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# Advancements in Microplastics Analysis: Emerging Analytical Techniques and Challenges

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***Abstract :***
*The widespread use of plastic has led to the pervasive presence of microplastics or plastic particles ranging from 1 to 5 mm in various environments such as freshwater, agricultural soil, and oceans. A significant portion of plastic waste comprises these microplastic fragments, posing emerging threats to ecosystems. Urgent action and comprehensive analysis are essential to track and understand the distribution of microplastics in the environment. Due to their polymeric nature, irregular morphology, and minuscule dimensions, quantifying microplastics presents significant challenges. This review delves into various analytical techniques employed for microplastic assessment, encompassing Fourier transform infrared spectroscopy, Raman spectroscopy, laser diffraction particle analysis, thermal analysis, scanning electron microscopy, pyrolysis gas chromatography, dynamic light scattering, and atmospheric solid analysis probe (ASAP) coupled with quadrupole mass spectrometry (MS). In summary, this review aims to outline the most advanced and effective methodologies for precise microplastic analysis in environmental samples.*
***Keywords:*** *Keywords Analysis, Environment, Microplastics, Quantification*

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## I. Introduction

Plastics have significantly enhanced modern life due to their lightweight, affordability, and durability (Gu et al. 2020). However, their non-biodegradable nature means they remain in the environment for extended periods, posing persistent pollution challenges. A study by Plastics Europe (2019) revealed that global plastic production has surpassed 350 million tonnes, with projections indicating a potential rise to 500 million tonnes by 2025 if unchecked (Geyer et al. 2017).

These plastics break down into various sizes, including nanoplastics, microplastics, mesoplastics, macroplastics, and megaplastics, dispersing across soil, air, water, and other environmental domains. They can also be transported by environmental currents. Microplastics are broadly categorized based on their origin into primary, from direct sources like sewage and rivers, and secondary, resulting from the fragmentation of larger plastic items through various processes (Guo and Wang 2019). Common microplastic forms include fragments, granules, threads, and films (Cózar et al. 2014; Huang et al. 2019; Guo et al. 2020).

Microplastics present significant environmental and health concerns. Their longevity and resistance to degradation result in adverse effects on ecosystems and wildlife (Wang et al. 2019; Guo et al. 2020; Queiroz et al. 2020; Mu et al. 2022). Additionally, these particles can release harmful additives and secondary pollutants into the environment (Liu et al. 2020). Given their vast surface area, microplastics can accumulate environmental toxins, posing risks to ecosystems and human health (Li et al. 2018; Naqash et al. 2020; Gaylarde et al. 2020).

Attributes such as size, composition, and structure influence the toxicity of microplastics. Certain forms, particularly fiber-shaped ones like PET, PE, and PP, present elevated risks (Pirsaheb et al. 2020; Lithner et al. 2011). Additionally, e-waste plastics often contain metals and flame retardants, further complicating their environmental impact (Li et al. 2019; Turner et al. 2019; Singh et al. 2020).

Microplastics encompass a range of polymers like PE, PP, PVC, PS, PA, and PET, each with varying environmental impacts (Zhu et al. 2019; de Souza Machado et al. 2018). Hence, understanding less common plastics' environmental impacts becomes crucial.

For effective microplastic monitoring, standardized sampling and analytical methods are essential (Galgani et al. 2013; Muller et al. 2020). Current methodologies, such as FTIR, SEM−EDX, thermal analysis, and Raman spectroscopy, aid in microplastic characterization (Song et al. 2015; Wagner et al. 2017; Majewsky et al. 2016; Araujo et al. 2018).

**Methods for identifying and measuring microplastics**

The visual identification of microplastics (MPs) in samples can be achieved using techniques such as light microscopy or polarizing microscopy, as observed in initial studies on MP quantification and characterization (Talvitie et al. 2017). Such visual methods typically categorize MPs into three primary groups: fibers, fragments, and pellets or microbeads. Using light microscopy, microplastics, which generally measure a few hundred micrometers, can be quantified. Given that these microplastics often lack distinct luminosity, their specific physical properties, like elasticity or hardness, become pivotal for their identification. Notably, fibers, fragments, and microplastic beads are commonly observed in environmental samples (Abadi et al. 2021). Approximately 70% of the time, microplastic samples are transparent in nature (Löder and Gerdts 2015). An optical microscope can rapidly identify colored polymers produced with specific dyes (Dehghani and Moore 2017). However, pinpointing colorless or shapeless plastic particles smaller than 100 µm remains challenging. Complicating matters, inadequate separation of sample particles can hinder accurate microscopic identification. The presence of sediments and organic components, resistant to chemical degradation, further complicates the microscopic distinction of microplastics. Prior research indicates potential misidentification rates exceeding 70% for transparent particles and over 20% for plastic-like particles. Distinguishing between synthetic and natural fibers solely through microscopy can be tricky due to their similar characteristics. For instance, cotton fibers might be misconstrued as plastic ones, but specific methods involving heated needles have been devised to differentiate them by melting the plastic particles (Shim et al. 2017; Hendrickson et al. 2018).Fluorescence microscopy, leveraging the inherent fluorescence of common plastic additives like bleaching agents, offers another avenue for microplastic identification. However, potential detection inaccuracies persist, particularly when some minerals possess self-fluorescent properties (Dehghani and Moore 2017)

**Thermal-based analyses of microplastics**

The properties of materials are significantly influenced by both time and temperature. Delving into this relationship, thermal analysis emerges as an indispensable technique within the realm of material science, shedding light on how materials evolve under varying thermal conditions (Majewsky et al. 2016). When it comes to environmental samples, particularly concerning the detection and study of microplastics, a meticulous approach is necessary. Prior to subjecting these samples to thermal analysis, they undergo a controlled heating process. This crucial step is instrumental as it sets the stage for understanding how microplastics react when exposed to temperature variations. As the temperature ascends, microplastics exhibit an intriguing behavior: they absorb significant amounts of thermal energy. This absorption leads to a transformative phase wherein these microplastics transition from their solid-state compositions. Depending on the magnitude of the temperature increase, these polymers can evolve either into a liquid or a gaseous state. This metamorphosis is not just a mere physical change; it's a phenomenon marked by distinct thermal reactions. Specifically, researchers observe an endothermic peak at a particular temperature threshold. This peak serves as a pivotal indicator, signaling the point at which the microplastic undergoes its transformative phase (Majewsky et al. 2016). However, it's crucial to understand that not all microplastics respond uniformly to temperature variations. The realm of polymers is vast, encompassing a myriad of materials, each with its unique thermal stability characteristics. This diversity necessitates a nuanced approach to analysis. Fortunately, the field benefits from established standards, such as thermograms of polymers. These standardized thermograms act as reference guides, providing invaluable insights into the composition, type, and even the additives present within microplastics (Majewsky et al. 2016). Moreover, the complexity of microplastics doesn't end with their thermal reactions. The challenge extends to the very nature of identifying these minuscule entities. Given their microscopic size, discerning microplastics from other environmental components under a microscope can be daunting. Issues arise when natural sediments and biological elements coexist, leading to potential misidentifications. Previous studies have highlighted the intricacies involved, revealing that over 70% of transparent particles and a significant portion of plastic-like entities are prone to misdiagnosis. Such challenges underscore the importance of refining detection methodologies (Shim et al. 2017; Hendrickson et al. 2018). Furthermore, the advent of sophisticated techniques has paved the way for innovative detection methods. For instance, the incorporation of heated needle tests has proven effective in distinguishing between synthetic and natural fibers. By subjecting particles to this method, the differential melting points between cotton fibers and plastics become evident, aiding in accurate identification. Another intriguing avenue in microplastic analysis pertains to the use of fluorescence microscopy. Given the prevalence of certain additives, such as bleaching agents in both textile and plastic synthesis sectors, fluorescence offers a promising detection pathway. These bleaching agents exhibit fluorescence under specific conditions, making them discernible under specialized microscopes. However, like all methodologies, this too comes with its set of challenges. Certain minerals possess inherent fluorescence properties, introducing potential inaccuracies in detection (Dehghani and Moore 2017).

**Thermogravimetric analysis**

Termogravimetric analysis (TGA) stands as a pivotal method within the domain of thermal analysis. This technique scrutinizes a sample's behavior concerning time and temperature. By monitoring the weight loss of a sample as it undergoes controlled heating under specific environmental conditions, TGA furnishes both qualitative and quantitative data regarding the sample's composition and properties (Ma et al. 2018). Historically, the examination of microplastics in wastewater has incorporated both TGA and Differential Scanning Calorimetry (DSC). Yet, the scope of differentiation has been somewhat limited. Notably, within these tests, only polypropylene (PP) and polyethylene (PE) could be distinctly identified. Curiously absent from clear identification were materials like polyamide (PA), polyvinyl chloride (PVC), and polyester (PES). Differentiating between materials such as polyethylene terephthalate (PET) and polyurethane (PU) presented particular challenges due to overlapping phase transition signals (Majewsky et al. 2016; Sun et al. 2019).

However, while thermal analysis offers immediate integration into analytical processes without demanding extensive sample preparation, it is not without limitations. One primary constraint stems from the intricate nature of polymer compositions. Polymer branching and the presence of impurities can significantly influence polymer transition temperatures, making certain copolymers elusive to thermal detection. Another noteworthy limitation lies in the destructive nature of thermal techniques. Given that these methods involve heating, they inherently compromise the physical integrity and appearance of the microplastic samples, hindering detailed morphological assessments. Consequently, the primary utility of thermal analysis lies in its capacity to elucidate the chemical composition of microplastics and quantify their presence. Yet, this inherently limits its applicability when seeking comprehensive insights into microplastic characteristics beyond chemical composition (Rocha-Santos and Duarte 2015; Majewsky et al. 2016; Shim et al. 2017; Huppertsberg and Knepper 2018; Silva et al. 2018).

**Atomic force microscopy-infrared and Raman**

The realm of nanoplastic research is vast and complex, requiring sophisticated techniques to uncover the intricacies of these minute particles. Among the arsenal of analytical tools available, Atomic Force Microscopy (AFM) stands out, especially when amalgamated with techniques like Infrared (IR) or Raman spectroscopy. This fusion offers unparalleled insights into the characteristics and compositions of nanoplastics.

At its core, AFM operates on the principle of a sharp probe interacting with a sample's surface. Unlike conventional optical microscopy, AFM doesn't rely on lenses or light. Instead, it employs a tiny cantilever with a nanoscale tip to "feel" the sample's surface, creating detailed topographic images. The flexibility of AFM extends beyond mere surface visualization; it allows for interaction modes, including contact and non-contact, providing versatile data acquisition methods.

When combined with spectroscopic techniques such as IR or Raman, AFM transcends its imaging capabilities. Spectroscopy, with its prowess in identifying molecular structures based on their interaction with light, complements AFM's topographical data. Together, they unveil not just the physical appearance but also the chemical makeup of nanoplastics. A fascinating interaction occurs when IR radiation is directed at a sample during AFM examination. The absorbed IR energy induces thermal expansion within the sample. This thermal effect becomes evident as vibrations in the AFM cantilever. By applying the Fourier transform method—a mathematical tool—the resulting vibration patterns can be analyzed. This intricate dance of interactions helps researchers quantify frequencies and amplitudes, providing deeper insights into the sample's molecular characteristics. Moreover, AFM's partnership with IR (AFM-IR) offers a dual advantage. Not only does it capture the topography with nanoscale precision, but it also records the sample's IR absorption spectra. This dual-data acquisition facilitates a holistic understanding, capturing both structural nuances and compositional details. For instance, pioneering studies have effectively harnessed this technique to analyze 100 nm PS beads, highlighting its potential and efficacy. However, as with all methodologies, challenges persist. One significant hurdle with AFM-IR emerges during the identification phase. Pinpointing individual nanoscale plastic particles within a milieu of unidentified materials remains a daunting task. Traditional microplastic analysis pathways often necessitate meticulous manual searches, a time-consuming and labor-intensive process. The efficiency of the preliminary treatment phase, where samples undergo separation and purification, directly impacts the subsequent analysis's success rate. Delicate, nearly transparent plastic particles can easily evade detection, particularly when relying solely on manual identification. Recognizing these challenges, the scientific community has explored alternatives. Automated techniques like FTIR/Raman mapping or continuous monitoring via Raman spectroscopy present promising avenues. These methodologies promise heightened accuracy, consistency, and reduced human-induced errors. By leveraging automation, researchers can streamline processes, potentially accelerating nanoplastic research advancements. Yet, a critical consideration looms large—the economic aspect. While automated solutions offer unparalleled advantages, they come at a considerable cost. Acquiring, maintaining, and calibrating sophisticated equipment demands substantial financial investments. For many microplastic research laboratories, especially those operating on constrained budgets, procuring such high-end instruments remains a formidable challenge.

**Near-IR spectra analysis method**

NIR spectroscopy is also being explored as a potential tool for assessing microplastics. FTIR examines the spectrum ranging from 600 to 4000 cm−1, while NIR focuses on the spectrum between 4000 and 15,000 cm−1, as highlighted by Zhang et al. (2018). NIR spectral studies often highlight chemical vibrations like X–H combinations, including C–H, N–H, and O–H. While NIR analysis may present challenges in precise quantitative evaluations, it excels in rapidly processing and evaluating extensive plastic sample datasets. For identifying the type of sample rather than merely quantifying it, this analytical method proves more advantageous, as noted by Paul et al. (2019).

**X-Ray diffraction**

XRD analyses were employed to scrutinize various microplastics. PET demonstrates notably low crystalline quality with prominent peaks; its peak intensity peaks at 25.7° with a value of 2 at 2 of 25.7°. PP exhibits robust crystalline characteristics with distinct, sharp peaks, showcasing a peak at 31.2° and a subsequent decrease at 34.3°. The highest diffraction peak intensities for PE, known for its intrinsic crystalline nature, are evident at 21.6°, 24.05°, and 27.5°, each displaying distinct and pronounced peaks. The broad and pronounced peaks observed in PS signify its inferior crystalline quality, capturing an intensity peak at 22.6°. Conversely, the XRD pattern of the PBT microplastic polymer appears erratic, lacking defined peaks due to its amorphous nature (Takur et al. 2023). Previous studies elucidate that the XRD patterns for three distinct microplastics—PE, PVC, and PS—reveal two robust and sharp peaks at 21.1° and 23.4° for PE, the absence of pronounced peaks for PVC, and wide peaks for PS, indicative of its suboptimal crystalline structure (Ezeonu et al. 2019; Liu et al. 2019; Moura et al. 2023).

**Vis–NIR analysis**

Vis-NIR spectroscopy measures the amount of light reflected from a sample's surface within the 350–2500 nm range to determine the reflectance for each wavelength. Given its capability to analyze the chemical composition of the sample, this method is effective for quantifying microplastics (Corradini et al. 2019). Common microplastics such as PET, low-density polyethylene (LDPE), and PVC can be identified utilizing an extensive Vis-NIR spectral database encompassing various polymers commonly encountered in environmental settings. Nonetheless, due to its reliance on optical detection, there remains a possibility of misidentifying biological particles as plastics, emphasizing the need for human interpretation.

**Cutting-edge technology**

Innovative techniques, stemming from advancements in analytical equipment and the fusion of novel detection technologies with established instruments, hold promise for addressing existing challenges in microplastic identification. A significant hurdle in microplastic analysis pertains to the lower limit of detectable size. Current analytical methods can identify particles only down to a few micrometers. Understanding the presence, fate, distribution, and toxicity of plastics at the nanoscale has become increasingly crucial, as minuscule plastic particles may pose heightened risks. Consequently, there's an imperative to devise new identification methods and establish protocols for the collection, extraction, purification, and concentration of nanoplastics.

**Nile red staining of plastics**

To address the challenge of detecting tiny, translucent particles, researchers have explored various staining methods. While some dyes like Eosin B, Rose Bengal Hostasol Yellow 3G, and Oil red EGN have limitations (Prata et al., 2019), the fluorescent dye 9-diethylamino-5H-benzophenoxazine-5-one has shown promise in selectively highlighting highly hydrophobic microplastics. Additionally, Nile red has been effectively employed for staining physiologically neutral lipids. Due to its strong affinity for neutral lipids, Nile red predominantly fluoresces in hydrophobic environments. This method offers benefits such as short staining durations (10–30 minutes) and impressive recovery rates reaching up to 96%. Moreover, a quick bleach wash can be applied as needed. Consequently, this staining approach serves as a valuable preliminary step, facilitating more in-depth spectroscopic analyses and effectively unveiling hidden microplastics (Erni-Cassola et al., 2017; Simmerman et al., 2020).

## II. Conclusions

The detection of microplastics within varied environmental matrices necessitates the integration of multiple analytical techniques. As the size of microplastics diminishes, their detection becomes increasingly challenging. Consequently, sub-micron analysis is gaining significance, especially when assessing the adverse impacts of microplastics on both the environment and human health. As the imperative for monitoring microplastic contamination intensifies, there's a pressing need to innovate and refine existing methodologies, aiming to streamline detection efforts.

To effectively locate and quantify nanoplastics within environmental samples, the development of reliable identification protocols is paramount. Prospective research endeavors should prioritize the formulation of either fully or partially automated analytical methodologies. Such innovative approaches could seamlessly incorporate image analysis techniques, enabling the identification of plastic constituents while concurrently discerning pertinent physical attributes like size and morphology of microplastics.

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