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A physical chemistry lens on environmental nanoplastics analysis challenges. Part I: why isolation is more complex than for microplastics?

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Nanoplastics (NPs) are emerging pollutants (EPs) which behave so differently to their micron-size counterparts that a new field of scientific interest is rapidly growing on the topic. The presence of these small plastic particles has been reported in a wide range of environments, from oceans to human organs. However, reliable quantitative data is sparse because their isolation from environmental matrices and subsequent detection faces technical limitation. Yet, baseline levels of NP occurrence are needed to understand their impact on the environment, on the biota at large, as well as on human health. This impact is feared to be more detrimental than that of microplastics, in part due to their high surface area to volume ratio which makes NPs, like other nanoparticles, very reactive. Their small size also means they can pass through living cell membranes and disrupt biological processes. To date, however, there is a lack of reliable information on the concentration and type of NP present in different environmental matrices to adequately study and address these issues. Most research has so far focused on micro-plastics whereas the isolation of sub-micron particles, and especially those smaller than 100 nm, remains a significant challenge. This review aims to highlight the current state of the art and the gaps in both knowledge and technology that are to be filled prior to a comprehensive study of NPs. Specifically, a focus on the unique physico-chemical properties of NPs is used to narrow down isolation methods most suited for subsequent NP detection, identification, and quantification. The expected outcome of this review is to provide researchers outside of the field of colloidal science a mechanistic explanation of the physico-chemical properties of common NPs necessary to achieve their selective capture.

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Environmental significance

Nanoplastics (NPs) are increasingly detected in the environment yet remain challenging to isolate and quantify due to their small size and complex behaviour. This review critically evaluates physical pre-treatment and separation techniques highlighting their operational limits and field applicability. By identifying practical recovery thresholds and matrix-specific constraints, it supports the development of robust analytical pipelines essential for environmental monitoring and risk assessment of NPs.

1 Introduction

Micro and nanosized plastics are found in a variety of environmental matrixes. They are known to be particularly prevalent in the marine environment due to their easy transportation *via* wind, coastal currents, and waves.¹ Microplastics (MPs, size range 1 μm to 5 mm) have also been found in wastewater, natural streams, and water reserves (rivers, lakes, oceans, *etc.*) as well as in soils.¹ The presence of nanoplastics (NPs, <1 μm in size) in the environment is not as

well documented, but they are expected to be present in the same locations as MPs, as well as in drinking water,² living organisms and even microorganisms.³ Emerging evidence further suggests that NPs occur in terrestrial and atmospheric compartments;^{4,5} however, their detection and quantification in soils and air remain technically challenging.^{6,7}

While all plastic pollution is problematic, different sizes of plastic particulates are associated with different mechanisms of environmental impact.⁸ To date, most studies have focused on macro- and micro-sized plastics. The literature is already rich in reports assessing the toxicological effects,³ pre-treatment methods^{9,10} and appropriate instrumentation¹¹ needed to detect MPs in aquatic environments. As a result, standard procedures and

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guidelines are becoming available – as detailed in part-II of this review –¹² and some industrial wastewater treatment plants (WWTP) already achieve up to 97% removal of >20 μm plastic particles using floatation, filtration, and membrane bioreactors.¹³ In contrast, data available on NPs' toxicity mechanism and their presence in commercial or environmental matrixes remain scarce.

In fact, the word “nanoplastic” only appeared in the literature in 1995 and while its occurrence has since increased exponentially (Fig. S1), the contribution of NPs in the overall plastic pollution issue remains difficult to estimate because of the significant analytical challenges faced when attempting to isolate, detect and characterize plastic particles at the nanoscale. Early studies have determined that NPs are potentially as dangerous to ecosystems as their larger counterparts, albeit *via* different transport and toxicity mechanisms.¹⁴ For instance, NPs may undergo long-range transport because of their small size and inflammatory responses have been reported in the lungs of rats exposed to small nylon fibres (2 μm).¹⁵ Together, these observations raise concern about inhalation exposure to NPs, particularly because inhalable nanoparticles are more broadly associated with adverse effects on the respiratory, cardiovascular and nervous systems, as well as with an increased risk of lung cancer.¹⁶ However, substantially more experimental data on NP inhalation are needed before robust conclusions can be drawn.¹⁷ Generating such data is challenging, because NP isolation from environmental samples is itself difficult. Failure to accurately isolate and analyse NPs in complex matrices such as air, soil, and biological samples can lead to significant underestimation of both environmental exposure and associated risks. Current limitation in NP isolation methods directly impacts our ability to assess the true scale of NP contamination and hinders the development of effective regulatory strategies. Without robust analytical techniques, remediation efforts may be misdirected or insufficient. Therefore, improving detection and quantification methods is crucial for both scientific understanding and policymaking.¹⁸ Yet, most experimental studies are still focusing on “model” purpose-made NPs which differ in shape and are not necessarily made of the same polymeric materials and additives as the most

common plastic in usage, and thus cannot be expected to exhibit the same behaviour as environmental NPs. The most common types of NP pollutants are given in Table 1, together with some of the commercially available “model” NPs.¹⁹ The former are made of the most commonly used polymers and can assume a wide variety of random shapes. In contrast, commercially available “model” NPs are typically spherical and cover a limited range of polymers, with polystyrene (PS) nanoparticles dominating the market. Their usefulness in helping our understanding of the environmental behaviour of NPs is not yet known. This limitation has increasingly been recognised by experts in the field and has prompted a growing body of work aimed at producing more realistic laboratory NPs. As these efforts mature, they may help bridge the gap between idealised model particles and environmentally relevant NPs, including through future commercial availability of improved reference materials.

Despite an increasing body of work, NPs are yet to be formally defined, with many scientists in the field ultimately debating “Do we really need to distinguish NPs from MPs?” and if yes, then on what basis is it appropriate to distinguish them? Should the separation between MPs and NPs be founded on physical or chemical properties (*e.g.*, structure, size, shape, composition, surface reactivity, *etc.*) or other factors such as degradation kinetics, toxicity, heterogeneity, and weight.^{25,26}

In the first section of this review, we discussed property-based distinctions between NPs and MPs (section 2). Next, we present methods that have previously been used for the isolation (section 3) of NPs along with their associated limitations. This leads us to identify a research gap which is described in (section 4) of this review followed by conclusions emphasizing the necessity for ongoing research to address environmental challenges and enhance accuracy in NP isolation and detection (section 5).

2 Nano-specific properties

Nano-specific properties relevant to the isolation and quantification of NPs are physical, chemical, mechanical, and optical in nature. All of which do affect the behaviour of NP particles in air/gas and fluid dispersions and other

Table 1 Common polymer types relevant to NP research, grouped according to their primary polymeric constituents. The table summarises the chemical composition of selected polymers, their commercial availability as model NPs (laboratory-produced), and their expected environmental occurrence based on the prevalence of corresponding macroplastic materials in the environment^{20–24}

Polymers (acronym)	Chemical formula	Commercially availability [example of suppliers]	Environmental occurrence
Polyethylene (PE): high density (HDPE)/low density (LDPE)	$(\text{C}_2\text{H}_4)_n$	Rare [CD Bioparticles]	High
Polypropylene (PP)	$(\text{C}_3\text{H}_6)_n$	Very rare [lab361; CD Bioparticles]	High
Polyethylene terephthalate (PET)	$(\text{C}_{10}\text{H}_8\text{O}_4)_n$		High
Polyvinyl chloride (PVC)	$(\text{H}_2\text{C}-\text{CHCl})_n$	Very rare [CD Bioparticles]	Moderate
Polisoprene	$(\text{C}_5\text{H}_8)_n$	No	Moderate
Polystyrene (PS)	$(\text{C}_8\text{H}_8)_n$	Common [Phosphorex Inc.; Abvigen; CD Bioparticles, Thermo Fisher]	Moderate
Poly (methyl methacrylate) (PMMA)	$(\text{C}_5\text{O}_2\text{H}_8)_n$		Rare
Polycaprolactone (PCL)	$(\text{C}_6\text{H}_{10}\text{O}_2)_n$		N/A

environmental matrixes, such as their tendency to adsorb other chemical contaminants, to resist sedimentation, to aggregate and to bind to lipid bilayers (Fig. 1a). Building on the property-based framework introduced by Gigault *et al.*,²⁶ this section focuses specifically on how nano-specific physicochemical properties constrain and inform isolation strategies. Rather than treating these properties as abstract descriptors, they are examined here as mechanistic drivers of isolation efficiency, selectivity, and failure modes, providing

the foundation for the isolation approaches discussed in section 3.

2.1. Physical properties

Size. NPs are often defined as plastic particles smaller than 1000 nm, with MPs consisting of plastic debris larger than 1 μm and smaller than 5 mm.²⁷ From an analytical perspective, this size difference alone already makes NP

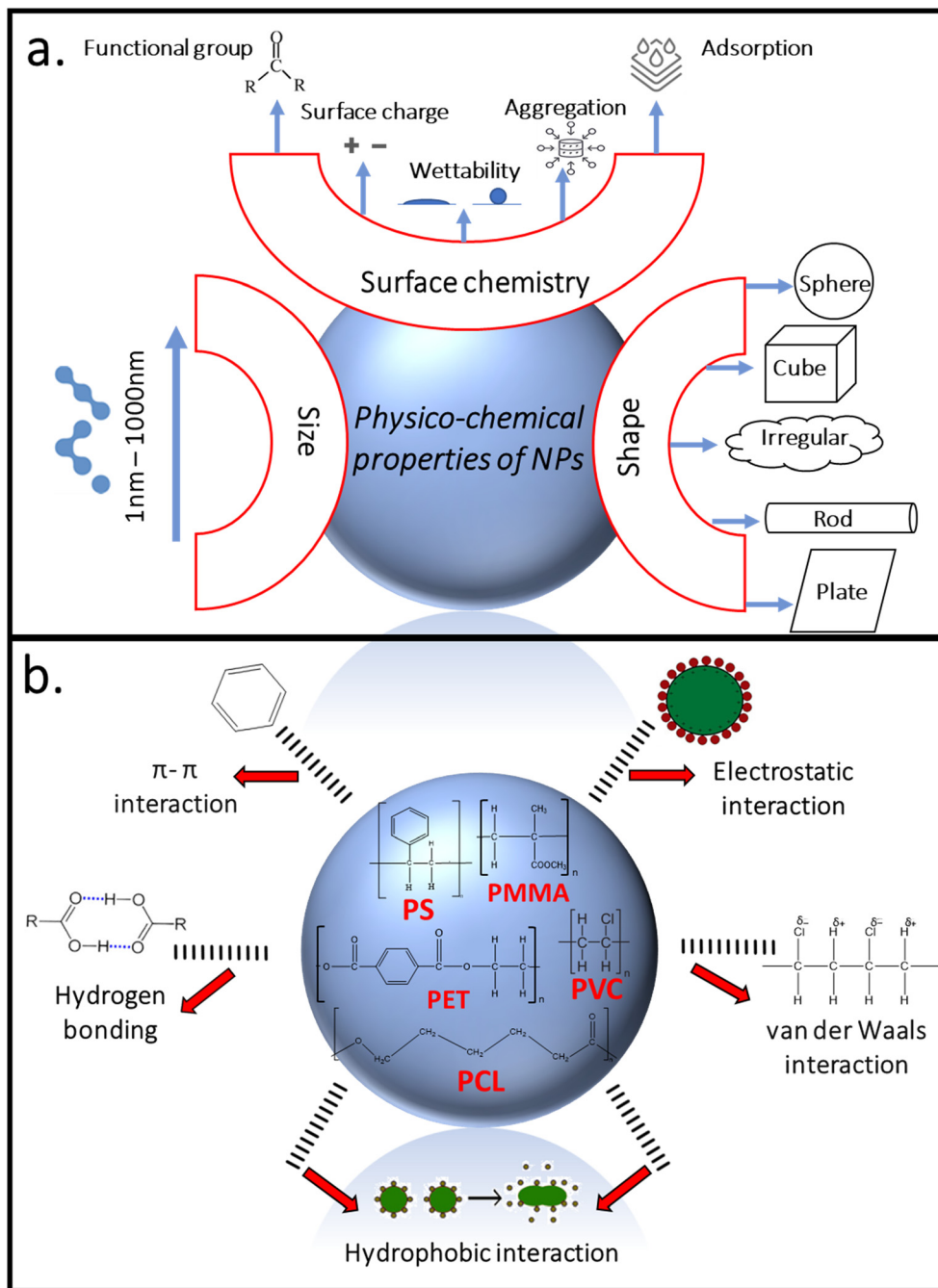


Fig. 1 a. Schematic of physico-chemical properties like size, shape, and surface chemistry of NPs adapted from ref. 32 with permission from MDPI, copyright 2022 and b. intermolecular interactions for most common NPs adapted from ref. 33 with permission from MDPI, copyright 2020.

isolation more difficult than MP isolation. Size-exclusion approaches such as filtration become increasingly demanding as particle size decreases, requiring finer membranes, higher operating pressures, and more advanced workflows, while the smallest fraction, especially below 100 nm, may be intrinsically under-recovered. In recent years however, researchers have started to differentiate MPs and NPs based on size-dependent properties rather than size alone. This is because their size imparts NPs with nano-specific chemical, mechanical, and optical behaviours that sets them apart from their micro-sized counterparts. Indeed, like other nano-objects, NPs 1) have a large surface to volume ratio, which increases their chemical reactivity, including their tendency to bind to contaminants, 2) have irregular and complex structures small enough to induce scattering of the light and 3) are subject to quantum effects rather than classical mechanics and thus act under different motion forces (*e.g.*, Brownian motion *vs.* gravity).²⁶ For these reasons, particles smaller than a few micrometres are very difficult to detect and isolate with the simple separation methods that are currently used for MPs. In natural environments, however, the expression of size-dependent physical properties of NPs is often moderated by low environmental concentrations and rapid heteroaggregation with natural colloids (proteins, polysaccharides, *etc.*), minerals, and biogenic particles, which can lead to the formation of eco-corona and reduce their effective mobility and persistence as discrete nanoscale entities. One could therefore wonder if truly nano – single and bare NPs – actually occur in the environment. To date this remains an open question.

Shape. Spherically shaped nanoparticles present comparatively fewer surface atoms than their rod-shaped counterparts. Surface atoms are more likely to engage in reactions with their surroundings, therefore elongated nanoparticles are typically more reactive than spherical nanoparticles. A study of three differently shaped silica nanoparticles (rod, short rod and spherical) demonstrated that rod shape nanoparticles had a longer residence time in the gastrointestinal tract than spherical nanoparticles.²⁸ Nanoparticle shape is also known to play a pivotal role in increasing their blood-circulation time through surface specific adsorption mechanisms.^{29,30} The implication for NPs, and more specifically for the oddly shaped secondary NPs, is that they have a higher tendency to stick to other contaminants than MPs, including natural organic matter, and biological material present in environmental matrices. Therefore, isolating NPs is even more difficult. It also means that they are potentially (depending on the actual chemical composition, as discussed in more detail in the section below on surface chemistry) more sensitive to chemical treatment commonly used to digest organic contaminants. While this sensitivity to chemical treatment adds challenges to the isolation of NPs for study purposes, it may well be an advantage in remediation context when it comes to treating the pollution itself. It is also worth noting that the shape

distribution of environmentally relevant NPs may itself evolve with decreasing size. Early studies on laboratory-generated secondary NPs suggest that the smallest particles may become increasingly rounded, which is consistent with the lower energetic cost and greater thermodynamic favourability of spherical morphologies relative to highly irregular shapes.³¹ If confirmed more broadly in environmentally weathered materials, this may partly moderate some shape-related effects at the very smallest end of the size range.

2.2. Optical properties

The detection of MPs currently heavily relies on their visualization *via* microscopy which is not easily transferrable to the detection of NPs that possess intrinsically different optical properties.³⁴ As the size of plastic particles, or indeed any particles, becomes of the same order of magnitude as the wavelength of light (400–800 nm), diffraction effects become more prominent, leading to increased scattering of light.³⁴ The scattering of light by nanoparticles in different directions creates a blurred or diffused appearance, reducing image clarity and resolution when using conventional optical microscopy.²⁶ This limitation is known as the diffraction limit, which refers to the bending of light waves as they encounter obstacles comparable in size to their wavelength. Diffraction effects can be particularly significant for irregularly shaped NPs because they provide many edges for the light waves to bend around and scatter in a range of directions. This phenomenon leads to changes in the intensity, polarisation, and phase of the scattered light.³⁵ As a result, nanomaterials and nanoparticles exhibit optical spectra at slightly shifted frequencies of emitted photons as compared to bulk materials.³⁵ This is one of the reasons NPs are particularly difficult to detect using standard optical microscopy.²⁶ In addition, unlike many inorganic nanoparticles, plastic particles are composed primarily of low atomic number elements, which limits intrinsic contrast not only in optical imaging but also in electron microscopy. This further complicates the visualization of environmentally relevant NPs, particularly when they are dispersed within complex natural matrices rich in heavy elements such as soils, as discussed further in part II.

2.3. Motion and interaction properties

The isolation of NPs from environmental matrices is difficult because for nanoparticles, gravitational and buoyancy forces are negligible compared to Brownian motion – the random motion of particles driven by thermal fluctuation and molecular collisions.²⁶ The dominance of thermal motion over gravitational settling at the nanoscale is not a new concept, but rather a well-established principle in colloid science. A detailed treatment is beyond the scope of this review, and interested readers are referred to the colloid literature;^{36,37} however, the key concepts most relevant to NP isolation are briefly summarised here for convenience. For NPs in environmental matrices, this means that conventional

separation approaches based on sedimentation or centrifugation are inherently less effective, particularly for the smallest fractions. At the same time, simple physical calculations remain useful for predicting behaviour such as settling velocity and therefore for guiding the design of isolation strategies.

A force balance between the downward gravitational force and the opposing viscous drag (Stokes drag) yields Stokes' law, which describes the terminal settling velocity of small spherical particles in a viscous fluid.

$$V = \frac{2(\rho_p - \rho_f)gR^2}{9\mu} \quad (1)$$

where V is the velocity at which particles sediment out of suspension, ρ_p is the particle density (kg m^{-3}), ρ_f is the fluid density (kg m^{-3}), g is the gravitational acceleration (m s^{-2}), μ is the dynamic viscosity of the fluid ($\text{kg m}^{-1} \text{s}^{-1}$) and R is the particle radius (m). A short scaling analysis of Stokes law, eqn (1), demonstrates how the settling velocity of particles in suspension is size-dependent: the smaller the radius of the particle, R , the smaller the velocity.³⁸ For instance, a 2 μm polystyrene bead (density $\rho \sim 1.05 \text{ g cm}^{-3}$) would settle in water (viscosity 0.001 Pa s) at a rate of approximately 0.4 mm per hour, while a 200 nm one would settle less than 0.1 mm in a day. Thus, the effect of gravity on small NP sedimentation is negligible. Instead, the aggregation behaviour of NPs is governed by other forces such as Brownian motion, which refers to the random movement of particles suspended in a fluid. It is driven by thermal fluctuation and results in collisions with surrounding molecules. Over time, this movement leads to diffusion. The magnitude of the Brownian motion is inversely related to the size of the particle and can be quantified by the Stoke–Einstein relation:

$$D = \frac{k_B T}{6\pi\mu R} \quad (2)$$

where the diffusion coefficient D ($\text{m}^2 \text{s}^{-1}$) represents how thermal fluctuations result in net particle displacement over time, k_B is the Boltzmann constant ($1.38 \times 10^{-23} \text{ J K}^{-1}$), T is the temperature (K), μ is the viscosity of the fluid ($\text{kg m}^{-1} \text{s}^{-1}$) and R is the radius of the particle (m). Comparing D with the gravitational settling velocity from the Stokes eqn (1), V , helps predicting the behaviour of NPs by determining which of the Brownian motion or sedimentation is dominant, through the Peclet number Pe :

$$Pe = \frac{V2R}{D} \quad (3)$$

When $Pe \gg 1$ settling dominates while Brownian motion dominates for $Pe \ll 1$, with both effects being comparable when $Pe \sim 1$, which often occurs for 1 μm plastic particles in water. In practice, calculation of the Peclet number can be useful during experimental design to determine whether settling is expected to occur under a given set of conditions. If so, eqn (1) can then be used to

estimate how rapidly settling may occur. Applied in this way, these concepts can help streamline experimental design and support a more rational selection of NP isolation approaches. Brownian motion also plays a significant role in providing NP stability in aqueous medium, together with other dominant nanoscale forces acting on nanoparticles such as van der Waals and electrostatic interactions which both affect surface adhesion, interparticle friction, and colloidal stability.³⁹ Although Brownian motion dominates NP behaviour in idealised systems, in environmental waters this effect is frequently superseded by NP aggregation, their attachment to suspended matter, or their incorporation into flocs, which shifts transport behaviour toward that of larger composite particles. Under conditions where NPs are brought into close proximity, such as during aggregation, filtration, or surface-based isolation processes, short-range structural forces can become significant. For instance, the structural force layer causes strong electrostatic repulsion between NPs when particle–particle separations fall below a few nanometres. This layer consists of ionic species that form around the NPs and come into existence when particle surfaces approach one another at very short distances. The estimated thickness of the structural layer present around NPs is between 11 and 18 nm.³⁹ At such separations, the arrangement of ions and molecules at the particle surface becomes increasingly important in determining interparticle interactions. These short-range forces are much stronger than the electrical double layer forces and can be either repulsive, attractive, or oscillatory which can create a barrier on the aggregation kinetics of NPs. In concentrated or confined systems, this phenomenon leads to a significantly higher critical coagulation concentration (CCC) for NPs than for MPs, that is the concentration below which particles are stable. High CCC increases their stability during transport.³⁹ Similarly van der Waals and electrostatic interactions are also expected to govern interactions between NPs and soil colloids or mineral surfaces, as demonstrated in laboratory studies using model nanoparticles.^{40,41} However, extrapolation to natural soils remains challenging due to the chemical and structural heterogeneity of environmental matrices. For example, Xu *et al.* showed that natural organic matter stabilises pristine NPs but destabilises photochemically weathered NPs,⁴² underscoring the difficulty of generalising the behaviour of model NPs to what takes place in complex environmental matrices. This complexity is reflected in several other recent soil-focused mechanistic studies. Astner *et al.* found that small 50 nm NPs remained more dispersed than larger 300–1000 nm NPs, which also agglomerated with a model vermiculite mineral phase.⁴³ Cai *et al.* showed that ionic composition, ionic strength and natural organic matter do not exert simple uniform effects on NP aggregation,⁴⁴ whereas Wu *et al.* reported a more direct trend in natural soils, with increasing ionic strength inhibiting NP transport and Ca^{2+}

promoting retention more strongly than Na^+ .⁴⁵ Chi *et al.* additionally demonstrated that NPs can become occluded within mineral phases, potentially limiting their accessibility and mobility in soil matrices.⁴⁶

Together, these studies are beginning to improve our understanding of NP behaviour in soils, but importantly they also show that variables such as particle size,⁴³ organic matter,⁴⁴ ionic strength and ion type influence how NPs adsorb to soil particles of different mineral compositions⁴⁶ in ways that are not straightforward, such that overall transport and retention cannot yet be predicted reliably. Specialist reviews covering the current state of the art for soil-focused research are available, although these generally address MPs and NPs together rather than focusing specifically on NPs.^{47,48}

2.4. Surface chemistry

Nanomaterials have a larger specific surface area compared to bulk, or even micron-size materials, resulting in a greater proportion of surface atoms. These surface atoms are comparatively unstable and reactive, making the surface chemistry of nanomaterials quite different from their bulk composition, due to surface oxidation, hydroxylation or other localised changes.⁴⁹ As a result, molecular interactions at the nanomaterial surface dominate interaction with the surrounding environment. The surface reactivity of nanomaterials, encompassing both atomic composition and chemical functions (*e.g.*, functional groups on the surface), is consequently a very important property that can be used to control the separation of NPs from mixtures containing larger particulates. Since NPs are polymeric nanoparticles, generally made from common organic monomers, their surface chemistry can be modified using various strategies to achieve functionalization (*e.g.*, change in surface charge) and potentially prompt aggregation to help with their recovery from environmental matrices.

Adsorption of contaminants (*e.g.* molecular species) to NPs can be classified based on the type of intermolecular interactions occurring between them. There are five main types of intermolecular interactions that enable NPs to act as effective sorbents. These are π - π interaction (*e.g.* interactions between aromatic rings), electrostatic interaction (interaction between charged molecules), van der Waals interaction (induced electrical interactions at a small distance), hydrophobic interaction (between nonpolar groups in an aqueous matrix), and hydrogen bonding (dipole-dipole attraction).³³ Fig. 1b shows how different classes of NPs are expected to exhibit the different types of interactions based on the chemistry of their constituting repeating units.

Like other colloids, plastics become more reactive and adsorbent when their surface to volume ratio is high. An interesting avenue for future work would be to gain a better understanding of NP surface chemistry and reactivity to control their specific binding to selected capture materials.⁵⁰ The high proportion of surface atoms is also the reason why

NPs are more difficult to isolate and potentially more dangerous than MPs, as it makes them more spontaneously reactive towards other additives and co-contaminants.²⁶

For instance, adsorption kinetics studies have shown that the adsorption of emerging micropollutants (ciprofloxacin [CIP] and bisphenol-A [BPA]) onto NPs occurs more rapidly than onto MPs under weathering conditions.⁵¹ In this work, the sorption capacity of polystyrene NPs (PSNPs) for both pollutants is higher than that of MPs, due to stronger hydrophobic interactions with these hydrophobic organic compounds. Aging was found to be another crucial factor boosting the affinity of both CIP and BPA for NPs. The mechanism underpinning adsorption in this case is intermolecular interaction, which is different for different pollutants. For BPA, hydrophobic interaction and π - π^* stacking are dominant, while the adsorption of CIP was governed by electrostatic interaction and hydrogen bonding. Aged-NPs exhibited greater interactions than both MPs and fresh NPs, because they presented more interfacial oxygen-containing groups. This example illustrates how interactions between micropollutants and plastic particles can differ substantially across MPs, NPs, and aged NPs, not only because of their inherent difference in size and shape but also their surface chemistry.

Aggregation is another key phenomenon often used for the isolation of MPs which could be extended to NPs. Aggregation is the result of particle-particle interactions, typically induced by an imbalance between the repulsion and attraction acting on these particles. As such, NP aggregation is governed by the same factor as adsorption kinetics, including shape, with spherical NPs having higher stability over anisotropic (direction-dependent) NPs.⁵² Other external factors affecting NP aggregation include ionic strength, pH, and temperature. According to the DLVO (Derjaguin-Landau-Verwey-Overbeek) theory, an increase in ionic strength leads to the compression of the electric double layer and decreases the zeta potential, rendering the particles less stable and more likely to aggregate.⁵² Singh *et al.* showed that PS NPs 240 nm in diameter are stable in a wide range of ionic strength in different environmental waters (ground, fresh and seawater).⁵³ They also found that an increase in temperature increases the kinetic energy of NPs, which in turn lowers their CCC. This decrease in CCC causes instability of the NPs and increases their tendency to aggregate in aqueous media.⁵³ Therefore, exploiting NPs' high surface reactivity and acting on external factors, such as ionic strength, through the addition of salt, or changes in temperature could be used as a strategy to induce aggregation where desired.⁵⁴ Neutralization of repelling charges can also be used to achieve aggregation easily.⁵⁴ Once aggregated, NPs are no longer nano in size and become easier to filter out from aqueous solution, or to isolate from soil and air matrices. While this can be a viable option to eliminate NPs from water samples, it is not a suitable approach to study the environmental occurrence of isolated NPs. It is also worth noting that in natural systems, intrinsic

NP surface chemistry is rapidly modified by the formation of organic and protein coronas, resulting in heterogeneous, environmentally conditioned surfaces that largely screen original surface charge and functionalisation, and govern subsequent interactions with biota and co-contaminants.

Overall, the forces affecting stability are somewhat different for NPs and MPs. For instance, structural layer forces, steric hinderance, intramolecular forces (not studied for NPs yet), and van der Waals repulsion forces become dominant for NPs because of their nano-specific physico-chemical properties described above, namely: shape, surface charge, functionality, structure and the most important one: size.

2.5. Bioavailability

Bioavailability refers to the extent to which particles can be taken up and accumulate in living organisms or ecosystems. NPs are more bioavailable than MPs due to their smaller size, higher surface area to volume ratio, and more reactive surface, as discussed in the previous section which together results in an increase in NP adsorption potential to toxic molecules, minerals, and microorganisms.¹⁹ The bioavailability of NPs reported in laboratory studies is therefore likely to be highly context-dependent in the environment, influenced by aggregation state, corona composition, and co-ingestion with organic matter, where NPs may act as incidental “fish-food” vectors rather than uniquely bioavailable particles.

MPs are of similar size to cells, while NPs are more closely scaled to proteins and organelles. As a result, the mechanism by which MPs and NPs interact with living organisms is different. MPs of polyethylene (PE), PS, and polymethylmethacrylate (PMMA) ranging from 1 to 10 μm were shown to attach to lipid membranes which caused destabilization of the lipid bilayer *via* mechanical stretching.⁵⁵ In contrast, NPs are small enough to penetrate cell membranes,⁵⁶ in particular, NPs smaller than 100 nm in size can be taken up by cells. This can occur through charge affinity destabilizing the lipid bilayer constituting the cell membrane, or other biological processes such as endocytosis. This is one of the reasons why nanosized plastics are more biologically active and capable of causing more adverse effects to living organisms than MPs.¹⁹

For instance, NPs < 50 nm transport into cells lining the intestinal membrane where they interfere with cell function.⁵⁷ Sub-micron plastic particles have been detected in human liver, brain and kidneys,^{58,59} and *in vitro* studies have reported that 20 nm PSNPs had detrimental effects on human liver cells and decreased cell viability.⁶⁰ Furthermore, studies have already shown that the uptake rate of NPs is dependent on their chemistry.⁶¹ Namely, 40 nm positively charged PS-NH₂ had higher uptake than negatively charged PS-COOH.⁶² Therefore, different classes of plastics could have different toxicity levels.

NPs can also breach the blood–brain barrier (BBB), as evidenced by two significant studies.^{63,64} The first study

demonstrated the rapid entry of polystyrene NPs (PS-NPs) (down to 0.293 μm) into the brain within 2 hours of ingestion by mice. Molecular dynamics simulations highlighted the role of cholesterol adsorbed onto NPs in enhancing BBB uptake.⁶³ The second study focused on 50 nm PS-NPs, revealing their potential to elevate BBB permeability and accumulate in the mouse brain, suggesting a propensity for neurotoxic effects through microglia activation. These collective findings underscore important aspects of NPs' bioavailability and uptake, however, direct comparison with MPs remains limited.⁶⁴

3 Isolation approaches

Current methods employed for the isolation of NPs are often the same as those used for MP isolation (Fig. 2), even though they may not be the most appropriate. While research efforts are ongoing to develop novel and efficient isolation methods specifically suited to the isolation of NPs from environmental samples, some pre-treatment approaches that have been proposed to date are reviewed in sections 3.1 to 3.3. These pre-treatments can be broadly classified into three categories (Fig. 2): purification (chemically remove bulk impurities such as organic matter), preconcentration (reduce the overall sample volume while maintaining the same number of NPs), and separation (uses physical methods to isolate NPs from other impurities). The advantages and limitations of each method described in section 3 are summarised in Table 2, together with the application range, reported enrichment factor and examples of the downstream analytical technique they are typically used with.

3.1. Purification

Digestion is the gold standard purification method for MPs. This process consists of dissolving the organic matter that is coexisting with plastic particles in environmental samples, including soil, plants and other biological samples.^{65,66} Digestion methods commonly use acidic (HCl, H₂SO₄, HNO₃), alkaline (KOH, NaOH), oxidative (NaClO, H₂O₂, FeSO₄),⁶⁷ or enzymatic (papain, trypsin, cellulose, collagenase, proteinase-K, Corolase 7089, chitinase) agents⁶⁸ to break large organic matter into smaller molecules and extract MPs from complex matrices like wastewater (Fig. 3). Alkaline and enzymatic digestion can isolate 97% of MPs from water.⁶ Due to its high recovery efficiency for MPs, digestion has also been attempted for NP extraction. For instance, enzymatic digestion has been employed to extract polyethylene terephthalate (PET), polyethylene (PE), polystyrene (PS) and polyvinyl chloride (PVC) particles from fish and oyster tissues.⁶⁹

There are significant advantages of using digestion as a primary treatment: it is affordable and relatively fast. However, digestion remains an intrinsically destructive pre-treatment method. Much like the target organic matter, plastics are made of organic repeating units which are also sensitive to acids and bases. Degradation is likely

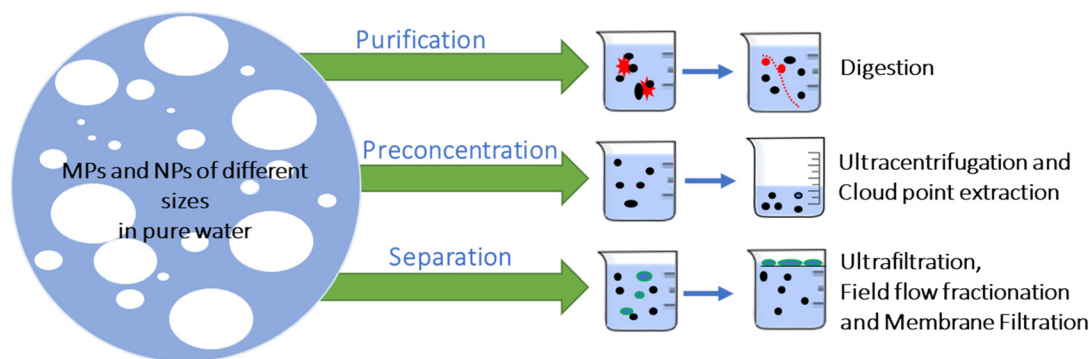


Fig. 2 Most common pre-treatment methods used for MP/NP isolation.

to occur during extended digestion times, which would result in sample loss. While sample loss may not be significant for MPs, it could become an issue with NPs, due to the higher surface area being directly exposed to the digestion agents.⁹ In practice, this means that NP loss during digestion cannot be assumed negligible, particularly if digestion conditions are not carefully optimised and controlled. Such losses could skew both particle counts and apparent size distributions. Recovery experiments should therefore be performed wherever possible, ideally using spiked samples containing model or laboratory-generated NPs with known properties. More comparative work reporting the extent and rate of NP damage under different digestion conditions would also be valuable for establishing more reproducible and transferable protocols. At the same time, a universal digestion workflow is unlikely to be achievable, as polymer-specific susceptibilities and matrix complexity will inevitably require some degree of case-by-case optimisation.

Enzymatic digestion, while more expensive, may be a more suitable option than chemical digestion for removing biological matter from samples containing NPs because it is less aggressive. This method of digestion is able to provide a better compromise between digestion efficiency and sample integrity, especially when dealing with samples containing high proportions of biological matter, such as sludges from water treatment plants.⁷⁰ Digestion can also be an effective way to separate micron-sized agglomerate of NPs, into smaller clumps, or individual particles. This aspect is often overlooked, yet a potentially essential step in studies concerned with determining the number and size of NP particles in complex environmental samples – where agglomerates could be mistaken for MPs. It should also be noted that digestion alone cannot remove all background materials. Inorganic particles such as sand, silts, and mineral colloids are not digestible and must instead be addressed through subsequent concentration or separation steps based on other properties, such as size or density.

3.2. Concentration

Ultracentrifugation (UC) is a preconcentration method used to increase the concentration of particles using rotational

speed. It accelerates the sedimentation of NPs by applying centrifugal forces through high-speed rotation. Just as gravity provides the driving force in the Stokes settling velocity equation (eqn (1)), the equivalent driving force in a centrifuge is the centrifugal force, given by:

$$F = m \times \frac{v^2}{r} \quad (4)$$

where m is the mass of the particle, v is its tangential speed (*i.e.* due to rotor rotation), and r represents the distance of the moving body from the centre of rotation. This expression describes the net outward force acting on the particle due to rotation.

To adapt the Stokes force balance for centrifugation, the gravitational acceleration g in eqn (1) is replaced with the equivalent centrifugal acceleration, expressed in terms of the relative centrifugal force (RCF) as $\text{RCF} \cdot g$. Substituting this into the original Stokes equation gives the modified expression for settling velocity under centrifugation:

$$V = \frac{2(\rho_p - \rho_f) \text{RCF} \cdot g R^2}{9\mu} \quad (5)$$

This equation illustrates that increasing the applied RCF allows even nanoscale particles to sediment at practical rates. For MPs (especially $>3 \mu\text{m}$) simple centrifugation with a speed of 2000 RCF is sufficient to settle particles in a reasonable time, but for NPs higher speeds are necessary. This requires the use of more expensive specialized instrumentation capable of reaching ultracentrifugation speeds. For example, spherical polystyrene particles in the 600–1000 nm range were efficiently sedimented using ultracentrifugation at 41 886 RCF over 2 hours. The method enabled ~ 50 -fold enrichment of fragmented NPs from river water, yielding a recovery rate of 87.1% and demonstrating its effectiveness for detection of NPs in environmentally relevant samples.⁷¹

In another instance, quantitative removal of ~ 200 nm particles from micrometre-sized suspended particulate matter has been recorded using 30 000 RCF in a continuous flow centrifugation (CFC) system (schematic CFC, Fig. 4(a)).¹⁰ CFC is a variant of centrifugal processes which has been reported

Table 2 Practical operating windows for NP detection methods. Abbreviations: IR/FTIR – Infrared/Fourier Transform Infrared Spectroscopy; SEM – Scanning Electron Microscopy; Raman – Raman Spectroscopy; Py-GC/MS – Pyrolysis Gas Chromatography/Mass Spectrometry; UV-Vis – Ultraviolet-Visible Spectroscopy; ICP-MS – Inductively Coupled Plasma Mass Spectrometry; TGA-MS – Thermogravimetric Analysis – Mass Spectrometry; TEM – Transmission Electron Microscopy; FESEM – Field Emission Scanning Electron Microscopy; DEP-Raman – Dielectrophoresis-Raman Spectroscopy; UV-SLS – UV and Static Light Scattering; MALS – Multi-Angle Light Scattering; DLS – Dynamic Light Scattering

Purification (digestion)	Technique	Medium			Reported enrichment factor	Advantage	Limitations	Examples of downstream characterisation technique	References
		Water	Air	Soil					
Purification (digestion)	Acidic	✓	✗	✓	—	<ul style="list-style-type: none"> Affordable Fast Effective removal of interfering organic matter 	<ul style="list-style-type: none"> Destructive Risk of sample loss Partial degradation or alteration of NPs 	<ul style="list-style-type: none"> IR/FTIR SEM Raman Py-GC/MS 	9, 67
	Alkaline Oxidative	✓	✗	✓	—	<ul style="list-style-type: none"> Suitable for biological samples 	<ul style="list-style-type: none"> Expensive Time-consuming 	<ul style="list-style-type: none"> FTIR Fluorescence quenching 	9, 76
	Enzymatic	✓	✗	✓	—	<ul style="list-style-type: none"> Simple Preserve NPs integrity Non-chemical method 	<ul style="list-style-type: none"> Limited sample volume Time and equipment-intensive Risk of incomplete separation Risk of aggregation 	<ul style="list-style-type: none"> SEM UV-Vis Hyperspectral imaging 	71
Concentration	Ultracentrifugation	✓	✗	✗	~50	<ul style="list-style-type: none"> Affordable Simple Non-chemical method 	<ul style="list-style-type: none"> Limited selectivity Requires optimization Not compatible with all NPs 	ICP-MS/(MS)	10
	CFC	✓	✗	✗	~10–16	<ul style="list-style-type: none"> Simple Non-chemical method 	<ul style="list-style-type: none"> Risk of sample loss/aggregation Limited selectivity Co-isolation of natural colloids 	<ul style="list-style-type: none"> Py-GC/MS TGA-MS Fluorescence microscopy 	72, 77
Separation	Centrifugation/density separation	✓	✗	✓	~3–20	<ul style="list-style-type: none"> Simple Non-chemical method Scalable 	<ul style="list-style-type: none"> Limited selectivity Surfactant interference Requires heating step 	<ul style="list-style-type: none"> Py-GC/MS TGA-MS Fluorescence microscopy Py-GC/MS TEM 	78
	CPE	✓	✗	✗	500	<ul style="list-style-type: none"> Affordable Simple Preserves NPs integrity Suitable for trace levels 	<ul style="list-style-type: none"> Risk of sample loss/adsorption Time-consuming Potential NP aggregation Risk of incomplete separation 	<ul style="list-style-type: none"> SEM Py-GC/MS 	79, 80
	Ultrafiltration	✓	✗	✓	—	<ul style="list-style-type: none"> Preserves NPs integrity Non-chemical method Scalable Reproducible Size-based separation 	<ul style="list-style-type: none"> Risk of sample loss/adsorption Requires optimization Sensitive to pH/ionic strength Complex setup Issues with sticky/polydisperse samples 	<ul style="list-style-type: none"> SEM Py-GC/MS UV-Vis/UV-SLS FESEM DEP-Raman 	5, 81, 82
Field flow fractionation	Field flow fractionation	✓	✓	✓	—	<ul style="list-style-type: none"> Fast Preserves NPs integrity Reproducible Detects aggregation/eco-corona Green method 	<ul style="list-style-type: none"> SEM Py-GC/MS UV-Vis/UV-SLS FESEM DEP-Raman MALS DLS 	<ul style="list-style-type: none"> SEM Py-GC/MS UV-Vis Laser light scattering Gravimetric analysis 	83–85
	Membrane filtration	✓	✓	✓	9–25	<ul style="list-style-type: none"> Affordable simple Non-chemical method Scalable Size-based separation 	<ul style="list-style-type: none"> Risk of sample loss/adsorption Membrane fouling issues Blank control challenges Matrix limitation 	<ul style="list-style-type: none"> SEM Py-GC/MS UV-Vis Laser light scattering Gravimetric analysis 	83–85

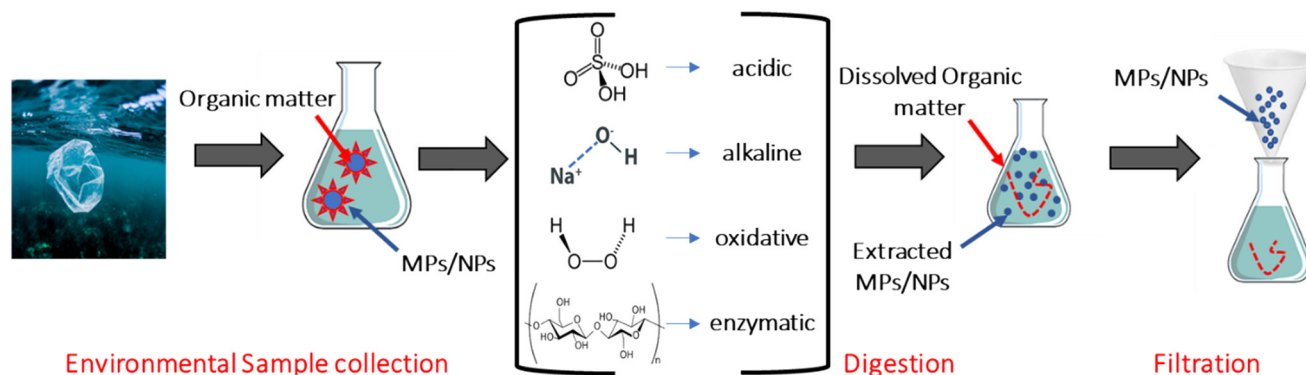


Fig. 3 Schematic representation of the digestion method for removing organic matter from environmental samples.

as an efficient method for NP concentration. CFC differs from UC in that large volumes of material can be centrifuged under high centrifugal forces without the need to repeatedly fill and decant centrifuge tubes, or frequently start and stop the rotor. Hildebrandt *et al.* demonstrated successful size and density based preconcentration and separation of Pd-doped polystyrene NPs from river water samples, using CFC operated at 2000 to 4000 rpm.¹⁰ It is worth noting that in this particular case, the presence of Pd increases the NPs' overall density, which would improve the separation efficiency of the centrifugal process. The closer the NP density to that of the surrounding medium, the less effective the centrifugation.

In fact, isopycnic (density gradient) centrifugation is yet another type of centrifugation that can also be applied to nanoparticles (NPs).⁷² It has been widely used in biology for isolating nanosized vesicles and exosomes and has shown promising results for MPs.⁷³ In this method, particles are separated based on their density by centrifugation through a fluid gradient (*e.g.*, sucrose, CsCl). Each particle moves until it reaches a position in the gradient where the fluid density equals its own – its isopycnic point – and remains there.⁷⁴ In one study, the impact of rotor geometry on vesicle sedimentation was determined and a practical method and online calculator for protocol standardisation were introduced.⁷⁵ This technique and similar calculation can be applied to NPs as it is capable of resolving materials that differ in density by as little as 0.0032 g cm^{-3} . This means that it could easily separate NPs made of PET ($1.34\text{--}1.40 \text{ g cm}^{-3}$), nylon ($1.13\text{--}1.15 \text{ g cm}^{-3}$), polystyrene ($1.00\text{--}1.06 \text{ g cm}^{-3}$), and polyethylene ($0.92\text{--}0.96 \text{ g cm}^{-3}$), as their density differences exceed this threshold by a substantial margin. With a careful experimental setup, it could potentially also be used to isolate more closely spaced materials such as PMMA from nylon ($1.18\text{--}1.20$ vs. $1.13\text{--}1.15 \text{ g cm}^{-3}$, $\Delta\rho_{\text{min}} = 0.03$) and maybe even polyethylene from polypropylene ($0.90\text{--}0.91 \text{ g cm}^{-3}$), or PVC from PET ($1.38\text{--}1.45$ vs. $1.34\text{--}1.40 \text{ g cm}^{-3}$) in some cases. This principle could also be advantageous for separating plastic NPs from non-plastic mineral particles that cannot be removed by digestion alone. For example, nanoscale sand, silicates, or other dense inorganic particulates should in theory partition differently within the

gradient than most common polymers, therefore potentially offering a route to reduce persistent inorganic background in environmental samples.

In essence, conventional centrifugation approaches are generally not suitable to isolate NPs from other nanoscale impurities of comparable density. Organic matter, such as protein aggregates, may co-sediment in the same volume or gradient fractions as NPs. Some valuable contributions have been made to alleviate this issue by integrating ultracentrifugation methods with chemical and enzymatic digestion for seawater or drinking water.¹⁰ For instance, microwave-assisted acid digestion (MWAD) has been used to trace and extract Pd-doped PS-NP particles ($\sim 160 \text{ nm}$) from ultrapure and river water, with 90% and 75% recovery efficiency respectively after CFC.¹⁰

Cloud point extraction (CPE) is another concentration approach which consists of adding surfactants to a suspension to lower the surface tension of the liquid and solid phases and induce phase separation. The term “cloud point” refers to the temperature at which a transparent suspension undergoes phase separation, thus becoming cloudy. In the context of achieving NP concentration, the cloud point corresponds to the stage where the organic/inorganic substances separate from the aqueous solution upon surfactant addition. Reportedly, Triton X-45 (TX-45) has been used to isolate trace PS (66.2 nm) and PMMA (86.2 nm) at $190 \text{ }^\circ\text{C}$ resulting in a 96.6% maximum average recovery of PS and PMMA (Fig. 4(b)). Herein, TX-45 was used as a non-ionic surfactant having polar heads which covalently bind with inorganic or organic compounds.⁷⁸ It is worth noting that while CPE may help concentrating NPs, it also introduces surfactants that may complicate subsequent chemical analysis of NPs. This is particularly relevant for mass spectrometry-based approaches, where residual surfactants or their degradation products could generate signals that overlap with, obscure, or be mistaken for polymer-derived fragments. CPE may therefore be most useful in workflows aimed primarily at particle enrichment prior to counting or sizing, rather than in methods where downstream chemical identification relies on clean molecular or thermal degradation signatures.

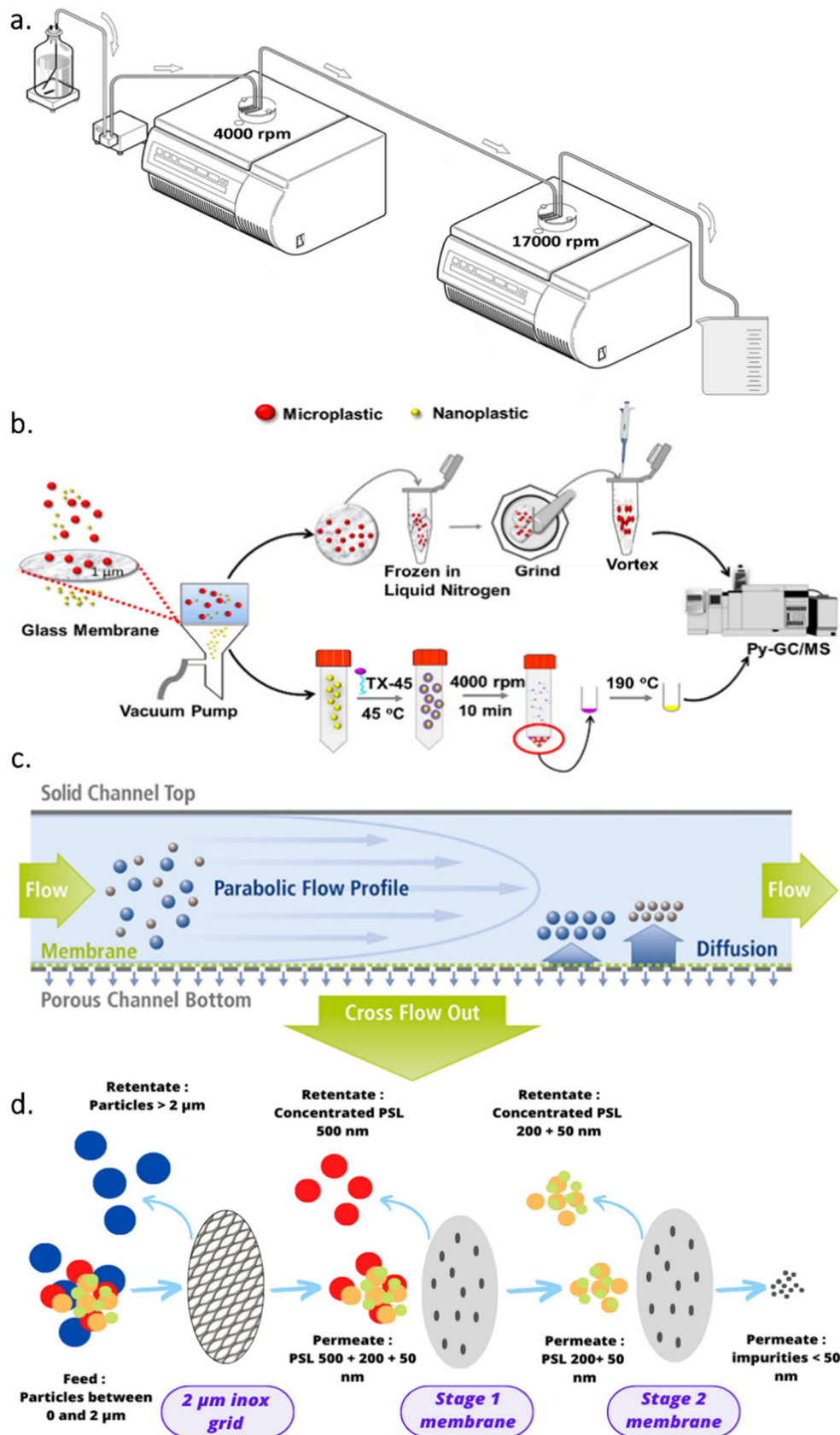


Fig. 4 Schematic of a. continuous flow centrifugation (CFC) method in suspension from low speed to high speed based on velocity settling reproduced from ref. 10 with permission from Frontiers, copyright 2020. b. Cloud point extraction using Triton X-45 for analysis reproduced from ref. 88 with permission from American Chemical Society, copyright 2021. c. AF4 reproduced from ref. 89 with permission from Frontiers, copyright 2015, and d. multi-stage filtration process reproduced from ref. 83 with permission from Elsevier, copyright 2025.

In a recent study, efficient preconcentration and matrix clean-up of NPs from samples were achieved in just 5 minutes using an asymmetric micro-electro membrane extraction method, referred to as μ -EME, that used a free liquid membrane in a conical micropipette tip. The enrichment factors reached over 20 and recoveries exceeded 70%. The technique was used to effectively remove interfering compounds from black tea while demonstrating good sensitivity (LOD: $6.00 \times 10^{-4}\%$ w/v) and repeatability in detecting NPs.⁸⁶

Although preconcentration methods are to some extent capable of enriching NPs into a smaller water volume, they have the disadvantage of 1) being reliant on heavy machinery and/or chemical additives; 2) requiring a time consuming sample handling procedure to limit cross-contamination, and 3) not effectively separating NPs from other like-sized contaminants.

3.3. Separation

Separation involves the removal of one substance from a mixture, most commonly using filtration through a porous membrane. Separating solids in suspension through a barrier can be driven by several forces, such as mechanical pressure used in membrane filtration, microfiltration, ultrafiltration, or diffusion used in field flow fractionation (FFF).⁸⁷ Here, the use of these filtration processes is discussed in the context of extracting NPs dispersed in fluids. However, separation techniques such as ultrafiltration and FFF, have also been applied to isolate NPs originating from solid matrices (e.g. soils and plant roots), following an initial water extraction or digestion step to transfer the NPs into a fluid phase suitable for separation.^{5,79}

Ultrafiltration (UF) is distinguishable from gravity driven filtration approaches in that it separates fine particles from water by exerting pressure on the water. Semipermeable membranes are typically used in UF and block all solid particles. The very small size of the pores of the UF membrane (less than 1 nm) demands additional pressure to force only water through. This type of membrane can be made of different types of materials depending on the type of target impurity, such as cellophane, polyvinyl alcohol, polyurethane, polytrifluorochloroethylene, *etc.*⁹⁰ UF has been successfully used to concentrate NPs from seawater. For example, a nano-porous membranes made of poly (ether sulfone) was used to remove NPs PET, PE, PS and PVC 5–50 nm, and a polysulfone-based cell was used to remove polyethylene NPs of 1 to 900 nm.⁸⁰

Field flow fractionation (FFF) was originally developed for separating and characterizing engineered micro- and nanoparticles. FFF separates particles based on their size, composition, and shape. A modern version of FFF, referred to as asymmetric flow field flow fractionation and commonly abbreviated as AF4, has been used for NP separation in environmental matrices.⁹¹ The setup used for AF4 includes two cover plates (top and bottom) made of stainless steel

separated by a flow channel. In the case illustrated in Fig. 4(c), the top channel and bottom channel were made of PMMA, and a cellulose membrane was placed on the bottom channel to separate the particles based on their size. The separation principle is diffusion based in which a parabolic field flow triggers the small particles to exit from the channel quicker than larger ones.²⁷ This method was used in association with enzymatic digestion for the separation of 1–100 nm PS beads from fish tissue samples.²⁷ While the method achieved good results for PS, it failed to detect polyethylene NPs in the same fish samples due to light scattering effects, which made the polyethylene NPs invisible to the multi-angle light scattering detector used. These results show that further research is needed towards novel methods for the identification of different polymer types.⁸²

Membrane filtration is a process widely used for the separation of NPs from complex matrixes based on their size. For example, a 2.5 μm cellulose filter has been used to separate nylon-66 and PET NPs (10–400 nm approx.) from micron-sized plastic obtained from the thermal decomposition of a teabag.⁹² In this study SEM, FTIR and X-ray photoelectron spectroscopy (XPS) were subsequently used to detect the NPs on the filter (see part II of this review for details on these detection methods).⁹² In 2017, the same research group used membrane filtration to separate MPs and NPs (PE microbeads <100 nm) from facial scrub; they reduced clogging using a sequential filtration process which consisted of membranes with progressively smaller pore sizes (*i.e.*, 25 μm , 2.5 μm , 0.45 μm and 0.1 μm).⁹³ In a different study on sequential filtration, a multi-stage filtration system was employed to retain particles ranging from 0 to 2 μm . This system used a 2 μm inox grid, a 0.45 μm PVDF membrane, and a 0.1 μm polyethersulfone (PES) membrane. While 52% of 500 nm PS NPs were recovered for first-stage filtering, with satisfactory repeatability (SD = 7%), 95% of 200 nm and 93% of 50 nm PS NPs were recovered for the second and third stage filtering, Fig. 4(d).⁸³ Beyond liquid-phase separations, filtration is also the primary approach for sampling airborne micro- and NPs. In atmospheric applications, however, filtration is not simply a separation step but also part of the sampling process itself, meaning that the properties of the sampler, sampling location and environmental setting can strongly influence what is ultimately detected. Higher particle counts are often reported in urban environments, indoors, or close to emission sources (e.g. near busy roads), and at lower sampling heights, than in more rural or elevated locations. The type of sampler—such as high-volume air pumps for active sampling or open funnels and Petri dishes for passive collection—also influences particle capture efficiency, as reviewed in detail elsewhere.⁹⁴ As a result, direct comparison between studies remains difficult unless these parameters are clearly described and, ideally, harmonised.

In airborne NP studies, particles are commonly collected by active air sampling using pumps coupled to porous membrane filters or size-segregating devices such as cascade

impactors, enabling isolation of particle fractions across defined aerodynamic size ranges.^{95–97} Filter choice is particularly important for the submicron fraction. For example, Rahman *et al.* showed that methods developed for larger airborne microplastics could not be transferred uncritically to particles below 2.5 μm , and that some filter substrates, specifically silver membrane filters, proved unsuitable for combined collection and spectroscopic analysis of the smallest particles.⁹⁷ Following collection, particles retained on filters often need to be transferred into a liquid phase for downstream characterisation, analogous to filtration-based workflows used for aquatic samples.

While membrane filtration is, in principle, applicable to the isolation of NPs from both liquid and airborne samples, the pore sizes required to retain the smaller NP fraction impose practical constraints. As pore size decreases, filtration throughput is reduced and susceptibility to fouling increases—effects that are considerably more pronounced for NPs than for MPs. Membrane fouling arises from the deposition and accumulation of particles smaller in size than the particles of interest in the pores of the membrane surface. One possible strategy to reduce pore blockages is to modify the surface properties of the filter material. In one example, cellulose filters were modified using heterogeneous polymer coatings to control wettability and improve mechanical strength.⁹⁸ In another instance, a polyacrylonitrile (PAN) membrane was modified with an electrospinning device using a layer-by-layer (LbL) assembly protocol. Layers of polyethylenimine (PEI) and poly (acrylic acid) (PAA) were deposited on the hydrolysed electrospun PAN membrane to obtain negative, neutral, and positive charges to improve its mechanical strength and hydrophilicity. The resulting maximum recovery on the positively charged membrane was 99% of PS-500 nm, 99.3% of PS-100 nm and 89.9% of PS-50 nm, compared to 92.4%, 12.3% and 2.3% respectively on the unmodified one.⁹⁹

Other reported strategies used to reduce membrane fouling effect include ozonation, coagulation and filtration,¹⁰⁰ granular filtration¹⁰⁰ and cetyltrimethyl ammonium bromide (CTAB – as a cationic surfactant) addition.⁹⁸ Yet, these methods did not prove to be very effective. Specifically, the formation of biofilms was reported, which caused significant blockages of the membrane pores.⁹⁸

Although membrane filtration is, to some extent, able to extract model NPs from water, controlling fouling of these membranes is an ongoing issue. More research into self-cleaning properties, controlled porosity and selective adsorption is warranted.

4 Knowledge gap

Isolating NPs from environmental samples still faces several significant bottlenecks. Most approaches used for NP isolation are adapted from techniques used for MPs and are not well suited to NPs due to their smaller size and large surface to volume ratio. For instance, the high surface area

and reactivity of NPs make them more susceptible to degradation during chemical treatments like digestion. While effective for removing organic matter, digestion can therefore lead to sample loss or alteration of NPs. This is not a desirable outcome in studies looking to quantify NPs in their environmental state; however, digestion, or equivalent green chemistry treatments could become a viable mitigation option to eliminate NP pollution by dissolution. Though research in this field would need to identify the products of NP degradation in such instance and confirm that they are not toxic.

Most current techniques attempt to isolate NPs “despite” their unique properties, rather than “exploiting” these properties to enhance the detection process. Exceptions to this are the asymmetric flow separation method, which takes advantage of the property of diffusion to effectively separate NPs and cloud point extraction which uses another nano specific property, to help with isolation: their tendency to aggregate. These liquid-phase extraction techniques have commonly been used for the extraction and preconcentration of trace metals in environmental matrices and are now being translated to NPs. Cases where surfactants are employed were discussed in section 3.2, but aggregation could also be induced *via* external factors, such as ionic strength, through the addition of salt, or changes in temperature as discussed in section 2.4. Isolating NPs by aggregation could help studies where chemical identification or total mass is sought. It could, for instance, be used to precipitate NPs into pellets large enough for IR or Raman analysis, and “heavy” enough to reach the LOD of TGA and Py-GS-MS. However, it would not be suitable for investigating the behaviours of single particles as re-suspension would be a challenge.

On the other hand, techniques like centrifugation can be effective in some cases, but they oppose the intrinsic behaviour of NPs, which often tend to float rather than settle in aqueous solvents. Efforts should therefore focus on developing innovative methodologies tailored specifically for NPs. Inspiration from other disciplines such as metal NP ecotoxicity, polymer chemistry, nanomaterial science, surface and interfacial physics could help inspire such advances to effectively enhance the accuracy and reliability of environmental NP analysis. Additional research on the natural state of NP occurrence in environmental samples is also warranted. Indeed, while NPs exhibit distinct size-dependent properties under controlled conditions, their behaviour in natural environments is expected to be shaped by low concentrations, aggregation with natural colloids, surface aging, and eco-corona formation. As a result, many nano-specific properties could be partially masked or transient, and environmental NPs are more likely to function as heterogeneous carrier particles whose interactions, transport, and biological effects are governed by surrounding matrices rather than intrinsic nanoscale features alone.

A further limitation, particularly acute for NPs, is that contamination artefacts and matrix interferences can significantly distort apparent recoveries. At the nanoscale,

where particle masses are extremely low and natural colloids are abundant, insufficient contamination control or inadequate background discrimination can readily produce false positives, false negatives, or substantial underestimation of the smallest fraction. This makes the use of appropriate internal standards or recovery tracers even more critical than in MP analysis, because unnoticed losses during isolation and transfer steps can have a proportionally much greater effect on the final result. This remains a major obstacle to comparing isolation efficiencies across studies and to translating laboratory workflows to real environmental samples.

Alongside improvements in isolation methods, adequate detection techniques using advanced analytical methods are also crucial to furthering our understanding of NP occurrence and impact. The current state of detection techniques available for NP analysis and their capabilities and limitations are covered in part II of this review.

5 Conclusion and future perspectives

Only a limited number of studies have addressed the significance of NPs' physicochemical properties in challenges faced by current isolation and quantification techniques. While the colloidal behaviour of nanoparticles has been well studied, knowledge of the physico-chemical properties of nanomaterials is not readily used to advance the field of NP analysis. Transferring this knowledge from colloid science to environment and health science is an essential step to expedite data collection and enhance mitigation strategies for this emerging pollutant issue.

Author contributions

MK contributed to writing – original draft and writing – review & editing, MM contributed to writing – review & editing, and provided financial support and supervision, Sophie Leterme, Sara J. Fraser-Miller and Christopher T. Gibson contributed to writing – review and editing. All authors read and approved the final manuscript.

Conflicts of interest

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

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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