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Sequential quantification of number and mass of microplastics in municipal wastewater using Fourier-transform infrared spectroscopy and pyrolysis gas chromatography-mass spectrometry☆

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ABSTRACT

Plastic pollution is a significant environmental concern because microplastics (MPs) accumulate in various ecosystems; therefore, the accurate identification and quantification of MPs in environmental samples is crucial. This study presents a new sequential analytical method that combines Fourier-transform infrared spectroscopy (FTIR) and pyrolysis-gas chromatography/mass spectrometry (Pyr-GC/MS) to characterize and quantify MPs. FTIR with a microscope allows the identification of the polymer type and physical dimensions of MPs, whereas Pyr-GC/MS enables determining the chemical composition of MPs with plastic additives. Pretreated wastewater influent samples spiked with reference MPs were filtered through an Al_2O_3 disk for FTIR analysis, and the surface contents were collected and subjected to Pyr-GC/MS analysis. The mass of the reference MPs estimated using FTIR were in good agreement but were slightly lower than those obtained using Pyr-GC/MS. This finding supports the notion that the proposed sequential method can be used to determine both the number and the mass of MPs in environmental samples.

1. Introduction

Plastic pollution in the environment is a major concern because the increasing production of plastics and mismanagement of their disposal led to the accumulation of microplastics (MPs) in oceans, freshwater, sediment, terrestrial ecosystems, and biota (Andrady, 2011; Meng et al., 2020; Zhou et al., 2020). Numerous researchers have highlighted the negative effects of MPs on the environment, animals, and humans, and isolating and identifying MPs in diverse environmental matrices has become particularly important (Hampton et al., 2022; Hidalgo-Ruz et al., 2012; Ivleva, 2021; Kwon et al., 2020; Sangkham et al., 2022; Silva et al., 2018). Various methods have been developed for pretreatment, separation, and quantification of MPs. Methods for separating MPs generally include chemical reagents (e.g., H_2SO_4, H_2O_2, and KOH) or enzymatic (e.g., proteinase-K and trypsin) digestion for removing organic matter other than plastics, density separation using dense salt solutions (e.g., NaCl, $ZnCl_2$, and NaI), and using separation methods such as filtration for the physical removal of other particles (Ivleva, 2021; Huang et al., 2023; Kwon et al., 2020; Silva et al., 2018). To quantify MPs, visual inspection, dye staining, spectroscopic methods such as Fourier-transform infrared (FTIR) and Raman spectroscopy, and mass spectrometric analysis after thermal decomposition, such as pyrolysis gas chromatography/mass spectrometry (Pyr-GC/MS) or thermal extraction and desorption (TED)-GC/MS are usually employed (Duemichen et al., 2019; Hermabessiere et al., 2018; Huang et al., 2023; Lenz et al., 2015; Mai et al., 2018; Tagg et al., 2015). Recent reviews of analytical methods for removing MPs provide good summaries and comparisons (e.g., Huang et al., 2023; Kwon et al., 2020; Lee and Chae, 2021).

FTIR analysis is one of the most widely used analytical methods for characterizing and quantifying MPs in environmental samples. This technique allows polymer identification by measuring the transmission or absorption of infrared radiation (IR) through the molecular vibrations of specific bonds and other functionalities. Furthermore, FTIR can be combined with optical microscopy to provide information on the chemical fingerprints and physical properties (e.g., color, size, and shape) of MPs. Although advances in instrumental technology, such as focal plane array detectors, have enabled fast scanning and automatic

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analysis of MPs, reducing the uncertainty and time spent quantifying MPs (Tagg et al., 2015), it still requires a few hours to scan whole area of one sample. Furthermore, it can be applicable for MPs greater than 10 μm and may be influenced by the presence of undigested organic matter and other interfering particles in the sample, which require extensive cleanup prior to the analysis (Primpke et al., 2020; Veerasingam et al., 2021).

In contrast, Pyr-GC/MS is a destructive thermoanalytical method. Samples containing MPs are thermally decomposed under defined conditions using a pyrolyzer and the pyrolysis products are analyzed using GC/MS. The advantages of this method are that chemical analysis of multiple particles can be conducted simultaneously and plastic additives can be detected with high sensitivity (Huang et al., 2023; Picó and Barceló, 2020; Santos et al., 2023). However, this method requires complex data processing and exhibits extremely variable limits of detection (LODs). Moreover, samples must be preconcentrated because very small quantities (usually less than 0.5 mg) are pyrolyzed to obtain reliable LODs (Dümichen et al., 2015; Santos et al., 2023).

The high diversity of the analytical methods for MPs with variable LODs causes significant discrepancies in the observed MP levels in similar sample matrices (Elert et al., 2017; Lu et al., 2021; Metz et al., 2020). The level of MP contamination can be expressed either as the number of MP particles in a sample (e.g., items L^{-1}) or as the mass concentration of the respective polymer (e.g., mg L^{-1}) depending on the purpose of the study. Generally, the results obtained using FTIR are expressed as the number of particles in the sample, whereas those obtained using thermal analyses, such as Pyr-GC/MS, are reported as the mass concentration of polymers. Moreover, FTIR analysis of MP particles is hampered by a size limitation (>10 μm particle size) owing to the wavelength range of IR, whereas there is no size limit for analyzing MPs using Pyr-GC/MS. Most earlier studies applied a single analytical method and the inherent differences between FTIR and Pyr-GC/MS could lead to inconsistencies in the calculations of the reported MPs levels.

Several studies have attempted to determine the reasons for discrepancies in the results obtained using spectroscopic and thermal analytical techniques. For example, Elert et al. (2017) compared the obtained results using Raman microscopy, FTIR, thermal extraction-desorption-GC/MS, and liquid chromatography for analyzing polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET); however, the quantities calculated using these methods were not compared. Primpke et al. (2020) compared the results obtained from Pyr-GC/MS and FTIR analyses of MPs in the same samples and found differences in the composition of polymer and disagreement between the mass calculated using FTIR data and Pyr-GC/MS, probably owing to variations in the levels and polymer compositions of the MPs in the samples.

Although non-destructive spectroscopic methods such as FTIR and destructive thermal analyses such as Pyr-GC/MS could be complementary, giving the number and mass of MPs in the collected samples, the sequential application of the two methods has rarely been attempted. Most proposed methods report MPs concentrations only in terms of their number or mass. A limited number of studies have reported both the mass and the number of MPs through parallel determination using replicate samples (Chouchene et al., 2022; Primpke et al., 2020). Because the sample preparation procedure depends on the terminal analytical instrument, pretreatment processes are usually not compatible with sequential analyses using FTIR and Pyr-GC/MS.

The primary objective of this study was to develop a sequential analytical method using FTIR and Pyr-GC/MS to quantify number and mass of MPs in environmental samples. A wastewater influent was chosen as a representative environmental matrix, and was spiked with a known amount (i.e., particle number and mass of MPs) of reference MP particles. The efficiency of extraction and quantification of MPs using the two analytical methods was determined. Finally, the proposed sequential determination method was applied to a raw wastewater sample to compare the mass of the MPs in the field samples obtained using FTIR and Pyr-GC/MS.

2. Materials and methods

2.1. Materials

Commercially available pellets of high-density polyethylene (HDPE), PP, and PS (LG Chem Ltd., Seoul, Republic of Korea), PET (Lotte Chemical, Seoul, Republic of Korea), and polyamide 6 (PA6) (Hyosung TNC Co., Seoul, Republic of Korea) were obtained (images of the raw materials are presented in Figure S1, Supplementary Material). The Korea Testing & Research Institute (KTR, Gwacheon, Gyeonggi-do, Korea) supplied the custom-made reference MPs of HDPE, PS, and PET (20–100 μm in size). These five types were chosen because of their high production volume (Plastic Europe, 2022) and abundance in wastewater (e.g., Park et al., 2020). Sodium iodide (99.5% purity) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Anthracene-d_{10} (98%) was purchased from Sigma-Aldrich Korea (Seoul, Republic of Korea).

2.2. Preparation and characterization of reference MP particles

A bench-top plastic extruder (LME-230, Dynisco, Franklin, MA, USA) was used to prepare cylindrical reference MP particles. The pellets of the five different polymer materials (HDPE, PP, PS, PET, and PA6) were molded into fibers of average diameter 74–133 μm. The fibers were cut into cylindrical reference MPs approximately 100 μm in length using a microtome (Microm HM200, Microm International, Walldorf, Germany), as described in our previous studies (Kim et al., 2022; Lee et al., 2022). The cross-sectional diameters and lengths of the cylindrical reference MP particles were measured using images captured with a BX51 microscope coupled with a DP20 camera (Olympus, Tokyo, Japan). The images were analyzed using ImageJ software version 1.53a (National Institutes of Health, Bethesda, MD, USA). The reference MP particles obtained from the KTR were fragmented particles with irregular shapes. Their sizes were calculated by measuring their fineness using a Partica LA-960 laser scattering particle size distribution analyzer (Horiba Ltd., Kyoto, Japan). The shapes of the reference MP particles were characterized using field-emission scanning electron microscopy (FE-SEM) (Quanta FEG 250, Thermo Fisher Scientific, MA, USA). The reference MP particles were attached to a scanning electron microscope (SEM) mount using carbon conductive adhesive double-sided tape (width 5 mm and thickness 0.16 mm; Woo Yang Chem, Seoul, Republic of Korea). After coating the surfaces of the MP particles attached to the SEM mount for 1 min at 20 mA, the surfaces of the reference MP particles were observed under FE-SEM in secondary electron mode with 5.00 kV of accelerating voltage.

The densities of the reference MP particles of the different polymer materials were measured employing a modified titration method (ISO, 2012) using 99% ethanol, distilled water, and NaI solution (density 1.58–1.60 g mL^{-1}). For measuring densities of HDPE and PP, the MP particles were added to 40 mL glass vials containing 10 mL of 99% ethanol. The air bubbles were removed. After the MP particles had sunk to the bottom, a small amount of distilled water was added using an automatic micropipette. After shaking carefully to mix the solution, the procedure continued until the MPs floated in the liquid for 1 min without rising or falling. Subsequently, to determine the solution density, 2 mL of the solution was placed in a balanced container using an automatic micropipette. The density measurements were conducted in triplicate for each reference MP. The measurement procedures for determining the density of PS, PER, and PA6 were the same as described above, except that NaI solution was used as the titrant instead of distilled water. The measured densities of the cylindrical HDPE, PP, PS, PET, and PA6 were 0.956 ± 0.003, 0.889 ± 0.009, 1.053 ± 0.005, 1.327 ± 0.003, and 1.121 ± 0.003 g cm^{-3}, respectively. The values of the fragmented

HDPE, PS, and PET were 0.954 ± 0.003, 1.049 ± 0.003, and 1.358 ± 0.003 g cm^{-3}, respectively.

Reference MPs for calibrating the Pyr-GC/MS method were prepared by grinding the molded fibers of HDPE, PP, PS, and PET using a freezer mill. Subsequently, the ground MPs were sonicated in methanol for 30 min and sequentially sieved through commercial-grade stainless steel sieves with pore sizes of 300 μm and 100 μm in sequence. The particles that passed through the 100 μm sieve were employed to derive the calibration curves of each polymer. In the case of PA6, calibration was performed using commercially available powder with a particle size of less than 50 μm.

2.3. Preparation and pretreatment of samples

To propose an optimal sequential method for MP particles using FTIR and Pyr-GC/MS, two methods for collecting MPs from municipal wastewater influent were compared. The wastewater was collected from the Jungnang wastewater treatment plant (WWTP) (Seoul, Republic of Korea) on September 4, 2021, using grab sampling. Stainless still device was used to collect wastewater influent in a retention tank and the collected sample was stored at 4 °C until use. Details of the sampling and storage of the WWTP influent are available in our previous study (Lee et al., 2022). Both methods analyzed MP particles on a filter using FTIR followed by mass analysis using Pyr-GC/MS. The detailed analytical procedure for both instruments is described in sections 2.4 and 2.5. The sample preparation and pretreatment processes were the same for both methods, except for the step of collecting MP particles from the filter that was used in the FTIR analysis.

For method development, water samples containing five cylindrical reference MPs (HDPE, PP, PS, PET, and PA6), two fragmented reference MPs (PS and PET), four mixtures comprising five cylindrical reference MPs, and five mixtures comprising three fragmented reference MPs were prepared. To evaluate the efficiency of collecting the MPs of the individual polymer materials, approximately 0.1 mg of each reference MP was weighed using a microbalance (model MCA3.6 P–2S01-M, Sartorius Lab Instruments GmbH & Co., Göttingen, Germany), and a weighing dish containing the weighed MPs was placed on the stage of an optical microscope. The number of reference MPs was counted, after which the MPs were added to a 74 mL glass test tube containing 30 mL of the wastewater influent. The samples for the polymer mixtures were prepared in the same manner, differing only in the composition of the injected MP particles. Each sample was prepared in duplicate. The compositions of all the mixtures used in method development are listed in Table S1 (Supplementary Material). Mixtures containing three fragmented reference MPs were prepared by adding <0.01 mg of each reference MP to water. The accuracy of the method was assessed by comparing the number and the mass of recovered reference MPs with those spiked in the FTIR and Pyr-GC/MS analyses.

For sample pretreatment, a wet peroxidation method using 30% H_2O_2 solution was employed to remove particles other than plastics (Al-Azzawi et al., 2022; Dyachenko et al., 2017; Park et al., 2020). First, 30 mL of 30% H_2O_2 solution was added to a test tube containing the reference MPs mixed with the WWTP influent. Subsequently, the test tube was placed in a dry oven at 60 °C for 3 h for wet peroxidation to occur. Afterward, the oven temperature was increased to 85 °C to evaporate the solution, and the test tubes were removed immediately after the solutions had dried completely to prevent potential melting loss of MPs on the surfaces. Although wet peroxidation and drying temperatures were not appropriate for PA6 considering its low glass transition temperature (Greco and Nicolais, 1976), they were chosen for efficient removal of organic matter and simultaneous recovery of other MPs (Karami et al., 2017). For density separation, 30 mL of NaI solution (1.6 g mL^{-1}; filtered through a Whatman grade GF/C glass microfiber filter with a pore size of 1.2 μm) was added to the test tubes and vortexed for 2 min to resuspend the particles present at the bottom of the test tube. The test tube was kept stationary for 3 h to allow the MP particles to float to the surface. The MPs on the surface of the solution were collected carefully and filtered through a Whatman Anodisc inorganic filter membrane with 25 mm diameter and 0.2 μm pore size. The obtained filters were placed in clean glass Petri dishes and dried in a vacuum desiccator. Three blank samples were prepared in three glass test tubes containing 30 mL of the WWTP influent. These samples were not spiked with reference MP particles but were treated using the same process as that used for the other samples.

For sequential quantification of MPs in the WWTP influent, 30 mL of well-mixed influent was added to a 74 mL glass tube, and peroxidation and density separation were performed using the same procedure described above. The particles in the two test tubes were collected using an Anodisc filter to concentrate the samples. Blank samples were prepared through the same pretreatment procedure and the same amount of distilled water.

2.4. Spectroscopic analysis of microplastics using FTIR

The particles collected on the Anodisc filter were analyzed using an FTIR spectroscope equipped with a dissection microscope (× 15) (Nicolet iN10 MX, Thermo Fisher Scientific, Waltham, MA, USA) by placing the filter on the FTIR mount. The spectral contributions of each reference MP were obtained. The collection time was 0.314 s at a resolution of 4 cm^{-1}, and these data were saved as reference spectra. The spectra of the whole filters were obtained by scanning the filters within the collection time of 0.314 s at a resolution of 16 cm^{-1} over a wavelength range of 4000–1400 cm^{-1}. The entire filter area was divided into several sections, and the scanned outcomes of each section were then combined to tally all MPs on the filter. Using OMNIC Picta Expert software (Thermo Fisher Scientific), suspected MPs were identified using correlation maps obtained by comparing the spectrum map of the filter with the reference spectra. To detect the MPs in the WWTP influent, the spectra of the reference MPs (Figure S2, Supplementary Material) and spectral libraries provided by the manufacturer were used as reference spectra. More than 100 libraries were provided by the manufacturer, of which 15 libraries were related to polymers or polymer-related materials and contained approximately 8500 spectra. To confirm the presence of MP particles, suspected particles were observed under the microscope of the FTIR instrument and their IR spectra were compared with those available in the library. Particles of the expected shapes showing a spectral similarity of >60% were determined as MP particles. A minimum similarity of 60% was selected, considering that the particle sizes were smaller in the current study than in previous studies using the same instrument, in which a similarity >70% was chosen (Park et al., 2020; Pham et al., 2023).

The masses of the identified reference MPs were estimated from their projected areas. For cylindrical reference MPs, the diameter and height of the cylinder were estimated from the projected area. The fragmented reference MPs were assumed to be ellipsoidal. The long and short axes identified from the projected image were used to estimate the volume of the ellipsoid (an illustrative example is shown in Figure S3, Supplementary Material) (Lee et al., 2022).

2.5. Mass analysis of MPs using pyrolysis-GC/MS analysis

The MPs collected on the Anodisc membrane filter were analyzed using the Pyr-GC/MS method after FTIR analysis using two preparation methods, namely the whole filter collection method (method 1) and the surface scratching method (method 2). In method 1, the supporting ring made of PP was removed carefully using a stainless-steel hole punch that fits with the area of aluminum oxide (Al_2O_3) part after measuring the mass of the filter (Figure S4, Supplementary Material). The remaining Al_2O_3 part of the filter was crushed gently using an agate mortar. The entire crushed sample was placed in a 20 mL glass vial and mixed using a vortex mixer. Approximately 1.5–2.5 mg of the crushed sample (weighed using a microbalance) was placed on pyrofoil (F590; Japan

Analytical Industry Co., Ltd., Tokyo, Japan). Anthracene d_{10} solution (0.02 g L^{-1} in *n*-hexnane) was used as internal standard for injection to pyrolyzer. After adding 40 μL of internal standard to pyrofoil and drying it, it was folded and placed inside the sample tube of a portable Curie Point Pyrolyzer (JCI−55, Japan Analytical Industry Co.). No PP and other MP particles were identified in three blank samples following the same procedure without filtration. In method 2, MP particles were collected to pyrofoil with a simple treatment. First, Scotch™ VHBTM double-sided tape of circular shape (diameter 2 cm, 3 M) was attached to a square aluminum plate (approximately 3 cm × 3 cm, 1 mm thickness), and the Al_2O_3 part on the back side of the Anodisc membrane, where the MP particles were collected, was attached to the other side of the tape. Because of the extremely small pore size of the Anodisc (0.2 μm), the filtered microplastics did not penetrate and remained on the surface. The surface of the Al_2O_3 part of the Anodisc membrane with the trapped particles was scraped off carefully with the wide end of a laboratory tweezer until the initial white surface of the Al_2O_3 part was re-exposed. The process of collecting the particles on the filter is illustrated in Fig. 1. The entire sample collected in powder form was transferred directly to pyrofoil. The sample collected on pyrofoil was treated with a tetramethylammonium hydroxide (TMAH, 25% methanol) solution and the internal standard, and subjected to Pyr-GC/MS analysis.

The pyrolyzed sample was injected into the GC/MS system using the portable injector of the pyrolyzer, after which mass analysis was conducted. The reaction conditions for the Pyr-GC/MS are presented in Table S2 (Supplementary Material). For the analysis of PET, 20 μL of the TMAH solution was added to the sample for thermochemolysis and allowed to evaporate at room temperature for 30 min prior to pyrolysis. The analysis was repeated four times for each sample, and the average of the quantified masses was used for data analysis.

The polymers were quantified by examining the specific indicator compounds of each polymer (Table S3, Supplementary Material) (Fischer and Scholz-Böttcher, 2017, 2019). Calibration standards were prepared by mixing kaolin and the reference MPs in a mass ratio of 10:1. Approximately 10–150 μg (1–15 μg in polymer mass) of the standard was weighed on pyrofoil and used to obtain calibration curves. Anthracene-d_{10} solution was added as an internal standard, and thermochemolysis was conducted using tetramethylammonium hydroxide (TMAH) to calibrate the PET. Multiple regression analysis was performed for polymers with various specific indicator compounds (HDPE, PP, and PS). The calibration parameters are listed in Table S4 (Supplementary Material).

2.6. Quality control

Metal or glass laboratory ware was used in the sample preparation and instrumental analysis processes to prevent possible contamination by the MPs. All sample preparation, pretreatment, and filtration steps were performed inside a laminar flow box (HSCV-1300) (Sinan Science Industry, Gyeonggi-do, Republic of Korea) to prevent contamination by indoor airborne MPs. All solutions used in the experiments were filtered using a metal filter (pore size 20 or 5 μm) before use. Filtered water and 99% ethanol were used to clean all the glassware prior to conducting the laboratory experiments. The samples were covered with a glass plate or aluminum foil when they were moved outside the laminar flow hood. The experimenters wore cotton coats and nitrile gloves during all the processing steps to minimize contamination. In addition, it was confirmed that MP particles were not observed in the blank samples (30 mL of distilled and filtered water) prepared in each batch, simultaneously treated with the WWTP influent samples.

3. Results and discussion

3.1. Characterization of reference MPs

The sizes and shapes of the reference MPs were characterized before analysis. Visual analysis of the cylindrical reference MPs was performed using an optical microscope. The average diameter range of the cylindrical MPs was 74.62–137.72 μm and the average length range was 112.29–133.34 μm (Table S5, Supplementary Material). Both the diameters and lengths of the HDPE reference particles exceeded those of the other particles. The size distribution of the fragmented reference MP particles obtained using a laser scattering particle size distribution analyzer is shown in Figure S5 (Supplementary Material), and the detailed size distributions are presented in Table S6 (Supplementary Material). The average diameters of the fragmented references HDPE, PS, and PET were 71.3, 36.8, and 79.5 μm, respectively. The fragmented reference PS was much smaller than other fragmented reference MPs. The particle size distribution of the fragmented reference HDPE was broader than that of other samples. The measured distribution of fragmented reference MPs was used to estimate the number of particles that had been injected during the recovery tests.

Most cylindrical reference MP particles had a regular cylindrical shape without any deformation, and their surfaces were smooth without any cracks or impurities (Figure S6, Supplementary Material). In contrast, the fragmented reference MP particles exhibited significant irregularities in size and shape, as well as greater surface roughness (Figure S7, Supplementary Material).

Table 1
Percent number and mass recovery of reference microplastics spiked in wastewater influent using FTIR and Pyr-GC/MS analysis.

Type of reference microplastics	FTIR (%)		Pyr-GC/MS (%)
	Based on number of particles	Based on the estimated mass	
Cylindrical HDPE	90.9 ± 8.2	86.4 ± 3.2	54.5 ± 0.6
Cylindrical PP	87.1 ± 4.5	83.8 ± 5.5	94.5 ± 5.4
Cylindrical PS	98.3 ± 7.8	87.5 ± 2.6	106.9 ± 7.8
Cylindrical PET	80.3 ± 9.9	73.6 ± 14.7	65.5 ± 6.1
Cylindrical PA6	73.6 ± 6.7	66.4 ± 7.6	68.4 ± 7.7
Fragmented HDPE	86.6 ± 1.4	58.3 ± 2.1	71.0 ± 1.8
Fragmented PS	49.4 ± 4.7	44.4 ± 4.3	88.6 ± 3.1
Fragmented PET	84.9 ± 9.6	76.9 ± 4.1	93.2 ± 3.6

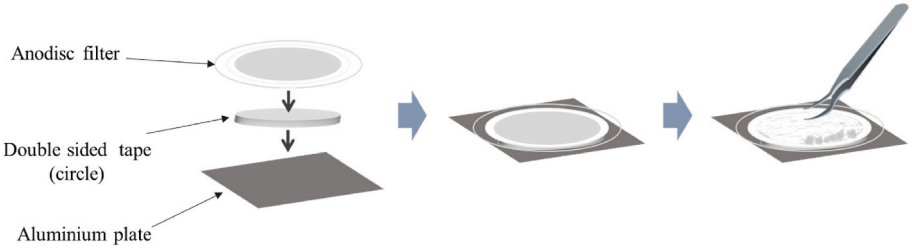


Fig. 1. Schematic illustration of the sample collecting method for mass analysis using Pyr-GC/MS after filtration.

3.2. Comparison of methods for collecting MPs from municipal wastewater

Table 1 presents the recoveries of spiked MPs in the municipal wastewater influent based on their number and estimated mass using FTIR and mass obtained using Pyr-GC/MS. Overall, the percent recoveries obtained using FTIR were greater than 70%, except for PA6. As noted in earlier studies, PA6 is likely damaged and adheres to the surfaces of laboratory ware during peroxidation or drying (Karami et al., 2017; Roch and Brinker, 2017). A significantly lower percent recoveries were observed for fragmented HDPE and PS MPs using FTIR than for cylindrical MPs. As shown in Figure S5 (Supplementary Material), the fragmented reference HDPE and PS contained a significant portion of particles smaller than 20 μm, which might be hardly detectable using FTIR analysis.

Cylindrical PP, PS, and PA6, and fragmented PET showed slightly higher mass recoveries using the Pyr-GC/MS method than using the FTIR method, although the differences were not significant. However, PA6 was not reliably quantifiable by either method. Cylindrical HDPE and PET showed higher recoveries using FTIR analysis than using the Pyr-GC/MS method, which could be ascribed to uncertainty in the analyzed mass of the MPs caused by the heterogeneity of the sample. In addition, both types of reference HDPE particles showed relatively low recoveries using Pyr-GC/MS analysis, indicating that FTIR is more suitable for analyzing HDPE particles.

Although the unreliability of using the estimated particle number to derive recovery values cannot be ignored, a comparison of the two analytical methods revealed the disadvantages of FTIR analysis, which have been indicated before (Primpke et al., 2020; Veerasingam et al., 2021). The importance of studying small (<20 μm) and irregularly shaped MP particles has been emphasized recently, as such particles abound in environmental matrices and weathering increases their abundance (Kooi and Koelmans, 2019; Menzel et al., 2022; Song et al., 2023). Therefore, FTIR analysis is not recommended in cases where irregularly shaped MP particles smaller than 20 μm dominate. However, this problem could be overcome by performing sequential analysis, as described in detail in the following section.

3.3. Application of the sequential determination method for environmental samples

In Fig. 2, a comparison is presented of the estimated mass of MPs spiked in the wastewater influent using FTIR and Pyr-GC/MS. Although the two identification methods were well in agreement for all individually spiked MPs (Fig. 2a), the estimation by Pyr-GC/MS showed a greater mass of all three individually spiked fragmented MPs (Fig. 2b). As discussed previously and shown in Figure S5 (Supplemental Material), the smaller MP particles in the fragmented reference MPs, which the FTIR method failed to identify, likely explain the significantly greater mass quantified by the Pyr-GC/MS method. This finding implied that the identification of MPs using only FTIR could underestimate the overall mass of MPs, particularly when highly fragmented particles of less than 20 μm dominate. The two methods exhibited larger deviations (Fig. 2c) when cylindrical MPs were spiked at lower concentrations in the mixture, although the total MP concentration was close to that in the individual spiking experiments. Significant dilution of a sample likely increased the detection limits of the individual MPs because the entire Al_2O_3 filter was crushed and introduced into the pyrolyzer, e.g., the indicator ions for PA6 were not identified under this condition. Therefore, the analytical procedure was improved by scratching the particles on an AlO_2 filter, as shown in Fig. 1 (method 2).

In Fig. 3, a comparison is shown of the masses estimated by employing FTIR and Pyr-GC/MS using method 2. All the masses of the spiked cylindrical MPs as a mixture, estimated using FTIR, agreed well with those estimated using Pyr-GC/MS, except for PA6 (Fig. 3a). Unlike in the case of cylindrical MPs, the masses estimated using Pyr-GC/MS for all three fragmented MPs were consistently greater than those estimated using FTIR. As discussed earlier and shown in Figure S5 (Supplemental Material), the significant contribution of smaller fragmented particles, which could hardly be identified using FTIR, explain these deviations. The most important advantage of method 2 is the lower detection limits for all tested MP materials because of the insignificant dilution. Moreover, the wastewater influent matrix could contain smaller MPs with irregular shapes, resulting in a greater mass in Pyr-GC/MS analysis. Therefore, this method is applicable to real wastewater influents without spiking with the reference MPs. Fig. 3c shows the masses of the MPs estimated by FTIR and Pyr-GC/MS using method 2 without spiking with the reference MPs. The masses of MPs in the wastewater influent estimated using Pyr-GC/MS were slightly greater than those estimated using FTIR, suggesting a notable loss of smaller microplastics in field samples when FTIR was employed as the primary detection method. The deviation from the 1:1 line was mostly within a factor of two. Accordingly, the sequential method proposed in this study provides valuable results, including the size, shape, number, and mass of MPs in environmental samples.

3.4. Environmental implications

The sequential determination procedure proposed in this study is very promising, as it provides the simultaneous measurement of both the number and the mass of the MPs within the same sample. Considering that the environmental impacts of MPs are likely influenced by factors

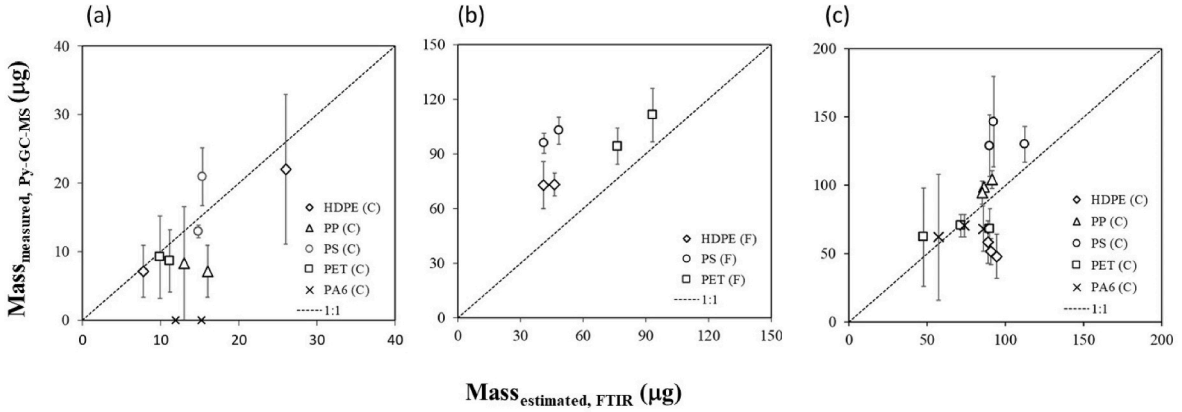


Fig. 2. Comparison of the mass of microplastics estimated using the FTIR and Pyr-GC/MS for (a) spiked cylindrical MPs, (b) spiked fragmented MPs, and (c) spiked cylindrical MPs as a mixture using method 1. Error bars represent standard deviations of four replicates in the Pyr-GC/MS analysis. C and F in parentheses denote cylindrical and fragmented reference MPs.

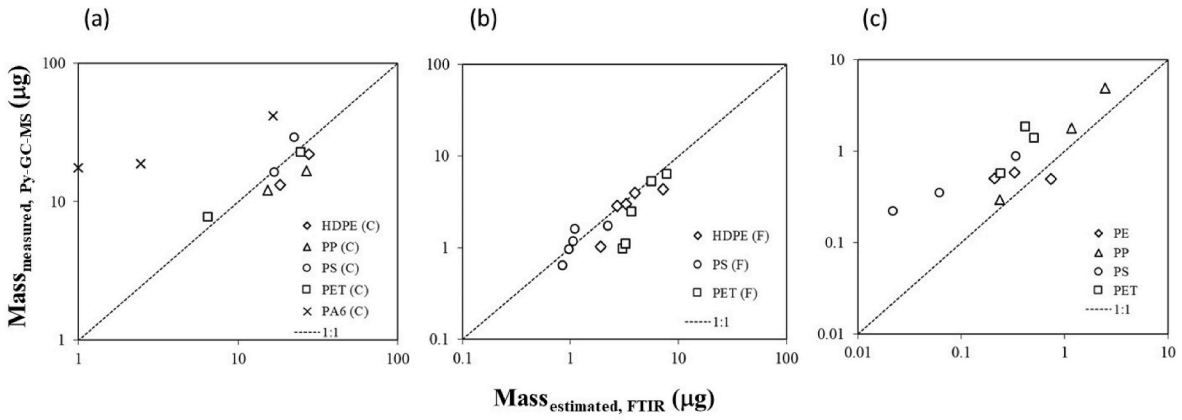


Fig. 3. Comparison of the mass of microplastics estimated using FTIR and Pyr-GC/MS for (a) spiked cylindrical MPs as a mixture, (b) spiked fragmented MPs as a mixture, and (c) the wastewater influent sample using method 2. C and F in parentheses denote cylindrical and fragmented reference MPs.

such as their number, type, and mass (Sun et al., 2022; Schwarzer et al., 2022), relying solely on methods utilizing a single instrument is insufficient for accurate assessment of the risks associated with these MPs. Sequential determination is, therefore, a more comprehensive approach. However, it is essential to extend the applicability of this method, particularly to samples with low MP concentrations. In addition, a clear definition of the detection limits for various MP materials in different matrices is necessary. Notably, the applicability of the sequential determination method could be limited to sample matrices containing brittle materials such as Al_2O_3 disks. The high pressure drop occurring during filtration, even after wet peroxidation and density separation, could constrain the effectiveness of this method for certain sample matrices. Nevertheless, the successful performance of the method was demonstrated in the case of WWTP influent.

4. Conclusions

The sequential analytical method developed in this study, which combines non-destructive FTIR and destructive Pyr-GC/MS techniques, effectively determined both the number and the mass of MPs present in WWTP influent samples. Estimation of MP mass using FTIR exhibited general agreement with the mass determined based on indicator ions using Pyr-GC/MS, particularly for reference MPs greater than 20 μm. However, slightly greater masses of MPs were quantified by Pry-GC/MS when the MPs were irregular and <20 μm. By harnessing the complementary nature of both instrumental methods for assessing the occurrence of MPs in field samples, the serial combination of these two analytical instruments holds significant promise for facilitating comprehensive evaluation of the toxicity and risk associated with MPs. This integrated approach provides a robust framework not only for accurately characterizing but also for understanding the potential environmental impacts of MPs. However, further validation is required using diverse environmental matrices.

Author contribution

So-Young Lee: Conceptualization; Methodology; Data curation; Investigation; Writing-original draft. Jiyul Ahn: Data curation; Investigation. Jung-Hwan Kwon: Conceptualization; Methodology; Funding acquisition; Supervision; Writing-review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2023.122452.

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