Effect of Agitation Method on the Nanosized Degradation of Polystyrene Microplastics Dispersed in Water

Harutaka Mekaru*

ABSTRACT: Reports of marine organisms ingesting microplastics—formed from large plastic litter drifting in the marine environment by mechanical forces such as waves and photochemical processes initiated by sunlight, particularly ultraviolet rays—are increasing. However, the degradation process from microplastics to nanoplastics that are easily consumed by plankton located in the lower part of the food chain is not clear. Therefore, 200 nm diameter polystyrene (PS) latex particles—nanoparticle tracking analysis (NTA) calibration particles—dispersed in ultrapure deionized water were subjected to three types of agitation: rotation mixing, shaking, and flowing in addition to standing without agitation, and the physical degradation caused by agitation for 1 week at room temperature (23 °C) was evaluated. The degradation of the particles into nanosized particles was assessed by particle size distribution measurement using NTA and shape observation using field emission scanning electron microscopy (FE-SEM). In addition, the ratio of particle aggregation during incubation was calculated from the number of particles present in the region exceeding the particle size distribution range of the monodisperse particles before agitation with respect to the total number of particles. A shape change to a particle size of 100 nm or less was observed for the rotating and flowing of samples, and the influence of aggregation was suppressed to within 21% of the total particles calculated by NTA at the maximum. These results show that chemically stable PS can be degraded from micro- to nanosize with simple agitation in ultrapure deionized water.

INTRODUCTION

Polystyrene (PS) does not degrade in the cellular environment and does not show short-term cytotoxicity. In addition, PS nanoparticles (NPs) can be easily synthesized in various sizes and have a clear surface functionality, making them a convenient platform for studying nanoparticle–cell interactions. However, harmful substances, such as environmental hormones adsorbed on microplastics (100 nm to 5 μm) and nanoplastics (≤100 nm), can migrate from the digestive organs of an organism to fat cells and bioaccumulate. Furthermore, there is concern that these chemicals may be concentrated up the food chain and eventually have a damaging effect on human health. In this process, nondegradable PS has a risk of being repeatedly acted as a transporter of harmful substances to the human body. Microplastics are generated from waste plastic as a result of wind and rain on land, wave forces in the ocean and on the shore, breaking or rubbing with sand and pebbles on the shore, and complex factors such as geological movements of mud and sand on the ocean floor. A calculation based on the approximated 79 000 tons of trash floating in the Pacific Ocean estimated 31% of it to be microplastic. The core of the microplastic problem is the adsorption and concentration of toxic chemicals on the microplastics, which are then incidentally ingested by marine organisms. Nanoplastics, in particular, are consumed by plankton located in the lower part of the food chain. Fish then eat the plankton, which concentrates harmful substances such as polychlorinated biphenyl (PCB) in the food chain.

PS is a common plastic in the marine environment, which drifts in the ocean because its specific gravity is lower than that of seawater, like polypropylene (PP) and polyethylene (PE). PS
and PE have been reported to adsorb polycyclic aromatic hydrocarbons (PAHs) at a higher concentration than PP. It has also been reported that oral administration of PS particles can adversely affect iron uptake and transport in human small intestinal epithelial cell models and live chicken small intestine models. It has been found that the proportion of PS particles taken up from the gastrointestinal mucosa of rats depends on the particle size and that smaller particles are more likely to be taken up. In addition, the distribution in the body after ingestion depends on the particle size, and the smaller the particle, the faster the distribution to each organ. Whether waste plastics can be degraded to nanosize particles in nature was under question; however, the formation of PS particles from disposable PS coffee cup lids by exposure to ultraviolet and visible light and mechanical grinding has now been shown in particle size distribution measurements by nanoparticle tracking analysis (NTA). However, there have been no reports on the degradation process from microplastics to nanoplastics. Therefore, measuring the concentration and size of microplastics and nanoplastics in the environment is essential for assessing the risks posed by plastic particles.

Figure 1. Particle size distributions and integrated particle number for PS particles (a) before and after incubating by (b) standing without agitation, (c) rotating, (d) shaking, and flowing using (e) tubing and (f) intelligent pumps for 7 days. Micro-/nanoparticles have a high tendency for adhesion and aggregation; therefore, to use these particles, aggregation and dispersion control are extremely important. For this purpose, it is first necessary to accurately grasp the aggregation state of the nanoparticles. Dynamic light scattering (DLS) and laser diffraction (LD)/scattering particle size analysis are qualitative methods, but have a wide dynamic range. We evaluated the aggregation of particles by analyzing the particle size distribution obtained by non-negative least-squares fitting of DLS data. Moreover, the size of aggregated particles was calculated from the delay in the observation time of the scattered light during DLS measurements. At present, there are no methods available that can quantitatively evaluate the nanometer to millimeter range; however, in the submicrometer range (0.1–1 μm), quantitative detection can be performed using NTA or resonance mass measurements (RMM). Each analysis method has advantages and disadvantages, and analysis using a single method involves a risk of error. In fact, it has been reported that the coefficient of variation (CV) of particle size distributions determined by NTA is not reliable. Therefore, a fitting analysis with a γ distribution determined by the shape and scaling parameters was proposed. Other methods to analyze aggregation have also been proposed. For example, it has been reported that electrospray-differential mobility analysis with single-particle inductively coupled plasma mass spectrometry
ES-DMA-spICPMS can be used to measure both the size and mass of particles independently and simultaneously to quantify the degree of particle aggregation. In addition, a method has been proposed to prevent erroneous measurement due to aggregation by measuring the ultraviolet absorption of samples separated by field-flow fractionation (FFF). However, the above methods are complex and require specialized training. Therefore, in the present study, we adopted a simple NTA method to evaluate degradation and aggregation. This involved a comparison of the number of accumulated particles by dividing the area into areas smaller and larger than the particle size distribution range before agitation.

In this work, which focuses on the physical degradation of nanosized PS from microplastics generated during agitation in aqueous solution, the influence of agitation is comprehensively investigated by agitating PS microplastics dispersed in water using three methods including rotation mixing, shaking, and flowing. Samples allowed to stand without agitation were also evaluated. In addition, the effects of liquid volume, rotation speed, and flow rate were investigated. As a result, an optimal agitation method and conditions that enabled physical degradation experiments of PS microplastics in the laboratory with appropriate particle size control are reported.

**RESULTS**

To confirm the presence or absence of microplastic degradation and aggregation simply by the particle size, it is necessary to use an initial particle sample that is monodisperse. Therefore, PS latex particles (average particle size = 204.0 ± 2.1 nm, National Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology), which are used as a certified reference material 570-a for calibration of NTA equipment, were chosen. As confirmed in Figures 1a and 2a, the average particle size of the PS particles measured five times by NTA was 195.9 ± 1.2 nm, and the field emission scanning electron microscopy (FE-SEM) image showed that the PS particles were spherical with relatively uniform particle size.

**Standing without Agitation.** A peak due to aggregation measured by various particle size distribution measurements may appear at the same position as a similar size large spherical particle. Furthermore, if the aggregation state varies, the reproducibility of the peak position also deteriorates. Therefore, as shown in Figure 1a, the accumulated number of particles calculated by NTA in the particle size range 10–600 nm was divided by the total number of particles and plotted as a percentage in relation to the right axis of the NTA data. Excluding the 150–350 nm range, the particle size range 50–150 nm, which was physically degraded during agitation and became smaller, was defined as “Area 1”, and the apparent particle size that increased owing to aggregation in the range

![Figure 2. FE-SEM images of PS particles (a) before and after incubating by (b) standing without agitation, (c) rotating, (d) shaking, and flowing using (e) tubing and (f) intelligent pumps for 7 days.](https://dx.doi.org/10.1021/acsomega.9b03278)
350–600 nm was indicated “Area 2”. The average particle size of the PS particles before agitation calculated by NTA was 195.9 ± 1.2 nm. Figure 1b shows the particle size distribution after resting 0.5 mL of PS particle dispersed water for 7 days in a microtube. Before standing, PS particles showed good reproducibility and a sharp trace. As shown in Figure S1a-1 of the Supporting Information, the measurements also showed good reproducibility after standing; however, the width of the peak was slightly broadened. Figure 2b shows the appearance of PS particles observed by FE-SEM. The shape remained spherical when compared with the PS particles before standing, as shown in Figure S2a-1 of the Supporting Information. At first glance, it appears that large-scale aggregation occurs in the particles after standing, however, this is a well-known phenomenon of the aggregation of particles caused by the drying of the suspension.20

The results of NTA measurements and FE-SEM observation when the liquid volume of the PS particle dispersion was changed to 0.5, 0.75, and 1 mL while using a 1.5 mL microtube are summarized in Figures S1 and S2 of the Supporting Information, respectively. Based on the particle size distribution data measured by NTA—performed five times in a row—accumulated numbers of particles in Areas 1 and 2 were calculated (Figure 3a,b) so that they could be compared before and after agitation. The percentage of the particle number in Area 1 before standing was 0.2%, and the ratio of the particle number belonging to Area 1 after standing was 0.2–4.5% owing to the difference in liquid volume. It was confirmed that PS particles were not degraded by simply standing in water. In addition, in Area 2, the percentage of the particle number was 0.4–8%, and the concentration of particles with a large diameter was slightly higher. However, in the case of the samples allowed to stand, the difference in volume did not appear to significantly affect the aggregation of the particles.

**Rotation Mixing.** Figure 1c shows the results of NTA measurement after rotation mixing of PS particles dispersed in water at 23 °C for 7 days at a rotation speed of 5 rpm. It can be confirmed that multiple peaks appear in the distribution. From the FE-SEM image in Figure 2c, it can be observed that the particles are generally nonspherical and that the sample contains many particles of relatively small size. Therefore, the diameter obtained from the NTA data of the PS particles after agitation is not the true diameter of these particles but that of spherical particles that have the same diffusion coefficient as the tracked particles. Based on the findings shown in Figure S3 of the Supporting Information, the effect of changing the speed of rotation to 5, 10, or 15 rpm when the liquid volume of the
sample was 0.5 or 0.75 mL was small. However, when the liquid volume was increased to 1 mL, particles with a diameter of 400 nm or more were not detected, and when the rotation speed was 5 rpm, the average particle diameter was ~182 nm, which supports a reduction in aggregation. However, it is understood from Figure S3 of the Supporting Information that although the PS particles appear rounded after rotation mixing, the shape is not a well-balanced sphere but distorted. The ratio of the particle number in Area 1 shown in Figure 4a increased with the liquid volume regardless of the rotation speed, except for the case where the liquid volume was 1 mL and the rotation speed was 10 rpm, which gave a ratio of 97%. In contrast, the proportion of particles present in Area 2 was 19% when the liquid volume was 0.75 mL and the rotation speed was 5 rpm, however, it was suppressed to a maximum of 4.4% or less under other conditions. These results indicated that agitating PS particles by rotating degrades them to nanosize without aggregation.

**Shaking.** Figures 1d and 2d show the results of shaking the PS particles dispersed in water (volume 0.5 mL) at 200 rpm, which is the minimum rotation speed that can be set by the block bath shaker used in this study. NTA measurement gave an average particle size of 182.5 ± 6.7 nm, and the peak shape was symmetrical. However, as is shown in Figures S4 and S5 of the Supporting Information, as the liquid volume increased to 0.75 and 1 mL, the NTA distribution also approached the shape of a single peak, and the particle shape remained spherical. In addition, the FE-SEM image showed generally larger particle sizes, however, the angular shape was similar to that of PS particles after rotation mixing. On the other hand, the number of particles classified as Area 1 shown in Figure 5 was 4% or less under most agitation conditions, which is relatively good in numerical terms. However, when the amount of liquid was 0.5 mL and the rotation speed of the block bath shaker was increased from 200 to 600 rpm, the number of particles classified as Area 1 rapidly increased from 14 to 53%, indicating that the degradation of the PS particles had occurred. In contrast, Area 2 caused by aggregation showed a maximum value of 19% when the liquid volume was 0.5 mL and the rotation speed was 600 rpm.
rpm, and the suppression of aggregation by shaking was diminished at this speed.

**Flowing.** Finally, the NTA measurement and FE-SEM observation results after circulating 0.5 mL of PS particles dispersed in water for 7 days in an ethylene tetrafluoroethylene (ETFE) tube at 0.05 mL/min—the minimum settable flow rate of the tubing pump used—are shown in Figures 1e and 2e, respectively. Figures 1f and 2f show the results of agitating PS particles using an intelligent pump under the same conditions. For both pumps, particles with an average particle diameter of 200 nm or less were measured at a high concentration before agitation. A small peak was also confirmed for diameters of 200 nm or more. When an intelligent pump was used, a broad and relatively strong peak appeared in the particle size range 250–500 nm. In addition, the FE-SEM image supports the presence of a mixture of particles with a diameter of approximately 170 nm (indicated by arrow A in the figure) and 580 nm (indicated by arrow B in the figure). However, the image also shows that these particles are spherical in shape with a smooth surface compared with PS particles after rotation mixing or shaking. In contrast, in Figure 2f, a square structure (indicated by arrows C and D in the figure, respectively) with square side lengths of roughly 450 and 500 nm were observed. More rectangular structures with lengths of 200 nm to 2.5 μm were observed; however, no correlation with the flow rate of the intelligent pump was found. Roughly estimated, a single square structure was observed per 2–5 μm² area. The NTA measurement results for samples for which the incubation flow rate was 0.05, 0.1, 0.5, and 1 mL/min, while the liquid volume of the sample was fixed at 0.5 mL, are shown in Figure S6 of the Supporting Information. In most conditions, the tendency for the number of particles with small particle size to be greater than the number of large particles is consistent with the numerical change in the ratio of the particles with respect to the total number, as shown in Figure 6. Although the percentage of particle number in Area 1 appears to decrease from 84% to around 50% with increasing flow rate, the FE-SEM observation results in Figure S7 of the Supporting Information show that the spherical shape was not maintained as the flow rate was increased and the shape became distorted. In addition, a large number of small fragments was observed. The ratio of particle number in Area 2 against the total was 17% at the maximum when the flow rate was 0.5 mL/min and 11% or less at other flow rates. In contrast, as shown in Figure S8, there was no significant change in the NTA distribution shape due to an increase in flow rate, even when the liquid was fed by the intelligent pump. In fact, the particle number ratio of Area 1 in Figure 6 was also stable in the range 41–46%, and in the case of Area 2, it changed from 21 to 5% as the flow rate increased.

**DSC Measurement.** There is tacticity in the structure of PS. Because isotactic PS, in which all asymmetric carbons have the same absolute configuration, and syndiotactic PS, in which absolute configurations are alternately arranged, have regular structures, a crystal structure exists, and a melting point (T_m) can be measured. The melting points of the crystalline PSs described are approximately 240 and 270 °C, respectively. In contrast, a structure in which the absolute configuration is irregular is called atactic PS. Atactic PS does not have a crystal structure and therefore has no melting point. Atactic PS is used in large quantities as a general-purpose plastic. The DSC measurement results shown in Figure 7 show an endothermic peak accompanying melting at around 274 °C. Based on this observation, the PS used in this experiment is believed to have contained syndiotactic PS. However, because the glass transition temperature (T_g) appears in the vicinity of 106 °C, which is higher than that of syndiotactic PS, there is a possibility that atactic PS with a rough molecular weight of 2 × 10^5 g/mol was also present. However, as the PS latex particles are believed to contain crystalline PS, the reasoning that links the square structure shown in Figures 2f and S9 with the recrystallization of PS is thought to be valid.

**DISCUSSION**

The aggregation and dispersion behavior of particles in a suspension strongly influences the particle size. Therefore, it is useful to understand the agitation conditions under which the particles are dispersed and agglomerated. Therefore, investigating the aggregation of PS particles dispersed in water is a valid approach for understanding the degradation process from microplastics to nanoplastics. In addition, since particles are usually much larger than single molecules, they are generally insoluble in solvents and unsoluble and nontoxic, and therefore, once they are aggregated, it is difficult to separate them using a uniform method (e.g., ultrasonication, grinding). Therefore, incubating samples while agitating using a method that suppresses aggregation is important for determining the exact biochemical degradation rate from microplastics to nanoplastics.

An initial finding showed that PS particles were aggregated after 7 days of storing the dispersion without agitation. Although this tendency was alleviated when the liquid volume of the sample was large, this finding suggested that some kind of agitation should be applied. Of the three agitation methods investigated, rotation mixing, shaking, and flowing, shaking was found to be the most effective for preventing aggregation of the particles. However, the shaking method also has limitations, and in the case of small sample volumes or high rotation speeds, aggregation was observed. In addition, an observation was made that the size of the PS particles decreased after agitation. Since PS [(C_3H_8)_n] has no OH group, it is not solvated by H_2O. Therefore, reductions in the PS particle size are not attributed to chemical decomposition but considered to be a result of physical damage to the particles during agitation. This physical damage effect was particularly observed in the case where the PS particles were circulated in an ETFE tube using a tubing pump. The effect can be likened to the observation in the natural world that many small, round stones are found downstream of the river and large, angular stones are often found upstream. This is because while the upstream stones are carried downstream by the river water flowing at a constant flow velocity, the stones collide with each other and with the riverbed, and the stones become separated or broken, their sizes are reduced, and the corners of the stones become rounded. Because of this, the small fragments that occur
are deposited as sand and mud at the bottom of the river.\textsuperscript{25} In the case of PS particles after flowing at relatively high flow rates (Figure S7c,d of the Supporting Information), it is possible to observe PS fragments that look like haze. Furthermore, in the case of using a tubing pump, which is often used as an artificial heart, there is also the possibility of causing particle breakage due to the so-called cavitation in which bubbles are generated and annihilated in a short time due to a pressure difference in the flow of liquid samples flowing in the tube as the roller rotates.\textsuperscript{26} Furthermore, when the liquid is fed by a linear driving pump with low pulsation, the cuboid structure observed by FE-SEM could be recrystallized PS. This means that PS particles were dissolved by ultrapure deionized water.

In the case of rotation mixing, when a microtube set on the disk-shaped holder approaches the top of the rotational movement, agitation accompanies a dynamic fall of the liquid sample in the microtube, which is orientated upside down, due to gravity. Such agitation, which is periodic but not constant in flow velocity, resembles a scenario in which stones are scraped as the waves are pushed and pulled on the shore. In fact, Figure S3 of the Supporting Information shows that once aggregated, particles are scraped off without separation, and as a result, they are observed as large, distorted shaped particles. Particle splitting due to external forces is most easily explained by shearing in the fluid. There are two cases in which this can occur. In the first case, splitting is due to shear stress in the fluid. The effect is small if the solvent has a low viscosity; however, if the flow rate is sufficiently high, the impact force per unit length can be increased. The second case involves solid–solid shearing between the particles and the walls of the container or tube. The contact surface areas of a 1.5 mL microtube and an ETFE tube filled with 1 mL of water are roughly 500 and 5000 mm\textsuperscript{2}, respectively. Therefore, the degradation of PS particles proceeds more efficiently in the continuous fluid in the ETFE tube than during agitation in the microtube. In this way, when the degradation process from microplastics to nanoplastics was investigated, the result depended greatly on the agitation method and conditions, and therefore, agitation conditions that can reproduce the exposure environment of particles must be selected as accurately as possible.

### Table 1. Experimental Conditions and Results

| Agitation Method                  | Temperature                  | Time   | Sample Volume (mL) | Rotation Speed/Flow Rate | Vailed Particle Tracks | Result                  |
|----------------------------------|------------------------------|--------|--------------------|--------------------------|------------------------|-------------------------|
| Standing                         | 23 °C (room temperature)     | 7 days | 0.5                | 6712                      | no aggregation         |                         |
|                                  |                              |        | 0.75               | 2519                      | aggregation            |                         |
|                                  |                              |        | 1                  | 5972                      | aggregation            |                         |
| Rotation Mixing                  | 23 °C (in constant-temperature oven) | 7 days | 0.5                | 4409                      | shape change           |                         |
|                                  |                              |        | 5 rpm              | 1127                      | shape change           |                         |
|                                  |                              |        | 10 rpm             | 2593                      | shape change           |                         |
|                                  |                              |        | 0.75               | 1464                      | shape change and aggregation |     |
|                                  |                              |        | 5 rpm              | 1474                      | shape change           |                         |
|                                  |                              |        | 15 rpm             | 1143                      | shape change and aggregation |     |
|                                  |                              |        | 1                  | 1448                      | shape change           |                         |
|                                  |                              |        | 5 rpm              | 1401                      | shape change           |                         |
|                                  |                              |        | 10 rpm             | 1698                      | shape change           |                         |
|                                  |                              |        | 15 rpm             | 1484                      | shape change           |                         |
|                                  |                              |        | 0.5                | 200 rpm                    | shape change           |                         |
|                                  |                              |        | 400 rpm             | 5622                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 8326                      | shape change and aggregation |     |
|                                  |                              |        | 0.75               | 200 rpm                    | shape change           |                         |
|                                  |                              |        | 400 rpm             | 4741                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 5815                      | no aggregation         |                         |
|                                  |                              |        | 1                  | 400 rpm                    | no aggregation         |                         |
|                                  |                              |        | 200 rpm             | 1248                      | shape change           |                         |
|                                  |                              |        | 400 rpm             | 4240                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 3857                      | aggregation            |                         |
| Shaking                          | 23 °C (under a temperature controller built in the block bath shaker) | 7 days | 0.5                | 7754                      | shape change           |                         |
|                                  |                              |        | 400 rpm             | 5622                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 8326                      | shape change and aggregation |     |
|                                  |                              |        | 0.75               | 200 rpm                    | shape change           |                         |
|                                  |                              |        | 400 rpm             | 4741                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 5815                      | no aggregation         |                         |
|                                  |                              |        | 1                  | 400 rpm                    | no aggregation         |                         |
|                                  |                              |        | 200 rpm             | 1248                      | shape change           |                         |
|                                  |                              |        | 400 rpm             | 4240                      | no aggregation         |                         |
|                                  |                              |        | 600 rpm             | 3857                      | aggregation            |                         |
| Flowing Using Tubing Pump        | 23 °C (room temperature)     | 7 days | 1                  | 1565                      | shape change           |                         |
|                                  |                              |        | 0.05 mL/min         | 1153                      | shape change and aggregation |     |
|                                  |                              |        | 0.1 mL/min          | 1717                      | shape change and aggregation |     |
|                                  |                              |        | 0.5 mL/min          | 1318                      | shape change and aggregation |     |
|                                  |                              |        | 1 mL/min            | 1266                      | shape change and recrystallization |     |
|                                  |                              |        | 0.05 mL/min         | 1421                      | shape change and recrystallization |     |
|                                  |                              |        | 0.1 mL/min          | 1396                      | shape change and recrystallization |     |
|                                  |                              |        | 0.5 mL/min          | 1451                      | shape change and recrystallization |     |
| Flowing Using Intelligent Pump   | 23 °C (room temperature)     | 7 days | 1                  | 1448                      | shape change           |                         |
|                                  |                              |        | 0.05 mL/min         | 1266                      | shape change and aggregation |     |
|                                  |                              |        | 0.1 mL/min          | 1421                      | shape change and recrystallization |     |
|                                  |                              |        | 0.5 mL/min          | 1396                      | shape change and recrystallization |     |
|                                  |                              |        | 1 mL/min            | 1451                      | shape change and recrystallization |     |
CONCLUSIONS

To understand the degradation process of PS microplastics to nanoplastics, the effects of four agitation methods including rotation mixing, shaking, flowing, and standing without agitation were compared by focusing on the decomposition and aggregation of PS particles dispersed in ultrapure deionized water. The degree of degradation and aggregation was evaluated by NTA measurements, and the degree of physical abrasion, leading to changes in particle shape, was assessed by FE-SEM observation. As a result, it was found that shaking prevented the aggregation and the physical damage of particles. However, the shaking method did have limitations depending on the agitation conditions. It was shown experimentally that aggregation suppression requires sufficient sample volume and that the lower the rotation speed, the more the aggregation of the particles can be suppressed. For rotation mixing, a relatively good aggregation reduction was only obtained for PS particles when the sample volume was 1 mL and the rotation speed was 5 rpm. When evaluating the biochemical degradability of particles in a solvent, shaking is the most gentle and suitable method for agitating particles; however, if physical degradability is of interest, flowing could be the most suitable method. In addition, it is possible that agitation by rotation mixing would be the most balanced method for evaluating the biochemical and physical degradabilities simultaneously. Therefore, it is necessary to select an appropriate agitation method and conditions depending on the natural environment degradation of PS microplastics to nanoplastics that is being simulated. The results of our experiments suggest that it is necessary to discuss plastic waste contamination in the marine environment not only from microto-nano-size degradation but also from recrystallization following degradation.

EXPERIMENTAL SECTION

NTA Measurements and FE-SEM Observation. The degree of aggregation of the particles before and after agitation was determined from the particle size distribution measured by NTA. A NanoSight LM10 (Malvern Panalytical) with a high-sensitivity complementary metal-oxide semiconductor (CMOS) camera was used for this measurement. The official detectable particle size by the manufacturer is 50–1000 nm for measurements of PS particles. The liquid sample containing the particles was drawn up in a 1 mL syringe and fed into the measuring cell via an ETFE tube with an internal diameter of 0.8 μm. When evaluating the biochemical degradability of particles in a solvent, shaking is the most gentle and suitable method for agitating particles; however, if physical degradability is of interest, flowing could be the most suitable method. In addition, it is possible that agitation by rotation mixing would be the most balanced method for evaluating the biochemical and physical degradabilities simultaneously. Therefore, it is necessary to select an appropriate agitation method and conditions depending on the natural environment degradation of PS microplastics to nanoplastics that is being simulated. The results of our experiments suggest that it is necessary to discuss plastic waste contamination in the marine environment not only from microto-nano-size degradation but also from recrystallization following degradation.

Preparation of Particle Suspension. The samples for FE-SEM observation were acquired by dropping each particle dispersion onto a 200-nm-thick Au/15-nm-thick Cr/525-μm-thick Si substrate. Since NanoSight LM10 recommends 10^7–10^9 particles/mL as the appropriate concentration for NTA measurements, the purchased PS particle suspension was diluted with ultrapure deionized water (resistivity = 18.2 MΩ·cm) 8 × 10^6 times and then dispersed by pipetting 50 times. It should be noted that some physically damaged PS particles were observed by FE-SEM when vortex mixing was used for 1 min or more in this dispersion step. Therefore, only pipetting was used to homogenize the dispersion.

Particle Incubation with Agitation. The incubation temperature and time were chosen as 23 °C and 7 days, respectively. In the aggregation experiment, we compared three methods: rotation mixing, shaking, and flowing. In addition, a sample was also prepared that was left to stand in a microtube holder without agitation (Figure 8a). The conditions for each agitation method are summarized in Table 1. Samples other than those agitated by flow were injected into a 1.5 mL microtube using a micropipette. Three different liquid volumes were evaluated, 0.5, 0.75, and 1 mL. For rotation mixing, the dedicated holder RVM-MT20 was fixed to a small rotation mixer RVM-101 (Sanyo) so that the rotation surface faced vertically, and the rotation speed was set to 5, 10, or 15 rpm. The rotation mixer was placed in a mini incubator MI-100G (Yonezawa) to maintain a constant temperature of 23 °C (Figure 8b). For shaking, a dedicated block C-100 was used with a block bath shaker MyBL-100CS (Matsusama SeisakuSu), the temperature was set to 23 °C, and samples in microtubes were shaken at rotation speeds of 200 (minimum settable value), 400, and 600 rpm. When evaluating the biochemical degradability of particles in a solvent, shaking is the most gentle and suitable method for agitating particles; however, if physical degradability is of interest, flowing could be the most suitable method. In addition, it is possible that agitation by rotation mixing would be the most balanced method for evaluating the biochemical and physical degradabilities simultaneously. Therefore, it is necessary to select an appropriate agitation method and conditions depending on the natural environment degradation of PS microplastics to nanoplastics that is being simulated. The results of our experiments suggest that it is necessary to discuss plastic waste contamination in the marine environment not only from microto-nano-size degradation but also from recrystallization following degradation.

Figure 8. Agitation methods for particles dispersed in water: (a) standing without agitation, (b) rotation mixing, (c) shaking, and (d) flowing.
rpm (Figure 8c). For agitation by flow, a tubing pump with relatively large pulsation and an intelligent pump for high-performance liquid chromatography (HPLC), which suppressed pulsation by linear driving, were compared. A digital metering tubing pump DSP-100SA (Asone) was used with an ETFE tube with an inner diameter of 0.8 mm and an outer diameter of 1.58 mm and a tube joint JHE312 (Eyela) with an inner diameter of 1.2 mm and a silicon tube with an outer diameter of 4.2 mm and an inner diameter of 2.15 mm. The silicon tube was connected to both ends of the custom tube cartridge L stored in the tubing pump. In the intelligent pump case, an ETFE tube with an inner diameter of 0.8 mm and an outer diameter of 1.58 mm was connected to a polyether ether ketone (PEEK) head of an intelligent pump UI-22-110 (Flom) via a tube joint JHE312 (Eyela) with an inner diameter of 1.2 mm and a ferrule integrated set screw TC-15 (Eyela) with an inner diameter of 1.58 mm. In both cases, after the ETFE tube and the silicon tube were filled with 1.5 mL of ultrapure deionized water, the ETFE tube was cut to length to give the same volume as the 1.5 mL microtube. The flow rate of the liquid sample from both pumps was set to either 0.05 (minimum settable value), 0.1, 0.5, or 1 mL/min. The volume of the sample inserted into the tube was 0.5 mL. The injection and removal of the sample into and out of the tube was performed using a 1 mL syringe via a manual three-way valve VF-5310 (Eyela) provided in the middle of the ETFE tube. The temperature during agitation for the standing and flowing samples was maintained at 23 °C using the laboratory air conditioner running for 24 h (Figure 8d).

Sample Preparation for DSC Measurement. The PS latex particles used were subjected to DSC measurement over the temperature range 50−300 °C at a ramp rate of 5 °C/min using a DSC-60 Plus instrument (Shimadzu). PS latex particles that had been naturally dried by evaporating the solvent were placed in a dedicated Al crimp pan, covered with an Al lid, and then sealed by pressing. α-Alumina was used as a reference sample for the DSC measurement.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03278.

Particle size distributions and integrated particle number and FE-SEM images of PS particles in ultrapure deionized water after incubation without agitation; particle size distributions and integrated particle number of PS particles in ultrapure deionized water after incubation with rotation mixing; FE-SEM images of PS particles after incubation with rotation mixing; particle size distributions and integrated particle number of PS particles in ultrapure deionized water after incubation with shaking; FE-SEM images of PS particles after incubation with shaking; particle size distributions and integrated particle number of PS particles in ultrapure deionized water after incubation under flow by tubing; FE-SEM images of PS particles after incubation under flow by tubing; particle size distributions and integrated particle number of PS particles in ultrapure deionized water after incubation under flow using an intelligent pump; FE-SEM images of PS particles after incubation under flow using an intelligent pump (Figures S1−S9) (PDF)

AUTHOR INFORMATION

Corresponding Author

Harutaka Mekaru — Sensing System Research Center (SSRC), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8564, Japan; orcid.org/0000-0002-1120-6018; Phone: +81-29-861-2431; Email: h-mekaru@aist.go.jp; Fax: +81-29-861-7225

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03278

Author Contributions

The manuscript was written with the contribution of only one author.

Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

We thank Ayako Irie of Quantum Design Japan, Inc. for giving us technical comments on the NTA measurements. The author received technical support for sample preparation for the DSC measurements from Kaori Kamata and Dr. Kazuma Kurihara of the National Institute of Advanced Industrial Science and Technology (AIST).

ABBREVIATIONS

PS, polystyrene; NTA, nanoparticle tracking analysis; FE-SEM, field emission scanning electron microscope; NPs, nanoparticles; PCB, polychlorinated biphenyl; PP, polypropylene; PE, polyethylene; PPAH, polycyclic aromatic hydrocarbons; DLS, dynamic light scattering; DL, laser diffraction/scattering particle size analysis; RMM, resonance mass measurement; CV, coefficient of variation; ES-DMA-spICPMS, electrospray-differential mobility analysis with single-particle inductively coupled plasma mass spectrometry; FFF, field-flow fractionation; ETFE, ethylene tetrafluoroethylene; CMOS, complementary metal-oxide semiconductor; HPLC, high-performance liquid chromatography; PEEK, polyether ether ketone

REFERENCES

(1) Loos, C.; Syrovets, T.; Musyanovych, A.; Maiänder, V.; Landfester, K.; Nienhaus, G. U.; Simmet, T. Functionalized polystyrene nanoparticles as a platform for studying bio-nano interactions. Beilstein J. Nanotechnol. 2014, 5, 2403−2412.
(2) Kluenker, M.; Kurch, S.; Tahir, M. N.; Tremel, W. Bio-nano: Theranostic at Cellular Level. In AAPS Advances in the Pharmaceutical Sciences Series 29, Particles and Nanoparticles in Pharmaceutical Products: Design, Manufacturing, Behavior and Performance; Merkus, H. G.; Meesters, G. M. H.; Oostra, W., Eds.; Springer International Publishing AG: Cham, 2018; pp 85−170.
(3) Murali, K.; Kenesei, K.; Li, Y.; Demeter, K.; Környei, Z.; Madarász, E. Uptake and bio-reactivity of polystyrene nanoparticles is affected by surface modifications, ageing and LPS adsorption: in vitro studies on neural tissue cells. Nanoscale 2015, 7, 4199−4210.
(4) Lunov, O.; Syrovets, T.; Loos, C.; Bell, J.; Dalacher, M.; Tron, K.; Nienhaus, G. U.; Musyanovych, A.; Maiänder, V.; Landfester, K.; Simmet, T. Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. ACS Nano 2011, 5, 1657−1669.
(5) Sakama, S.; Hayashi, M.; Akashi, M. Design of nanoparticles composed of graft copolymers for oral peptide delivery. Adv. Drug Delivery Rev. 2001, 47, 21−37.
(6) Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E. Lost at Sea: Where Is All the Plastic? Science 2004, 304, 838.
(7) Lebreton, L.; Slat, B.; Ferrari, F.; Sainte-Rose, B.; Atken, J.; Marthouse, R.; Hajbane, S.; Cunsoleo, S.; Schwarz, A.; Levivier, A.; Noble, K.; Debeljak, P.; Haral, H.; Schoeneich-Argent, R.; Brambini, R.; Reisser, J. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Sci. Rep.* 2018, 8, No. 4666.

(8) Cole, M.; Lindeque, P.; Halsdand, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* 2011, 62, 2588–2597.

(9) Ribeiro, F.; Garcia, A. R.; Pereira, B. P.; Fonseca, M.; Mestre, N. C.; Fonseca, T. G.; Ibarco, L. M.; Belbianno, M. J. Microplastics effects in *Scrobicularia plana*. *Mar. Pollut. Bull.* 2017, 122, 379–391.

(10) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* 2011, 62, 1596–1605.

(11) Rochman, C. M.; Manzano, C.; Hentschel, B. T.; Simonich, S. L. M.; Hoh, E. Polystyrene Plastic: A Source and Sink for Polycyclic Aromatic Hydrocarbons in the Marine Environment. *Environ. Sci. Technol.* 2013, 47, 13976–13984.

(12) Mahler, G. J.; Esch, M. B.; Tako, E.; Southard, T. L.; Archer, S. D.; Glahn, R. P.; Shuler, M. L. Oral exposure to polystyrene nanoparticles affects iron absorption. *Nat. Nanotechnol.* 2012, 7, 264–271.

(13) Jani, P.; Halbert, G. W.; Langridge, J.; Florence, A. T. Nanoparticle Uptake by the Rat Gastrointestinal Mucosa: Quantitation and Particle Size Dependency. *J. Pharm. Pharmacol.* 1990, 42, 821–826.

(14) Lambert, S.; Wagner, M. Characterisation of nanoplastics during the degradation of polystyrene. *Chemosphere* 2016, 145, 265–268.

(15) Elkvall, M. T.; Lundqvist, M.; kelpsiene, E.; Šileikis, E.; Gunnarsson, S. B.; Cedervall, T. Nanoplastics formed during the mechanical breakdown of daily-use polystyrene products. *Nanoscale Adv.* 2019, 1, 1055–1061.

(16) Weinstein, J. E.; Crocker, B. K.; Gray, A. D. From macroplastic to microplastic: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat. *Environ. Toxicol. Chem.* 2016, 35, 1632–1640.

(17) Filipe, V.; Hawe, A.; Jiskoot, W. Critical Evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the Measurement of Nanoparticles and Protein Aggregates. *Pharm. Res.* 2010, 27, 796–810.

(18) Cavicchi, R. E.; Meier, D. C.; Presser, C.; Prabhu, V. M.; Guha, S. Single Laser Pulse Effects on Suspended-Au-Nanoparticle Size Distributions and Morphology. *J. Phys. Chem. C* 2013, 117, 10866–10875.

(19) Murphy, B. J.; Pritinski, D.; Migler, K.; Douglas, J. F.; Prabhu, V. M. Dynamic light scattering investigations of nanoparticle aggregation following a light induced pH jump. *J. Chem. Phys.* 2010, 132, No. 194903.

(20) Matsuura, Y.; Ouchi, N.; Nakamura, A.; Kato, H. Determination of an accurate size distribution of nanoparticles using particle tracking analysis corrected for the adverse effect of random Brownian motion. *Phys. Chem. Chem. Phys.* 2018, 20, 17839–17846.

(21) Tan, J.; Liu, J.; Li, M.; Hadri, H. E.; Hackley, V. A.; Zachariah, M. R. ElectroSpray-Differential Mobility Hyphenated with Single Particle Inductively Coupled Plasma Mass Spectrometry for Characterization of Nanoparticles and Their Aggregates. *Anal. Chem.* 2016, 88, 8548–8555.

(22) Mintenig, S. M.; Bäuerlein, P. S.; Koelmans, A. A.; Dekker, S. C.; van Wezel, A. P. Closing the gap between small and smaller: towards a framework to analyze nano- and microplastics in aqueous environmental samples. *Environ. Sci. Nano* 2018, 5, 1640–1649.

(23) DeCarlo, P. F.; Slowik, J. G.; Worsnop, D. R.; Davidovits, P.; Jimenez, J. L. Particle Morphology and Density Characterization by Combined Mobility and Aerodynamic Diameter Measurements. Part 1: Theory. *Aerosol Sci. Technol.* 2004, 38, 1185–1205.

(24) Chiu, F.-C.; Peng, C.-G. The atactic polystyrene molecular weight effect on the thermal properties and crystal structure of syndiotactic polystyrene/atactic polystyrene blends. *Polymer* 2002, 43, 4879–4886.