Preparation of synthetic micro- and nano plastics for method validation studies

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HIGHLIGHTS

- Detailed procedure for cryogenic milling of microplastics to replace standardized beads is proposed.
- Misidentification issues, particularly with PVC were found in FTIR and Raman measurements.
- Milled microplastics represent real environmental samples much better than standardized beads.
- New imaging experiments present possibility for industrial scale in-situ microplastic monitoring.

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ABSTRACT

Microplastic (MP) pollution is a persisting global problem. Accurate analysis is essential in quantifying the effects of microplastic pollution and develop novel technologies that reliably and reproducibly measure microplastic content in various samples. The most common methods for this are FTIR and Raman spectroscopy. Coloured, standardized beads are often used for method validation tests, which limits the conclusions to a very specific case rarely observed in the natural environment. This study focuses on the preparation of reference micro- and nanoplastics via cryogenic milling and shows their use for FTIR and Raman method validation studies. MPs can now be reproducibly milled from various plastics, offering the advantages of a better representation of MPs in real environment. Moreover, this study highlights issues with the current detection methods, up to now considered as the most reliable ones for MP detection and identification. Such issues, e.g. misidentification, will need to be addressed in the future. Additionally, milled MPs were used in experiments with commercial high-resolution imaging device, enabling a possible in-situ optical detection of microplastics. These experiments...
1. Introduction

Plastics are often inexpensive, strong and durable mixtures of polymers and small-molecule additives that resist corrosion and environmental degradation (Thompson et al., 2009). Microplastics (MPs) with particle sizes of 1 μm–5 mm (Frias and Nash, 2019) and nanoplastics (NPs) ranging from 1 nm–1 μm (Gigault et al., 2018) both emerge from the breakdown of plastic materials. In the environment, this fragmentation is triggered by environmental stressors, such as photo-oxidation and mechanical abrasion (Andrady et al., 2011; Browne, 2015). Due to their insolubility in water and slow biodegradation, plastics persist as environmental pollutants (Kutz, 2012). Because MPs are globally spread pollutants with varying chemical and physical characteristics, their analysis requires robust and reliable methods to ensure the quality of research and to follow concentration in different streams and efficiencies of purification technologies. Accurate and consistent analyses are crucial for evaluating MPs’ environmental and health risks.

Several technologies have been used for identifying polymer types of MPs, such as Fourier transform infrared spectroscopy (FTIR) (Higgins, 2013; Løder et al., 2015; Tagg et al., 2015; Chen et al., 2021), Raman spectroscopy (Pezacki et al., 2011; Watts et al., 2014; Sobhani et al., 2019; Jin et al., 2022; Nava et al., 2021; da Silva et al., 2021; Lu et al., 2021), and pyrolysis gas chromatography mass spectrometry (Hermabessiere and Rochman, 2021; Fischer and Scholtz-Böttcher, 2019; Fischer and Scholtz-Böttcher, 2017). The validity of these methods often hinges on the use of positive control samples, which contain a known quantity or mass of MPs. These controls enable the assessment of accuracy, recovery, and repeatability. The most common materials for positive controls have been fluorescent commercially available MP beads, such as polystyrene (PS) (Uurasjärvi et al., 2021) or polyethylene (PE) (Mari et al., 2021). Further, Lares et al. (Lares et al., 2019) used polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP) and styrene butadiene rubber (SBR) along with PE and PS. The standardized sizes of these beads were reported as 90 μm (Uurasjärvi et al., 2021) or 300 μm or larger (Mari et al., 2021; Lares et al., 2019).

However, this approach to analysis has limitations. Recovery rate studies may not accurately reflect environmental conditions if they include particles of only one polymer type and shape. Synthetic MPs should better represent real environmental samples, which vary in shape and size. Currently, commercial reference materials for MPs are limited in polymer types, sizes, and shapes. Consequently, there is an urgent need for reproducible methods to prepare synthetic MPs for reference materials. Such reproducibility is vital for enabling study comparisons and meta-analyses. One method to produce representative synthetic MPs is cryogenic milling. While some methods for preparation of synthetic MPs via cryogenic milling exist already (Srinivas and Lyons, 2005; Kefer et al., 2021; Anuar et al., 2021), detailed parameters are often unreported or limited to a single type of plastic. However, the preparation parameters should be reported for different polymer types, because they have different properties, which may affect the grinding process. For instance, glass transition temperature ($T_g$) affects the milling process with respect to the plastic (Srinivas and Lyons, 2005). As plastic particles become more brittle at low temperatures (below $T_g$) (Kalia, 2010), they can be ground into finer powders, down to NP size range. Thermal properties, sizes, shapes, molecular composition and surface area of MPs prepared this way have also been characterized via scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), Brunauer-Emmet-Teller surface area measurement, laser diffraction, and differential scanning calorimetry (Kefer et al., 2022).

The primary objective of this study was to create synthetic microplastics (MPs) and nanoplastics (NPs) by grinding larger beads and subsequently employing these particles in a range of method validation studies. We describe in detail the process parameters for cryo-milling and characteristics of yielded MPs and NP to provide a reproducible method for preparing representative reference MPs. Moreover, we provide example data how the synthetic MPs were used to validate various pre-treatment and measurement methods including Raman micro spectroscopy, imaging Fourier transform spectroscopy, and in situ imaging of MPs from water using ultra high-definition imaging (UHD).

2. Experimental

Polymers were obtained from several sources (Table 1).

The polymers were ground using Retsch ZM200 Polymer mill as in (Anuar et al., 2021; Kefer et al., 2022). A respiration mask was worn at all times due to easily airborne particles. Plastic beads were cooled in liquid nitrogen for about 10 min before processing. Samples should not be inserted into the chamber with liquid nitrogen due to risk of explosion. Speed of the grinding was adjusted based on the polymer’s behaviour. Normal sieves were used at first (pictures of sieves can be found in the supporting information), a 500 μm distance sieve replaced the regular 500 μm sieve eventually. Distance sieves leave a gap between the rotor and the sieve which reduces the heating rate for plastic particles in the chamber, enabling faster grinding with less jams. This way, all plastics could be ground straight to at least the 500 μm level and then the obtained powder was ground stepwise through a 250 μm, 120 μm and 80 μm sieve. With some polymers, 120 μm sieve can be skipped because grinding the 250 μm powder straight to 80 μm does not cause much agglomeration under certain grinding conditions. However, the drawback of distance sieves is the amount of plastic material getting stuck to the sides of the sieve that does not grind, which causes large amounts of losses in yield. Sieves were cleaned with organic solvents and wiped with a brush and a vacuum cleaner to avoid cross-contamination.

Yields of each polymer after grinding are relatively consistent across different types, at the 80 μm sieve the remaining MP content is about 10–20 % of the initial mass. Losses are attributed to the MPs spreading across the device due to escaping sealing, losses during collection and the several sieving steps (material stuck to sieves for example). In the case of PA, a large portion of the dust is simply lost by becoming airborne. Distance sieves allowed for skipping of more sieves in most cases, so eventually, all sieves down to 120 μm were replaced with distance sieves.

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Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Reference number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear-low density polyethylene</td>
<td>FB4230</td>
<td>Borealis, Austria</td>
</tr>
<tr>
<td>High density polyethylene (HDPE)</td>
<td>CG9620</td>
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</tr>
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<td>Polystyrene (PS)</td>
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<td>RAMAPET R182</td>
<td>Indorama, Indonesia</td>
</tr>
<tr>
<td>Polyamide (PA) 66</td>
<td>Zytel 101 F N</td>
<td>DuPont, United States</td>
</tr>
<tr>
<td>Styrene butadiene rubber (SBR)</td>
<td>Etra standard SBR</td>
<td>Etra, Finland</td>
</tr>
<tr>
<td>Plasticized polyvinyl chloride (PPVC)</td>
<td>Etradur</td>
<td>Etra, Finland</td>
</tr>
<tr>
<td>Unplasticized polyvinyl chloride (UPVC)</td>
<td>Etraplast</td>
<td>Etra, Finland</td>
</tr>
<tr>
<td>Post-consumer recycled polyethylene (PE)</td>
<td>Mixed sample</td>
<td>Fortum, Finland</td>
</tr>
<tr>
<td>Post-consumer recycled PP</td>
<td>Mixed sample</td>
<td>Fortum, Finland</td>
</tr>
</tbody>
</table>
2.1. Grinding parameters for different polymers

This section describes the grinding parameters and processes used for various polymer types with references to the glass transition temperature \( T_g \) and the melting temperature \( T_m \) (obtained from https://resources.perkinelmer.com/corporate/cmsresources/images/44-74863tech-mptgandstructureofcommonpolymers.pdf, n.d.)).

2.1.1. PE (HDPE: \( T_g = -125^\circ \text{C} \), \( T_m = 130-140^\circ \text{C} \); LDPE: \( T_g = -130^\circ \text{C} \); \( T_m = 85-125^\circ \text{C} \))

No particular issues arose for both LDPE and HDPE while using distance sieves. A 250 \( \mu \text{m} \) distance sieve can be used on starting pellets without heating or clustering problems. However, it is recommended to add a maximum of 4–5 pellets to the grinding chamber at once. Time lapse between adding pellets into the grinding chamber was approximately 10–15 s. On the 80 \( \mu \text{m} \) sieve, the time lapse needs to be increased to avoid a melted PE film formation on the sieve, which requires heated organic solvents to remove, such as trichlorobenzene. A small amount of powder (approximately the size of maximum 1 starting pellet) is lifted directly from liquid nitrogen into the chamber at each time lapse. This not only prevents jams, but also makes the lump heavy enough to fall into the grinding chamber and not stick to the walls of the mill instead, increasing the yield. Grinding must be finished before addition of further material into the chamber. Grinding speed was 10,000 rpm for the starting pellets, 8000 rpm or less for sub-250 \( \mu \text{m} \) sieves. Grinding speed does not need to be changed with using distance sieves compared to normal sieves.

2.1.2. PP (\( T_g = -20 \text{ to } -5^\circ \text{C}; T_m = 165-175^\circ \text{C} \))

Due to low \( T_g \) and the stark increase of temperatures in the device during the grinding, PP readily melts in the grinding chamber. This makes PP the most difficult plastic to grind. Grinding is to be conducted very slowly, 1–2 beads at a time with 10–20 s waiting intervals to allow for grinding of the added beads, even with distance sieves. The ground powder on smaller sieves also needs to be processed in very small amounts. Only a few grains of PP powder are to be lifted directly from liquid nitrogen into the chamber to prevent jams. At lower sieve sizes than 250 \( \mu \text{m} \), the process slows down even further and is not very feasible, as 1 g of such a powder would take days to prepare. Very small amounts (in the scale of \( \sim 0.1 \text{ g} \)) of PP could be prepared with the 120 \( \mu \text{m} \) distance sieve. Grinding speed should be always the lowest possible for avoiding jams (6000 rpm), although 8000 rpm is applicable with distance sieves sufficiently reduces the heating of the material. Processing of PS tends to collect on the edges of the sieve and is not ground properly. This polymer did not present any other issues, so it can be worked with using proper respiratory protection. Grinding speed can be high in this case but should be reduced on small sieves (<10,000 rpm). Addition of beads can be fast, like PET.

2.1.3. PS (\( T_g = 90-110^\circ \text{C}; T_m = 240^\circ \text{C} \))

Sometimes long fibres are formed during the grinding. PS also jams often on normal sieves, but not nearly as often as PP. It does not cause issues when using distance sieves, which means the air gap in distance sieves sufficiently reduces the heating of the material. Processing of PS should be very slow on lower size sieves. Initial grinding step can have grinding speeds of up to 10,000 rpm and several beads (\( \sim 6 \)) can be added at a time, at smaller sieves the addition and parameters need to be same as for PE. When using distance sieves, 8000 rpm can be used throughout the entire grinding process and the initial grinding step can even be performed with a 120 \( \mu \text{m} \) distance sieve.

2.1.4. PET (\( T_g = 70-80^\circ \text{C}; T_m = 245-264^\circ \text{C} \))

PET has high temperature resistance (high \( T_g \) and \( T_m \)), therefore it is the easiest polymer to grind. Jams are not very likely, but they can occur when grinding powder. PET jams are very difficult to clean. They solidify quickly and are resistant to most chemicals. Hot toluene solution for several days can soften PET enough for it to be scrapped off the sieve with some effort. Grinding speed in this case does not affect the grinding significantly, but at lowest sieve sizes it is recommended to use speeds lower than 10,000 rpm. Many beads (\( \sim 10 \)) can be inserted into the chamber at once without issues. Powder addition can also be faster than for other polymers. This polymer can be grinded with the 120 \( \mu \text{m} \) distance sieve as the initial step.

2.1.6. SBR (\( T_g = -6 \text{ to } -13^\circ \text{C}; T_m = 160-200^\circ \text{C} \))

Initial particles added to the mill were small pieces cut from an SBR sheet. The rubber was ground with no issues jam-wise, but it stained the polymer pool, as the small particles instantly agglomerated into very large particles obtained by larger sieves. It is not recommended to grind this polymer below the 1 mm sieve.

2.1.7. PPVC/UPVC (\( T_g = 80^\circ \text{C}; T_m = 85^\circ \text{C} \))

PPVC (yellow) and UPVC (transparent) pieces were cut from a larger, thicker sheet. Pieces were rectangular, \( \sim 3 \text{ mm} \) wide and \( \sim 5 \text{ mm} \) long. Those pieces were cooled in liquid nitrogen and grinded as other polymers. Initial grinding can be done at 12000 rpm, but for the 80 \( \mu \text{m} \) sieve the speed should be reduced to 8000 rpm or less. Grinding PVC is easy to carry out and several sheets (\( \sim 10 \)) can be inserted into the chamber at once. The powder can be ground quickly as well, the addition of powder can even be continuous. With distance sieves, grinding is easy, but PVC tends to collect on the edges of the sieve and is not ground properly. This could be due to high density of PVC, making it heavier. Normal sieves are therefore preferred, since they provide higher yields. The initial grinding step should therefore be with the 500 \( \mu \text{m} \) sieve.

2.1.8. Recycled PP, PE

Recycled MP beads were obtained from Fortum’s plastic recycling
facilities located in Finland and were tested in grinding procedures. Recycled plastics seem to be easier to grind than pure ones, which could be due to aging of the plastics or additives in the commercial products. These beads were made from, as that is the only difference between the beads used and their pristine counterparts. Grinding speeds and other parameters remain the same as with their pristine counterparts, but jams were less likely.

A summary of all grinding parameters developed in this study is given in Table 2.

### 2.2. Digestion protocol

Recovery rate tests were conducted with the grinded polymers. Digestion procedure was tested for studying effects on the MPs during a purification process commonly employed before MP analysis. Grinded MPs were subjected to an enzymatic digestion treatment based on the protocol developed by Lüder et al. (Lüder et al., 2017). 20 mL of ultrapure water were spiked with a known amount of grinded MPs under an optical microscope (10–20 particles per sample). 30 mL of H2O2 was added and shaken at 50 °C and 100 rpm for 24 h. The product was filtered through a steel filter (pore size 15 μm) and the filter was placed in 30 mL of phosphate buffer with pH adjusted to 9. Afterwards, 5 mL of protease A-01 was added and the mixture was shaken for 24 h at 100 rpm and 50 °C. The product was collected on a silver filter (Sterlitech Co, Kajaani, Finland). The method was developed and validated using a real sample from industrial wastewater effluent. In this case, effluent water samples of various volumes (50–100 mL) were imaged without pretreatment and particle numbers in the samples were analysed using corresponding Valmet FS5 software.

### 2.3. Characterization of grinded MPs

#### 2.3.1. Morphology − scanning electron microscope (SEM)

The size and shape of grinded MP particles were analysed using the Hitachi S-4800 SEM device at 5 kV, 5 μA (further adjusted based on the sample) and a working distance of 8 mm. Powder was attached to a double-sided tape and coated with 2 nm of gold.

#### 2.3.2. Imaging FTIR

The device used for FTIR imaging was Agilent Cary 670/620 equipped with a 128 × 128 FPA detector and a pixel size of 5.5 μm. The filter area of 12 mm diameter was scanned in reflection mode with 15× objective, in a 3800–800 cm⁻¹ spectral range and 8 cm⁻¹ spectral resolution. Results were analysed using SIMPe software, developed by Primpke et al. (https://simple-plastics.eu, n.d.), using common libraries for the grinded polymers. The software analyses Pearson correlation coefficients between sample and reference spectra. The threshold for particle recognition was adjusted to detect the most particles possible with correct identification. Information acquired from SIMPe contains particle number, sizes and types. The particle size was limited by the steel filter pore size (15 μm), which was used during the enzymatic treatment protocol.

### 2.3.3. Raman

Grinded pristine MPs were examined with Raman in two techniques to validate the method, i.e. firstly to study recovery rates after the digestion protocol and secondly, as a reference to ultra-high-definition imaging (see Section 2.3.4).

The Raman spectra were obtained with two different Raman imaging microscopes (ThermoScientific DXR2xi and DXR3xi). With DXR2xi, samples on gold filters were placed on a glass substrate for recovery rate studies. In addition, 785 nm laser wavelength was used in 5 cm⁻¹ resolution and 50–3300 cm⁻¹ range. The other settings were aperture 50 μm confocal pinhole, a grating 400 lines/mm, 10×/0.25 NA objective, 10 mW laser power, a 0.14 s exposure time and 30 scans per measurement. The analysed particles were selected manually.

With DXR3xi, a method utilising particle selection was used as described in Tsering et al. (2022) with some improvements. Instead of gold-coated filters, Anodisc (Whatman, 0.25 mm, 0.2-μm pore size) filters were used, placed on a stainless-steel plate with an 18 mm hole below the filter. In addition, 10×/0.25 BD objective was used for both imaging and detection. The resulted spectra were compared with libraries from Slopp and Slopp-E (Munno et al., 2020) for the spectral range of 600–1800 cm⁻¹. This characterization method was used only for pristine grinded MPs without any pretreatments to assess the suitability of these MPs for particle selection.

In the aim of development of novel sensors for in situ identification and ultra-high-definition imaging (Section 2.3.4) of MPs, preliminary measurements were performed for film-type non-grinded plastic samples. These reference measurements for in-situ imaging of MPs in water were done by placing a plastic film into a sample holder with an opening. Samples were measured above the opening to prevent any background signal from other materials than the measured ones. Only UPVC and LDPE films were measured first. Afterwards, cover glass was placed on the plastic films and the measurement was repeated. Finally, two droplets of distilled water were pipetted onto the plastic film, cover glass was placed on top of the sample and the measurement was repeated for a third time. The measurement parameters were same as mentioned above for DXR2xi. The setup simulates a cuvette used with the Valmet FS5 device. The aim of this study is to identify the plastic type of a rough MP through a probe window and a water film between the window and the MP. LDPE, in this case, presents a strongly translucent object with low surface roughness, while UPVC presents a semi-transparent higher surface roughness particle.

#### 2.3.4. Ultra-high-definition imaging of MPs directly from water

In this study, grinded MPs were imaged using a commercial, ultra-high-definition imaging device Valmet FS5 (Valmet Automation, Kajaani, Finland). The method used was a previously developed method for screening the presence of MPs in a flowing water sample that contains organic materials as well as MPs (Peiponen et al., 2023). The imaging time was 10 ms, with an image obtained every 0.1 s from a vertical cuvette flow. The volume of sample was 0.5 L, diluted with tap water during measurement. 0.5 L was suggested as the appropriate volume in a previous study (Prata et al., 2020). The detection window is 16.2 mm × 13.5 mm. The diluted sample can also be recirculated within the device for additional measurements. A more detailed description of the device can be found in Peiponen et al. (2023). The minimum detected size with this method is very close to 1 μm. The detection is based on a narrow band light source in the spectral region of white light. This avoids strong absorption of infrared radiation typically observed in this method. MPs can be differentiated from other particles using the light intensity bitmap of the Valmet FS5, described in more detail in Peiponen et al. (2023).

Further, the method was tested using a real sample from industrial wastewater effluent. In this case, effluent water samples of various volumes (50–100 mL) were imaged without pretreatment and particle numbers in the samples were analysed using corresponding Valmet FS5 software.

### Table 3

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Recovery rate (%)</th>
<th>Recovery rate (adjusted for misidentification) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>PVC</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>PS</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>PET</td>
<td>93</td>
<td>90</td>
</tr>
<tr>
<td>PP</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>PE</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Rec. PP</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>Rec. PE</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>Average recovery rate</td>
<td>61.1</td>
<td>75.8</td>
</tr>
</tbody>
</table>

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### Table 2

<table>
<thead>
<tr>
<th>Recovery rate test results on FTIR</th>
<th>Recovery rate indicates the percentage of initially added particles that were detected by the device.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rec. PE</td>
<td>60</td>
</tr>
<tr>
<td>Average recovery</td>
<td>61.1</td>
</tr>
</tbody>
</table>
3. Results and discussion

3.1. Grinding results

MPs were successfully prepared from all polymers. Resulting powders were analysed under SEM to determine the sizes of particles (Fig. 1). Particle size distribution at specific sieves did not differ significantly between different polymer types, except for SBR. The SBR particles immediately agglomerated and formed large balls of several micrometers, which requires longer grinding time for small sieves. This may come from the brittleness of the material, or particle sizes at the 80 μm sieve were mostly between 25 and 200 μm. As such, those particles cannot be separated and properly used in experiments. Therefore, SBR rubber is not as useful for analysis and grinding as other polymer types. Size range of particles at 250 μm sieve was mostly between 25 and 200 μm and particle sizes at the 80 μm sieve were in the nanoparticle range (as low as 40 nm) (Fig. 1). Shapes of MPs were irregular in all cases, except for the case of rubber, where particles were mostly spherical. More examples of SEM images can be found in the supplementary information. One can notice that the shape can be either rounded and angular depending on the plastic type, as illustrated by SEM images of PS and PVC in Fig. 1. This may come from the brittleness of the material, or from the heat generated by the grinding (in relation with glass transition temperature), which requires longer grinding time for small sieves. Overall, MPs of a wide variety of shapes and were successfully prepared. The method was reproducible and reliable. Thus, these kinds of MPs can offer an option for commercial reference materials for future studies, as they are more representative for the MPs in the real environment. Biofilm deposition or other surface phenomena may occur in natural environments, which is a broad study on its own, beyond the scope of this article. Various surface effects, aging and weathering tests should be conducted to account for these factors. However, mechanical grinding introduces surface roughness that would also be found in environmental samples. Furthermore, in the EU, clear, round PET beads are still used for standardization (Betz, 2021), thus, the approach proposed in this work with various shapes, polymer types, sizes and mechanical surface damage is a significant improvement over current method validation protocols.

3.2. Recovery rate results

Recovery rate tests were conducted on all the usable prepared MPs. The results for FTIR and Raman recovery rate tests are given in Tables 3 and 4, respectively.

3.2.1. FTIR

The results with FTIR contained many examples of misidentification. SiMPle can for instance identify a particle as a polymer that was not originally added to the sample. Agglomerates of two or more particles can be identified as one particle. Manual corrections to account for those issues allows the recovery rate to reach 75.8 %. However, in a real sample, manual corrections are not feasible due to lack of knowledge regarding the sample’s contents. Thus, this research highlights an issue in the current common identification methods used to detect MPs. Particularly PVC was difficult to identify, as without manual corrections, almost none of its particles were identified correctly. A blank sample of only ultrapure water was also prepared to eliminate the possibility of laboratory contamination and no MPs were detected from it. Examples of misidentified particles can be found in supporting information.

3.2.2. Raman

In the case of Raman, the same samples were measured with different instruments (DXR2xi and DXR3xi), by using different settings and filters to assess the suitability of these MPs to different types of Raman analyses.

Raman spectroscopy is one of the most common methods for MP identification. Usually, organic materials must be removed from the dry sample before analysis, to ensure no overlap between the spectral signature of MPs and, for instance, wood components having rich absorption bands within the same spectral range (Schwanninger et al., 2011). Such an overlap would cause misidentification.

In case of Raman, recovery rates were comparable between different methods. The difference in the average recovery rates is largely attributed to two factors. Studies of recycled MPs were not done with DXR3xi and the enzymatic treatment protocol was not applied with that device.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Recovery rate (%)</th>
<th>Recovery rate (%)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DXR2xi (Gold)</td>
<td>DXR2xi (Anodisc)</td>
<td>DXR3xi with particle selection (Anodisc)</td>
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<tr>
<td>PA</td>
<td>65</td>
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<td>80</td>
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<tr>
<td>PVC</td>
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<td>65</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Rec. PP</td>
<td>40</td>
<td>30</td>
<td>/</td>
</tr>
<tr>
<td>Rec. PE</td>
<td>43</td>
<td>20</td>
<td>/</td>
</tr>
<tr>
<td>Average</td>
<td>48.8</td>
<td>31.9</td>
<td>52.2</td>
</tr>
</tbody>
</table>

**Table 4**

Raman recovery rate test results on gold and Anodisc filters, measured with DXR2xi, and on anodisc filters measured with DXR3xi. The enzymatic treatment described in Section 2.2 was not done before analyses with DXR3xi.
which reduces losses of MPs in the process. An obvious discrepancy between the detectability of the plastic types is clearly visible from Table 3. The particular case of PVC is interesting since it raises the problem of transparency of the MP particle. It is known that transparent PVC particles are difficult to analyse by Raman spectroscopy (Tsering et al., 2022; Fernandez-Gonzales et al., 2022). However, white PPVC MPs showed the same issue. Therefore, PVC seems to be a problematic material that cannot be correctly identified by both methods explored in this study. In the case of recycled MPs, the colouring, additives, and aging might affect the spectra and thus their acquisition, which leads to lower detection rates. This comes with a decrease of the peak intensities per particle, resulting in failures in matching spectra with the database. The threshold for matching spectra was 60 % for all other MPs, but it seems PVC might require lower match percentage thresholds. Examples of misidentified spectra of PVC are included in the supplementary information.

Overall, one can conclude that transparency, combined with a large size and shape range makes the grinded MPs excellent reference materials. In addition of being better representatives of real environment, they cannot be seen during the sample pretreatment phase. Therefore, they do not prompt possibility of more precise handling of the samples. It results in a more reliable measurement than with standardized, large, coloured MP beads that are regular in size and shape. The results also highlight issues with the current detection methods that need to be further addressed.

3.3. Raman plastic film measurements

Fig. 2 shows the results of the second part of Raman experiments. On the left of Fig. 2, confocal Raman spectra are shown. They are obtained from pristine translucent LDPE film (with inhomogeneous volume and constant average surface roughness Ra = 0.3 μm). On the right, spectra obtained from a pristine semi-transparent UPVC (Ra = 0.25–0.80 μm) film are shown. As explained in the experimental section, plastic samples were measured only in air, then with a cover slip above plastic sample, and finally, cover slip-purified water film-plastic sandwiched

![Fig. 2. Raman spectra of LDPE and UPVC measured in air (top), with a cover slip (middle) and with water and a cover slip (bottom).](image)

![Fig. 3. Images of MP particles a) LDPE particle; b) LDPE particle 0.5 s later; c) UPVC particle; d) UPVC particle 0.5 s later.](image)
system. In all cases, the same spectral features appear despite the measurement conditions. This suggests that MPs could be observed if they are near the cuvette wall.

Although the intensity of LDPE Raman spectra was strongest for LDPE in air, weaker for cover slip-LDPE and weakest for cover slip-water film-LDPE system, no drastic changes in the strength of the spectral signals occurred. This simply comes from the fact that glass, water, and LDPE have different refractive indices that will cause losses of probe light. The order of magnitude of intensities was opposite for UPVC. This is due to the variation of local surface roughness of the UPVC particle, which was not exactly in the same position during the measurements. Moreover, the roughness is smoothed by the water film in the third experimental condition. Additionally, semi-transparency of the particle causes loss of intensity of the probe laser beam. Nevertheless, it is obvious from the spectra shown in Fig. 2 that presence of UPVC with considerable microroughness can be revealed through glass and a water film by confocal Raman point measurement. Raman spectroscopy is suitable for monitoring spatial refractive index changes and surface roughness from mechanical stress, because Raman response is strongly affected by light scattering characteristics of a material (MacDonald et al., 2003). Additionally, in principle, micro-Raman spectroscopy could offer a method to detect contaminants of a MP in aquatic environment by means of a 3D refractive index map (Azkune et al., 2020).

### 3.4. UHD Imaging with Valmet F55

The preparation method of MPs presented in this paper enables the simulation of the formation of MPs having different degrees of translucency. Typically, external mechanical forces and photodegradation of MPs can increase the haziness of MPs. This haziness, related to the translucency of the sample, can be detected with the Valmet F55 UHD device.

Typically, a stereomicroscope would give 3D information of MPs by imaging a stationary MP from two different angles. The size limit detection in this case would be 100 μm, but by taking UHD images from moving irregular-shaped MPs in water flow, much smaller particles can be observed (Peiponen et al., 2023). In the images obtained in this study on grinded MPs, particles under 1 mm were studied. Additional observable factors in these measurements were surface roughness and volume inhomogeneity, which could be seen from rotation of the MPs in water flow. Some results are shown in Fig. 3.

An example on an LDPE particle is illustrated in Fig. 3a and b. The image in Fig. 3b was acquired about 0.5 s later than the image in Fig. 3a. Both figures show completely bright projection areas, and if only the

---

**Table 5**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Particle count</th>
<th>Mass of fraction 1 (0-0.2 mm)</th>
<th>Mass of fraction 2 (0.2-0.4 mm)</th>
<th>Mass of fraction 3 (0.4-0.6 mm)</th>
<th>Mass of fraction 4 (0.6-0.8 mm)</th>
<th>Mass of fraction 5 (0.8-1.2 mm)</th>
<th>Mass of fraction 6 (1.2-2 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>405,716</td>
<td>93.2</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>50 mL</td>
<td>545,801</td>
<td>96.5</td>
<td>3.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>60 mL</td>
<td>650,240</td>
<td>96.8</td>
<td>3.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>100 mL</td>
<td>8804</td>
<td>93.2</td>
<td>6.8</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Rec. rate</td>
<td>PS_PP</td>
<td>8804</td>
<td>93.2</td>
<td>6.8</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>
while the recovery rate sample had a different size distribution. The samples had consistent size fractions throughout the three samples, are detected and recognized as a false positive. However, the effluent ing of MPs is another method with which to estimate their size and The first interesting result is that besides the MPs, many other features shape. An excerpt of the conducted measurements is given in Table 5 .

Fig. 5. Examples of detected MPs using the Valmet FS5 imaging device. Panel a) is from an industrial wastewater sample, Panels b, c and d are from the recovery rate sample.

image in Fig. 3a would be available, the size of the particle would be underestimated since the projection area seen in Fig. 3b is much larger than in Fig. 3a, due to rotation of the irregular-shaped MP in the water flow. From Fig. 3c and d, one can observe similar change in the projected surface size but also a strong variation in the contour and sidewalls of the particle. While the LDPE in Fig. 3a and b shows round features, the UPVC in Fig. 3c and d shows sharp edges. The UPVC also has bright and dark grooves, which are finishing marks observed already in the pristine plastic.

Rising air bubbles play an important role in vertical microplastic transport (Lehmann et al., 2023). Microbubble MP interactions in batch air flow have been studied before (Swart et al., 2022). During measurements of grinded MPs, a small size irregular LDPE MP and an attached air bubble were observed (Fig. 4). The central part of the air bubble is seen as a bright spot (in supplementary data there is a video of the particle in motion). The hydrophobic LDPE has captured a single air bubble on its surface.

In addition, imaging tests were conducted with a recovery rate microplastic sample prepared specifically for this experiment (20 PP and 20 PS particles were dispersed in ultrapure water), as well as a real industrial wastewater effluent sample with the Valmet FS5 device. Imaging of MPs is another method with which to estimate their size and shape. An excerpt of the conducted measurements is given in Table 5. The first interesting result is that besides the MPs, many other features are detected and recognized as a false positive. However, the effluent samples had consistent size fractions throughout the three samples, while the recovery rate sample had a different size distribution. The spiked particles were large (>100 μm), so despite the number of identifications, the size and mass fractions were correctly determined. While this method does not give quantitative results regarding the MP number or type, it gives good quantitative data for size and shape. Some examples of recorded particles are shown in Fig. 5.

From Fig. 5 it can be observed that many shapes of microplastics can be detected from the spiked microplastic sample with the Valmet FS5 device, as well as from the industrial samples. Fig. 5a presents a MP fibre (distinguished from biological fibres by its homogeneity and rounded edges), Fig. 5b shows a more rectangular particle, Fig. 5c shows a complex shape that can be a cluster of several particles and Fig. 5d shows an example of a spherical particle with a fibrous “tail”. The thorough and systematic analysis of such images will lead to a better database of shapes and sizes for MP that will be used for machine learning-based recognition algorithm. However, such detailed study does not belong to the scope of this paper. At this stage, it seems imaging with the Valmet FS5 or similar optical imaging device is promising for qualitative detection of MPs in continuous water flow and with the use of machine learning (already present in the device), it could offer an industrial scale option to detect and sort microplastics out of a water flow prior robust analysis by Raman and FTIR to confirm complex cases, e.g., false positives, and aggregates.

The high sensitivity of Valmet FS5 is demonstrated in the case when a single drop of detergent Triton X-100 was introduced in 0.5 L sample volume containing LDPE MPs. In the image shown in Fig. S1, a large LDPE MP and smaller ones can be observed, but importantly also light interference fringes. These rather straight fringes are due to density variation in cross direction of the water flow. The reason of the formation is the refractive index difference between water (1.33) and Triton X-100 (1.490–1.494) (https://www.deltamicroscopies.com/en/products/triton-x-100/, n.d.). The regularity of the fringes along the water flow direction indicates nature of laminar flow. This example highlights the sensitivity and future potential of the Valmet FS5.

4. Conclusions

A cryogenic milling protocol for several types of plastics was successfully developed and MPs, as well as NPs were prepared. It resulted in MPs with irregular shapes and sizes, allowing the preparation of artificial samples very close to the MPs in the real environment, as confirmed by the imaging of real wastewater samples. These particles are hence considered more representative than currently available commercial reference materials and better for developing analytical methods for MP detection. The same method is also applicable for older and aged plastics, so control samples can be made much more accurately for many types of analyses. The MPs already offered insight into the measurement issues that still plague current detection methods (such as misidentification). Synthetic MPs prepared this way could certainly be used to further improve current methodology and develop an efficient standardized analysis technique. Moreover, it can speed up the development of databases for MP and NP recognition by automated devices.

In terms of application of these synthetic MPs, in the realm of optical detection given as an immediate example in this paper, the capture of an
MP close to cuvette wall for imaging it, obtaining a light intensity bitmap, that can be used to identify MPs among organic particles to assure the identification of plastic type by Raman signal, and hence identification by point measurement of Raman spectrum by inexpensive confocal Raman device is a possible solution for qualitative and quantitative analysis of MPs in water. Currently, analysis of water samples is possible in a laboratory environment with a combination of Valmet FSS and a confocal Raman microscope. However, simultaneous performance of these two measurements could be design for in-situ measurements. Commercial field-portable Raman spectrometers are already available (Asamoah et al., 2021) and they could in principle be combined with a device like Valmet FSS or other portable imaging device. The combined instrument would provide images, a light intensity bitmap and spectral information about plastic type, particle size, and shape more rapidly and directly from a complex water sample than current microscopic and spectroscopic methods, without interference from water.

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CRediT authorship contribution statement

Blaz Hrovat: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. Emilia Uurasjärvi: Writing – original draft, Validation, Supervision. Mirka Vitalic: Writing – review & editing, Validation, Methodology, Investigation. Ana Franco del Pino: Writing – review & editing, Investigation. Mika Manttari: Writing – review & editing, Supervision, Project administration. Nikolaos Papamathaiakis: Investigation. Anti Haapala: Supervision, Methodology. Kai-Erik Peiponen: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. Matthieu Roussey: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology. Arto Koistinen: Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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