mass spectrometry (Py-GC/MS) identifies polymers through characteristic thermal degradation products and can be adapted via sequential pyrolysis to improve detection of more polar polymers [233–235]. This approach is particularly suited for environmental samples, as it reduces matrix interferences and enables the analysis of polymers together with their additives using multi-shot programs [233,236,237]. In such workflows, an initial thermal desorption step allows selective analysis of plastic additives and co-extracted organic compounds, followed by a high-temperature pyrolysis step dedicated to characterisation of the polymeric matrix [233,236,237]. Although robust, the method relies on selective pyrolysis markers that are not always unique and may be affected by matrix components, complicating the interpretation of mixed or environmental samples.

Thermogravimetric analysis (TGA) monitors mass loss during heating and, when coupled with GC-MS or FTIR (TGA–GC/MS, TGA–FTIR), provides additional chemical information. However, automated spectral matching remains challenging in complex matrices due to overlapping signals and deviations of environmental spectra from reference profiles. This may lead to false positives or false negatives when inappropriate matching thresholds are applied [238]. These limitations reduce representativeness and hinder comparability across environmental datasets [229,239].

To address some of these challenges, several volatile-focused extensions have been developed. Thermo-extraction and desorption GC-MS (TED-GC/MS) provides more detailed compositional profiles of volatile degradation products, offering higher chemical resolution than Py-GC/MS, albeit with longer runtimes and greater expertise requirements [28,240]. Thermal desorption proton transfer reaction MS (TD-PTR-MS) further increases sensitivity, enabling near-real-time monitoring of volatiles with detection limits below 1 ng for polystyrene. Nonetheless, both techniques face issues such as low recoveries, environmental interferences and the absence of standardised reference materials for validation [22,241].

Additional MS-based techniques extend analytical capability beyond thermal decomposition. Matrix-assisted laser desorption ionisation time-of-flight MS (MALDI-ToF MS) enables rapid detection of high-molecular-weight polymers but provides no direct information on particle size or morphology. Single-particle ICP-MS (spICP-MS) enables particle-by-particle analysis for metal-doped NPs, yielding size and concentration data, although its use is restricted by the requirement for uniform doping and the lack of harmonised protocols [22]. Time-of-flight secondary ion MS (ToF-SIMS) provides nanoscale chemical imaging with very high spatial resolution, but it is resource-intensive and highly sensitive to matrix effects, limiting its suitability for routine analysis.

Overall, thermal and MS-based methods provide high chemical specificity and can partially mitigate matrix effects through programmed thermal desorption of surface-bound contaminants. However, destructive workflows and overlapping degradation profiles limit their standalone applicability for complex environmental MP/NP mixtures. Their analytical value increases substantially when integrated with spectroscopic, imaging or fractionation techniques and supported by appropriate reference materials and standardised protocols.

4.3. Spectroscopy and imaging integration

Hyperspectral imaging extends spectroscopic information into the spatial domain, generating chemical maps that enable morphological assessment comparable to SEM while reducing artefacts caused by uneven particle distribution [32]. Because most instruments cannot provide both morphology and chemical identity simultaneously, correlative imaging approaches that align μ-FTIR or μ-Raman with SEM in the same field of view are increasingly adopted, although they are not yet widely available or standardised [32].

Beyond visualisation, spectral decoding enables discrimination of chemical signatures in MPs and NPs. Classification can be achieved using artificial intelligence and machine learning (AI and ML), either through supervised approaches using labelled datasets or unsupervised methods such as principal component analysis (PCA). Deep-learning models are also being explored to improve robustness and accuracy. However, performance remains strongly dependent on large and diverse training datasets and on curated, standardised spectral libraries [242,243].

In laboratory evaluations, hyperspectral imaging applied to hydrophobic ES extracts exhibited several artefacts, including particle accumulation near filter edges, uneven distribution across the surface and losses during drying or transport (Text S1 in the Supplementary Material; consistent with laboratory trial observations, 2025). Manual counting of dyed particles proved unreliable due to inconsistent dye uptake and subjective classification criteria [96]. Automated analysis using Microplastics Visual Analysis Tool (MP-VAT) 2.0 reduces subjectivity and increases throughput, but may still introduce misidentifications on heavily stained filters, consistent with reports showing that fluorescent signals from particles can exceed those of alumina filters retaining them (Fig. S4 and Table S9 in the Supplementary Material) [89,244]. At low particle concentrations, μ-Raman spectroscopy remains the most reliable confirmatory method.

Continued progress will require improved dyes or alternative filter substrates, more robust algorithm training with benchmarked datasets and curated spectral libraries to support reproducible, high-confidence identification.

4.4. Challenges in the identification and quantification of micro- and nanoplastics

The identification and quantification of MPs and NPs in environmental matrices remain challenging due to sample complexity and intrinsic limitations of available analytical methods [227]. Critical constraints include matrix interferences, particle aggregation, limited nanoscale sensitivity and the absence of harmonised protocols [227].

Matrix interferences continue to represent a major obstacle. Natural organic matter, salts and mineral particles frequently overlap or obscure spectroscopic signals (e.g., FTIR, Raman, XPS, EDS), lowering sensitivity and increasing the likelihood of false positives [227]. Pretreatment steps such as digestion, filtration and liquid–liquid extraction are indispensable but introduce risks including polymer degradation, particle loss, morphological alteration and added analytical complexity [24,130]. Balancing matrix removal with polymer preservation remains essential.

Aggregation and colloidal instability pose particular challenges for NPs. Their high surface energy promotes clumping during sampling, pretreatment and analysis, which distorts results from dispersion-based techniques such as DLS and NTA [245,246]. Vibrational spectroscopies (μ-FTIR, μ-Raman) remain effective for MPs but cannot reliably detect particles below ~1 μm. Advanced nanoscale spectroscopies (AFM-IR, nano-FTIR, SERS, TERS) extend detection to 10–100 nm, although high costs, limited sampling areas and restricted availability still constrain widespread use [22,28,226].

Thermal and MS-based techniques (Py-GC/MS, TED-GC/MS, TD-PTR-MS) offer high chemical specificity but are destructive, suffer from overlapping degradation profiles and often require representative standards that are not widely available [233,238,247]. Recent ISO drafts define key quality control indicators for chromatography–mass spectrometry workflows, including calibration accuracy ($R^2 > 0.99$), method detection limits (MDLs), LOD, LOQ, recovery rates (80–120%), reproducibility (RSD <15%), and procedural blank verification to control cross-contamination [248].

Faster, lower-resolution techniques such as UV–Vis spectroscopy are sensitive to aggregation artefacts and lack molecular specificity. Imaging-based approaches, including hyperspectral imaging and correlative workflows, integrate chemistry and morphology but are limited by the absence of curated spectral libraries and validated algorithms [242,249].

Across all analytical modalities, three systemic gaps still persist: (i) the lack of standardisation, reflected in inconsistent pretreatment cut-offs, variable QA/QC practices and heterogeneous reporting units [44,51,250]; (ii) the absence of certified reference materials (CRMs), which are essential for calibration, recovery validation and inter-laboratory comparability [74,251,252]; and (iii) data-integration bottlenecks, as widespread adoption of artificial intelligence and machine learning depends on large, diverse and well-curated training datasets supported by robust external validation [45,253,254].

Table 2 and Fig. 10 summarise the principal analytical techniques, together with their spatial resolution, advantages and limitations. Because quantitative performance depends on the analytical technique, polymer type and matrix composition, direct comparison across methods remains challenging. To complement this overview, Table S10 provides a comparison of reported quantitative performance metrics, including limits of detection (LOD), limits of quantification (LOQ) and relative standard deviation (RSD) for selected analytical techniques. Future progress will require coordinated development of CRMs, international reporting standards and validated hybrid workflows that integrate high-throughput separation tools with high-precision chemical characterisation [16,255]. Such integration will be essential to improve reproducibility, comparability and the regulatory acceptance of MP/NP data in environmental monitoring [22,256,257].

Table 2
Summary of relevant techniques for MPs and NPs identification and quantification.

Category	Methods (examples)	Primary Use	Spatial Resolution/Applicability	Advantages	Limitations	Ref.
Light scattering	DLS, NTA, MALS	Size distribution and colloidal stability	Variable	Rapid, non-destructive	Sensitive to polydispersity, limited in complex matrices	[218,258–261]
Microscopy	SEM, TEM, AFM	Morphological analysis	Variable	High-resolution imaging	Labour-intensive, extensive preparation required	[247,249,262]
Spectroscopy	μ-FTIR, μ-Raman	Chemical identification	μ-FTIR: ~10 μm -hundreds μm μ-Raman: ~300 nm –1 μm	Reliable for MPs	Limited sensitivity for particles <1 μm	[263–266]
	AFM-IR	Nanoscale chemical + morphological analysis	10–100 nm	High resolution, dual functionality	Limited sampling area, high cost	[267,268]
	Nano-FTIR	Nanoscale chemical analysis	~50 nm	Enhanced sensitivity	Sensitive to sample geometry, expensive	[179,269]
	UV–Vis spectroscopy	Concentration assessment	Proxy for concentration in stabilised suspensions, requires calibration	Rapid, scalable for monitoring	Sensitive to polydispersity, limited in complex matrices	[133,270]
	XPS, SEM-EDS	Elemental/surface chemistry	~10–100 nm	Bonding + elemental detail	Weak sensitivity for light elements, matrix overlap	[271–273]
	NMR	Polymer structure (bulk)	Bulk	Functional-group insights	Low sensitivity at NP loadings, long acquisitions	[124,274]
Thermal analysis	DSC, TGA (TGA–GC/MS, TGA–FTIR), Py-GC/MS, TED-GC/MS, TD-PTR/MS	Crystallinity, stability, decomposition profiles	Bulk	Simple, robust, compositional fingerprints	Overlapping signals, destructive analysis, requires standards	[35,87,125,196,275–278]
Mass spectrometry	MALDI-ToF-MS, spICP-MS, ToF-SIMS	Polymer composition, surface chemistry	Variable	High chemical specificity, particle-level data (spICP-MS)	Destructive (Py/TED/TD), matrix overlap, labelling required, resource-intensive	[279–281]
Imaging	Hyperspectral imaging (AI/ML, PCA, DL), correlative workflows (μ-FTIR/μ-Raman +SEM)	Integrated morphology + chemistry	~100 nm–μm	Combines morphology & chemistry, higher throughput	Requires spectral libraries, high data complexity, not yet standardised	[282–287]

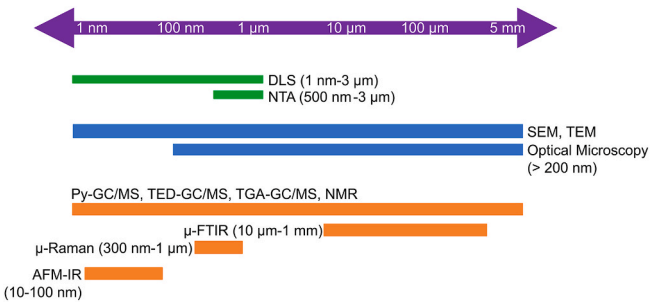

Fig. 10. Typical size ranges of analytical techniques used for the characterization of MPs and NPs.

5. Conclusions and perspectives

Despite substantial methodological progress since 2019, the analysis of microplastics (MPs) and nanoplastics (NPs) still faces important limitations. Improvements in sample preparation, particularly oxidative and enzymatic digestion, have enhanced matrix removal while reducing polymer degradation, yet maintaining a consistent balance between digestion efficiency and particle preservation remains difficult. These issues, together with persistent QA/QC constraints, continue to affect data quality. Although analytical workflows have evolved, separation remains the most critical bottleneck. Conventional approaches such as membrane filtration, ultrafiltration and density-based methods are widely used but show reduced efficiency for particles below 100 nm due to fouling, adhesion and aggregation. More advanced strategies, including field-flow fractionation (FFF), cloud-point extraction (CPE), hydrophobic eutectic solvents (ES) and electro-assisted methods, offer higher separation resolution, yet their performance remains limited by scalability, operational cost and robustness in complex matrices. Identification and quantification methods have also expanded, but no single technique can simultaneously capture size, morphology and chemical composition. Multi-technique and correlative approaches are increasingly adopted, although broader implementation is hampered by the lack of certified reference materials (CRMs), heterogeneous sampling and reporting practices and the scarcity of validated spectral libraries for AI-assisted analysis. Addressing these challenges will require harmonised procedures for sampling, pretreatment, reporting and QA/QC, supported by wider access to CRMs that cover environmentally relevant polymers and size ranges. Progress in separation will benefit from hybrid workflows that combine high-throughput preconcentration with size-resolved fractionation, ideally using greener and more robust media such as recyclable membranes, bio-based sorbents and hydrophobic ES. Advances in nanoscale identification are likely to come from integrating vibrational spectroscopies with emerging techniques such as AFM-IR, nano-FTIR and SERS or TERS, combined with correlative imaging to strengthen chemical assignment. Routine use of data-fusion and machine-learning tools will require curated spectral libraries and transparent model validation, while translation to environmental and regulatory contexts will depend on simpler, lower-cost screening tools coupled to confirmatory methods validated across diverse matrices. Together, these priorities outline a clear path for moving MP and NP monitoring toward reproducible, scalable and environmentally relevant analytical frameworks.

CRediT authorship contribution statement

Francisco T.T. Cavalcante: Writing – original draft, Visualization, Investigation. **Ana M. Ferreira:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Teresa Rocha-Santos:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Rilvia S. De Santiago-Aguiar:** Writing – review & editing, Funding acquisition. **João A.P. Coutinho:** Writing – review & editing, Supervision, Funding acquisition.

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Declaration of generative AI use

The authors used ChatGPT (OpenAI) for language editing and text polishing only. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the integrity and accuracy of the manuscript's content.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trac.2026.118823.

Data availability

No data was used for the research described in the article.

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