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Evaluating the Effect of Chemical Digestion Treatments on Polystyrene Microplastics: Recommended Updates to Chemical Digestion Protocols

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Establishing the toxicity and exposure consequences of microplastics (MPs) on marine organisms relies on the nondestructive isolation of plastics from biological matrices. MPs are commonly extracted from these matrices by chemical digestion using alkali (e.g., potassium hydroxide (KOH) and sodium hydroxide (NaOH)), oxidative (e.g., hydrogen peroxide (H_2O_2)) and/or acidic (e.g., nitric acid (HNO_3)) reagents. Although these digestion conditions can be highly effective for MP extraction, they can also react with the plastics. This can attribute an inaccurate representation of plastic contamination by altering MP visual characteristics (size, shape, color), thereby impeding identification and potentially returning erroneous numbers of ingested particles. In this study, the degradative impacts are assessed of the routinely applied digestion reagents (i) KOH, (ii) NaOH, (iii) H_2O_2, and (iv) HNO_3 on polystyrene (PS) based MPs sized between 200 µm and 5 mm. Degradation of the PS MPs is evaluated using FT-IR, gel permeation chromatography, NMR, photoluminescence spectroscopy, and microscopy. These studies reveal HNO_3 to be the most destructive for PS MPs, while the alkali and oxidative reagents result in negligible changes in plastic properties. These results are recommended to be used as a guideline to update current protocols to ensure the nondestructive treatment of MPs.

1. Introduction

The cost effectiveness and easy manufacturing of synthetic polymers has seen disposable plastic products permeate every aspect of our daily lives. Because of this, plastic pollution is fast becoming one of the most degradative anthropogenic processes of all time, and is continuing at an alarming rate due to the catastrophic, global mismanagement of plastic materials.[1–4] Polypropylene (PP), polyethylene (PE), and polystyrene (PS) account for an estimated 42% of all plastic products ever made, and unsurprisingly, these materials are the most prevalent plastic pollution worldwide.[5,6] The impacts of plastic pollution in the marine environment has been apparent for decades (e.g., entanglement and ingestion),[7] however, research is yet to reveal the full scope of environmental impacts caused by smaller plastic fragments less than 5 mm in diameter, referred to as microplastics (MPs). MPs can now be found in almost every environmental matrix worldwide, sparking a plethora of studies investigating the impact of their long-term exposure and toxicity towards marine life.[1–5,8,9] A critical aspect of these studies relates to the separation and isolation protocols used to retrieve the MPs from biological matrices.[10,11] Protocol performance, including the accurate quantification of MP loadings in marine organisms, hinges on the nondestructive retrieval of MPs from biological matrices.

Existing methods for isolating MPs from biological materials include manual visual sorting, density floatation in supersaturated saline solutions, elutriation, and chemical digestion treatments.[12,13] Although manual visual sorting and density floatation techniques are widely applicable for most polymer and environmental sample types, they do not offer the same recovery efficiency as chemical digestion treatments, particularly for complex tissue matrices (e.g., fatty fish tissue).[11] For this reason, chemical digestions in combination with high temperatures and prolonged treatment times are commonly utilized to achieve more robust and efficient MP recovery.[11,14–22] However, these digestion treatments often use alkali (e.g., alkali hydroxides including potassium hydroxide (KOH) and sodium hydroxide (NaOH)),

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oxidative (e.g., hydrogen peroxide (H_2O_2)) and/or acidic (e.g., nitric acid (HNO_3)) reagents which can react with, deform and/or completely deteriorate the MP.[15,18–20,23] The use of particularly harsh conditions, e.g., full immersion in 69% HNO_3 at high temperatures (>80 °C) and/or for prolonged exposure times (>1 month), to digest tissues such as mussels, whole juvenile fish as well as tissue and stomach samples from invertebrates has been reported.[12,21,23,24] Furthermore, the choice of digestion methodology (e.g., reagent type and concentration, digestion temperature, and time) is often at the discretion of the researcher, and thus can vary significantly between studies.[11] Allowing for such variability in treatment methodology can lead to inconsistent comparisons between studies and an inaccurate determination of the level of contamination within a sample (e.g., number of ingested MPs).[11] Despite the importance of these MP studies, a standardized digestion method that prescribes a suitable and nondestructive, chemical treatment remains elusive and is an area that needs critical evaluation to prevent further inconsistent reporting.

To establish the extent of degradation of MPs recovered after chemical digestion, visual assessment of the item's morphology (e.g., size, shape, and color) is commonly done by microscopy, however, this analysis is qualitative, and often inconclusive or even misleading.[11] Quantitative spectroscopic methods offer a more accurate means of measuring MP reactivity (e.g., degradation), the most common being Fourier transform infrared spectroscopy (FT-IR) and photoluminescence spectroscopy (PL).[25–28] FT-IR captures the spectral profile of the isolated plastic, which can then be compared against a database of reference spectra to identify changes in the functional groups on the polymer chain, i.e., oxidation and/or polymeric chain scission.[28] Similarly, PL has been used to infer polymer degradation by measuring fluorescence quenching of the incorporated dye.[17] Another lesser used quantitative method, often employed by polymer scientists and engineers, is gel permeation chromatography (GPC), which analyses the average molecular weight distribution. This technique provides direct evidence of polymer chain degradation. The application of such methods in combination should be considered when developing a nondestructive chemical digestion method to recover MPs from biological materials.

This study will evaluate the impacts of routinely applied chemical digestion reagents (i) KOH, (ii) NaOH, (iii) H_2O_2, and (iv) HNO_3 as a function of digestion temperature and time,[11] on Nile red (NR)-stained PS MPs. PS products contribute significantly to aquatic plastic pollution and are one of the three most prevalent marine plastic contaminants.[29–31] PS frequently interacts with wildlife and ecotoxicity studies have revealed they adversely impact many marine species.[32–35] Additionally, PS is highly susceptible to chemical reaction. Treated PS MPs will be analyzed using both qualitative (microscopy) and quantitative (FT-IR, PL, NMR, and GPC) methods to accurately evaluate the effect these digestion methods have on the physical and chemical properties of the MP, which may impact accurate characterization and recovery. The results from this study will guide recommendations for the chemical digestion of biological materials to minimize MP degradation during processing.

2. Experimental Section

2.1. Materials

PS (Sigma Aldrich, average molecular weight = 192K g mol^{-1}, batch number MKCL2807), NR (Sigma Aldrich), tetrahydrofuran (THF) (Unichrom, HPLC grade), deuterated chloroform ($CDCl_3$) (Sigma Aldrich, 99.99 atom%), KOH (AnalaR; 85% grade), NaOH (Univar; 97% grade), HNO_3 (RCI Labscan Limited; 32% grade), and H_2O_2 (Univar; 32% grade) were used as received. Dichloromethane (DCM) (Univar, ACS grade) was distilled prior to use.

2.2. Microplastic Preparation

Additive free and virgin PS was chosen since it is commercially available, allowing for easy reproduction and chemical manipulation. Virgin PS is representative of the variety of environmental PS contamination and is a well-defined control standard, thus allowing for a comprehensive assessment of its reactive pathways and impacts of chemical digestion, decoupled from additive presence and/or weathering effects. Additionally, previous reports have shown that the degree of polymer degradation was not molecular weight dependent,[12,20,21] therefore PS with an average molecular weight was chosen to represent both high and low molecular weight PS.

PS was dissolved in DCM at ambient conditions with constant stirring until homogenous. Once dissolved, a 4:1 solution of THF and NR (0.5 μg mL^{-1}) was added and allowed to stir until homogenous in color.[26] Staining of the PS MPs with NR was done to monitor for polymer degradation using PL.[26,27] This solution was casted on a watch glass, where the solvents were evaporated to form a white plastic membrane. The dried membrane was then processed into MPs using a Magic Bullet—Nutribullet 900 Series blender and sieved over a stainless-steel screen sieve (Glenammer Sieves) to afford MPs within the size range 300 μm to 1 mm.

For the size-dependent study, pure PS was processed using a Magic Bullet—Nutribullet 900 Series blender and sieved over stainless-steel screen sieves (Glenammer Sieves) to afford 200 to 300 μm (small), 400 μm to 1 mm (medium), and 5 mm (large) MPs. Particles were irregularly shaped with a smooth surface (Figure S1, Supporting Information).

2.3. Digestion Conditions

MPs (0.5 g, 5–200 fragments depending on size) were exposed to nine different digestions conditions using KOH (10% w/w), NaOH (40% w/w), and H_2O_2 (30% w/v), and six different digestion conditions using HNO_3 (69% w/v) (**Table 1**). Due to HNO_3 being a potent oxidizer and unstable at high temperatures (boiling point of 83 °C), HNO_3 digestions at 90 °C were not conducted. Samples were maintained at constant temperature using a controlled water bath (Grant JB Instruments). Following chemical digestion, MPs were washed with deionized water to remove excess reagent and air dried overnight under ambient conditions.

Table 1. Digestion conditions used in this study (reagent concentration, digestion time and temperature).

Reagent	Digestion Time [h]	Digestion Temperature [°C]
KOH (1.8 M)	12, 24, 48	30, 60, 90
NaOH (10 M)	12, 24, 48	30, 60, 90
H_2O_2 (9.8 M)	12, 24, 48	30, 60, 90
HNO_3 (15.8 M)	1, 2, 12	30, 60

2.4. Sample Analyses

2.4.1. Spectral Analyses

Infrared spectra of the neat samples were collected using a Thermo Scientific Nicolet iS5 FT-IR spectrometer equipped with an attenuated total reflectance crystal head attachment. Spectra were analyzed using PerkinElmer Spectrum Software (V11.0). Spectra were baseline corrected using default PerkinElmer parameters. The PerkinElmer COMPARE algorithm was run to establish the similarity between spectral profiles of fragments after digestion treatments. The following filters were applied to the COMPARE mathematical correlation: resolution, intensity and noise weighting; CO_2 blanking and H_2O weighting. Infrared profiles were also systematically searched (SEARCH PerkinElmer) using Euclidian distance against a commercially available NICODOCOM IR spectral library (Polymers and Additives; NICODOM Ltd., Czech Republic) to evaluate whether fragments could be confidently identified as PS postdigestion. The search was conducted in the 3900–650 cm^{-1} region, however, the region between 2500 and 1900 cm^{-1}, with a −0.01 to 0.1 noise in baseline was excluded since it may still be contaminated by small features from atmospheric absorptions. A percent match ≥70% is considered acceptable for accurate polymer identification.[36] ^{1}H-NMR of the samples were recorded as solutes in deuterated chloroform ($CDCl_3$) at 298 K on a Bruker 400 MHz NMR spectrometer using standard Bruker pulse sequences and were referenced to the residual solvent peak ($CDCl_3$, δ_H 7.26). Only peaks diagnostic for PS bonds susceptible and indicative of polymer reactivity (i.e., aromatic and aliphatic bonds) were monitored.[1,17,26,28] PL was used to analyze the fluorescence intensity of MPs pre- and postdigestion. MPs ($n = 3$) were dissolved in THF in a ratio such that the optical density (OD) of the solution was 1 ± 0.01 at an absorbance wavelength 257 nm. The OD was measured on a Shimadzu UV-2600 spectrophotometer using Shimadzu UV probe 2.61 software. The PL intensity of NR in solution was measured using a Shimadzu RF-6000 spectrofluorophotometer with an excitation wavelength of 530 nm, emission ranges between 545 and 800 nm and emission slit bandwidths of 10 nm. The emission peak intensity at 677 nm was compared in all samples.

2.4.2. Molecular Weight Analysis

Weight-average molecular weight (M_w) (i.e., the average molecular weight in g mol^{-1}), and polydispersity index (PDI) (i.e., polymer length heterogeneity) gives information on the M_w distribution of a sample, and was used to estimate the extent of polymer degradation. Changes to the M_w and PDI of PS before and after treatment was evaluated using GPC. MPs (2 mg) were dissolved in THF (1.5 mL) and mixed until no solid remained. The solution (50 µL) was injected onto a 1260 Infinity II Multi-Detector GPC (Agilent Technologies) equipped with an ultraviolet (UV) absorbance and refractive index detector. The two PLgel 5 µL MIXED-C columns (300×7.5 mm) (Agilent Technologies) were calibrated using PS narrow standards in THF at 35 °C.

2.5. Microscopy

A Leica MZ26A microscope fitted with a Leica DFC 600 camera was used to assess any discoloration and/or changes to MP fragment morphology. Images were analyzed using the Leica Application Suite LAS 4.4.0 software. To further observe changes to the surface morphology (i.e., cracking and porosity) MP fragments were examined using a Jeol Superprobe JXA-8200 scanning electron microscope (SEM).

2.6. Statistical Analysis

The chemical and physical properties of treated MPs were compared to unprocessed, control MPs to reveal impacts. Standard deviations for OD were calculated in Microsoft Excel. Standard error margin for GPC was calculated in Microsoft Excel, as a sum of the instrument and experimental error.

3. Results

Microscopy of the untreated MPs revealed the particles to be of irregular shape within the size range of 300 µm to 1 mm, with pitting of the surface (**Figure 1**). The FT-IR and ^{1}H-NMR spectra of the untreated, control fragments were consistent with those of pure PS,[26,28] (Figures S2 and S3, Supporting Information, respectively). The M_w of the MPs particles was the same as the virgin PS (M_w = 174K g mol^{-1}, PDI = 2.5).

3.1. Alkaline Digestion

Chemical analyses and microscopy of MPs exposed to KOH (1.8 M) and NaOH (10 M) at 30, 60 and 90 °C for 12, 24, and 48 h, revealed these digestion protocols did not alter any of the physical or chemical properties of the MPs compared to control. FT-IR revealed spectral similarities of 95% (KOH, 48 h and 90 °C) and 97% (NaOH, 48 h and 90 °C) to PS in the infrared spectral library (Polymers and Additives; NICODOM Ltd., Czech Republic) (Table S1, Supporting Information). Similarly, ^{1}H-NMR and PL spectral profiles were consistent with control MPs (**Figure 2**; Figure S3, Supporting Information). Lastly, no differences were observed in the appearance of MPs treated with KOH and NaOH reagents at 30 and 60 °C even under extended exposure times. At 90 °C, the particles expanded in size after treatment for 48 h (Figure 1c,d).

3.2. Oxidative Digestion

No significant differences in the ^{1}H-NMR spectra and PL intensities were observed for MPs treated with H_2O_2 (9.8 M) at 30, 60,

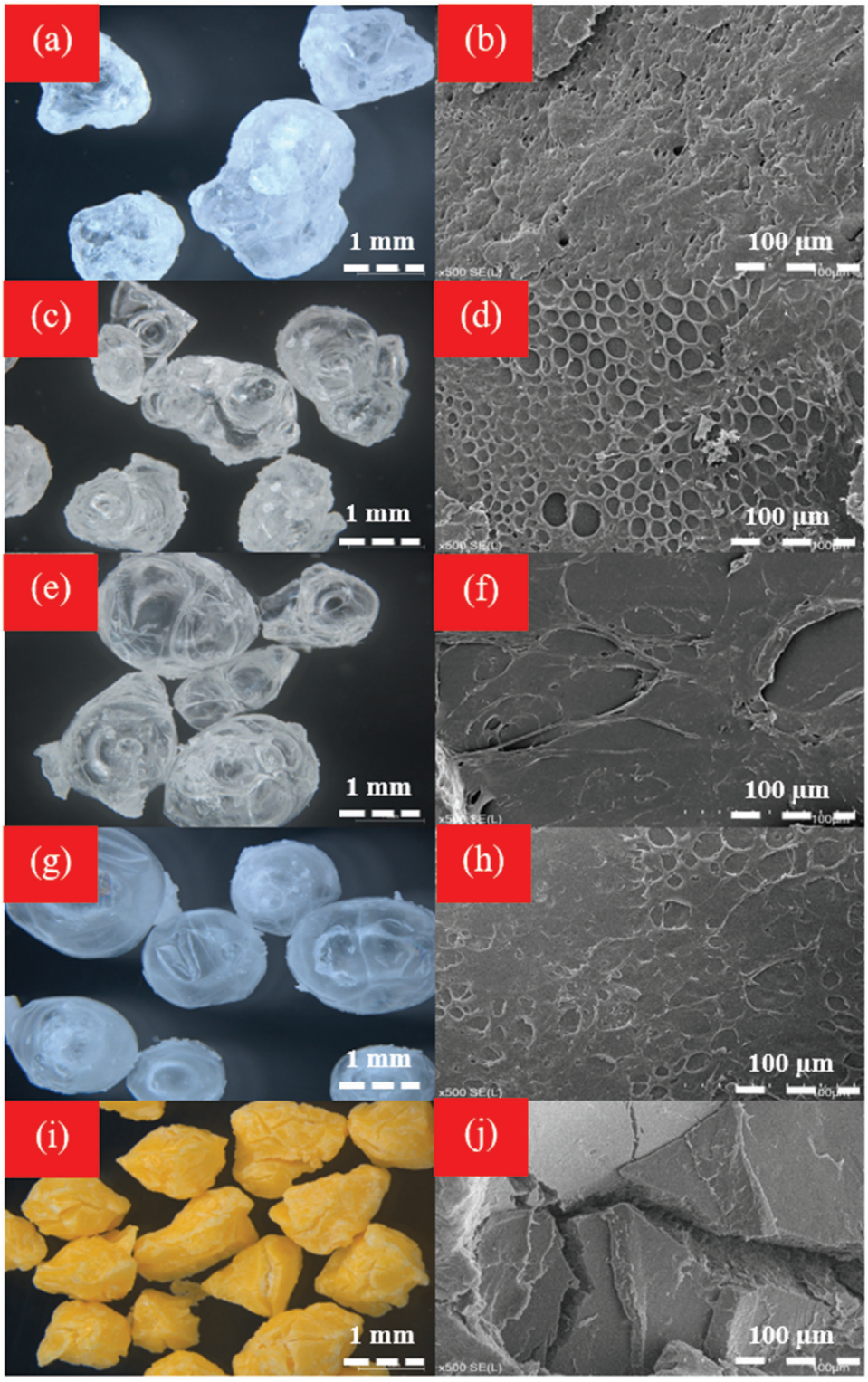

Figure 1. Microscope images of the untreated polystyrene (PS) microplastics (MPs) a) 2.5× and b) 500×; PS treated at 90 °C for 48 h with 1.8 M potassium hydroxide at c) 2.5× and d) 500×; PS treated at 90 °C for 48 h with 10 M sodium hydroxide at e) 2.5× and f) 500×; PS treated at 90 °C for 48 h with 9.8 M hydrogen peroxide at g) 2.5× and h) 500×; and PS treated with 15.8 M nitric acid for 60 °C for 12 h at i) 2.5× and j) 500×.

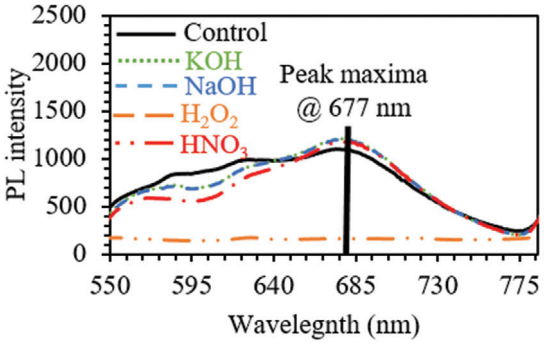

Figure 2. Relative photoluminescence spectra for untreated polystyrene (PS) (control), and PS treated under the following digestion conditions: 90 °C for 48 h with 1.8 M potassium hydroxide (KOH) solution, 10 M sodium hydroxide (NaOH) solution, 9.8 M hydrogen peroxide (H_2O_2), and 60 °C for 12 h with 15.8 M nitric acid (HNO_3).

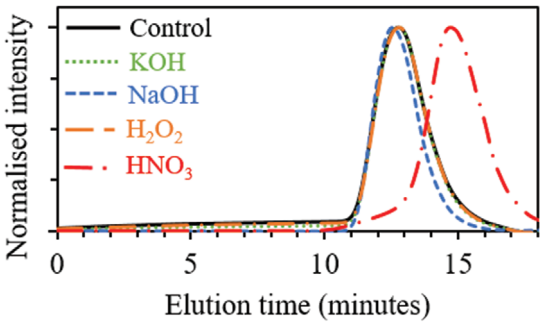

Figure 3. Gel permeation chromatography traces for untreated polystyrene (PS) (control), and PS treated under the following digestion conditions: 90 °C for 48 h with 1.8 M potassium hydroxide (KOH) solution, 10 M sodium hydroxide (NaOH) solution 9.8 M hydrogen peroxide (H_2O_2), and 60 °C for 12 h with 15.8 M nitric acid (HNO_3).

and 90 °C for 12, 24, and 48 h, as compared to controls (Figure 2; Figure S3, Supporting Information). However, GPC showed a small decrease in the M_w from 174K to 171K g mol^{-1} after exposure to the most extreme digestion condition (48 h and 90 °C) ($p > 0.05$) (**Figure 3**). Likewise, FT-IR revealed structural changes under this extreme condition, with the formation of broad hydroxyl (–OH) bands at 3364 cm^{-1} (**Figure 4**; Figures S4 and S5, Supporting Information), although, there was a spectral similarity of >86% to control PS (Table S1, Supporting Information). Finally, for this extreme condition, optical microscopy revealed a significant expansion in particle size (up to 5 mm) (Figure 1g).

3.3. Acidic Digestion

HNO_3, a strong oxidizing acid that facilitates efficient digestion of biological materials,[11,14] significantly altered the chemical and physical properties of the PS MPs. Processing the samples at 30 °C for 12 h, or 60 °C for more than 2 h, resulted in the formation of new peaks in the FT-IR spectra at 1680, 1650, and 1330 cm^{-1} (Figure 4; Figures S4 and S5, Supporting Information), indicating the formation of aromatic-N=O and C–N bonds. These peaks became more prominent when MPs were treated at higher temperatures, exposed for longer treatment

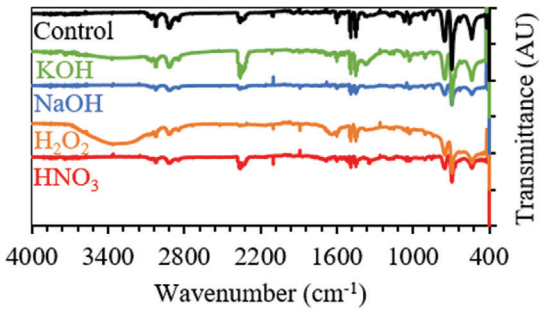

Figure 4. Infrared profiles of untreated polystyrene (PS) (control), and PS under the following digestion treatments: 90 °C for 48 h with 1.8 M potassium hydroxide (KOH) solution, 10 M sodium hydroxide (NaOH) solution 9.8 M hydrogen peroxide (H_2O_2), and 60 °C for 12 h with 15.8 M nitric acid (HNO_3).

Table 2. The degree of aromatic nitration (Ar-NO_2) of polystyrene (PS) treated under 15.8 M nitric acid digestion conditions. Ar-NO_2 (%) was calculated by comparing the integrated resonances from 7.30 to 6.25 ppm and 1.95 to 1.15 ppm.[28]

HNO_3 treatment	Ar-NO_2 inclusion [%]
30 °C for 1 h	No nitration
30 °C for 2 h	No nitration
30 °C for 12 h	2.5
60 °C for 1 h	No nitration
60 °C for 2 h	1.5
60 °C for 12 h	6

times and/or as particle size decreased. These changes were supported by a decrease in spectral similarity to control PS (75% at 12 h and 60 °C) (Table S1, Supporting Information). The ^{1}H-NMR spectra also revealed a downfield shift of characteristic aromatic peaks at ≈7.70 ppm (Figures S6 and S7, Supporting Information), with the shift increasing with temperature and time, as evidenced by the integration of the relevant regions; 7.30–6.25 and 1.95–1.15 ppm (**Table 2**; Table S2, Supporting Information). GPC traces showed a significant decrease in the M_w from 174K to 48K g mol^{-1} and an increase in PDI from 2.5 to 5.7 (12 h and 60 °C) (Figure 3; Figures S8 and S9, Supporting Information). The PL intensity did not change significantly at 30 °C; however, at 60 °C it decreased with extended exposure time (**Figure 5**). Moreover, significant physical deteriorations were evident by microscopy and is consistent with the aforementioned chemical properties of the MPs: deep surface cracking, embrittlement, and an intense yellowing discoloration was visible after 12 h at 30 °C and increased in severity with temperature (**Figure 6**). The deterioration of the particles was also more pronounced as the size of the particles decreased (Figure S10, Supporting Information).

4. Discussion

This study tested the suitability of four routinely used digestion reagents, KOH, NaOH, H_2O_2, and HNO_3 for the nondestructive isolation of PS MPs. **Table 3** summarizes our findings. HNO_3 was found to be destructive to PS, causing swelling, degradation, and

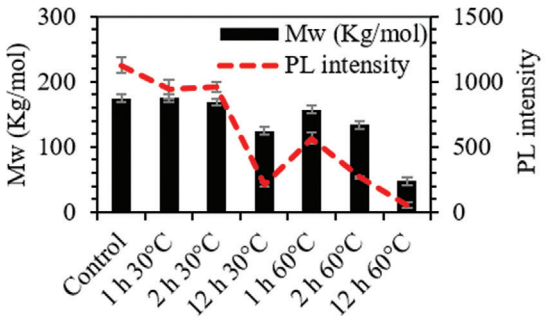

Figure 5. The weight-average molecular weight (M_w) of polystyrene (PS) samples after treatment under 15.8 M nitric acid digestion conditions (bar graph). The average photoluminescence (PL) intensity (dashed red line) is graphed for comparison.

nitration of the polymer. H_2O_2 was found to cause mild effects, specifically swelling and minor changes in the infrared spectral profile. Exposure of PS to KOH and NaOH under the most extreme conditions tested (90 °C and 48 h) proved nondestructive. Caustic reagents should therefore be considered in preference to oxidative and acidic reagents to digest biological materials for the recovery of MPs.

4.1. Caustic Digestion using NaOH and KOH

Of the four digestion reagents investigated, KOH and NaOH exacted the least effect on the chemical and physical properties of PS. Based on our detailed studies, these caustic digestion reagents are ideal as there was no polymer degradation regardless of treatment time or temperature. This lack of degradation is evidenced through the minimal change in the molecular weight distribution (GPC), minimal change in the spectral profiles (FT-IR, PL, ^{1}H-NMR) and no change in the morphology up to 60 °C. While the spectral properties of the PS were not altered after heating at 90 °C, particles were noticeably swollen (Figure 1), which could be attributed to the polymer's thermal properties. For PS, the glass transition temperature is ≈90 °C,[37] and coupled with the porous nature of the particles, has been shown to result in thermal expansion (blowing).[38] To support this theory, non-porous (smooth) PS MPs were analyzed under the same temperature regimes with no thermal expansion observed, confirming the blowing agent (NaOH or KOH solution) could not enter the MP (Figure S1, Supporting Information). When plastics are recovered from the environment, they often contain pits and aberrations due to weathering,[1] therefore we recommend high temperatures be avoided when recovering soft plastics using chemical digestion.

4.2. Oxidative Digestion using H_2O_2

Oxidative reagents are often used for the digestion of bivalves and fish.[11] The spectroscopic signatures of the polymer structure was found to vary when exposed to heated oxidative digestion (H_2O_2, 9.8 M). No change in the polymer structure was observed at 30 °C for 12, 24, and 48 h, however, upon increasing the temperature to 60 °C, the appearance of a –OH band in the FT-IR spectra emerged, and its increasing intensity correlated with longer treatment time. ^{1}H-NMR, PL, GPC, and microscopy found no change in the polymer structure, molecular weight, or physical structure, so it is unlikely the appearance of the –OH band is related to degradation, but rather to the degradation products of H_2O_2 (i.e., water).[39] These observations are consistent with polymer properties observed upon increasing the processing temperature to 90 °C, whereby an increase in the intensity of the –OH band and a more pronounced blowing effect were observed. The GPC, ^{1}H-NMR, and PL data revealed only minor changes in the molecular weight and spectral profiles. This H_2O_2 digestion methodology is therefore nondestructive (chemically and physically) when using temperatures 60 °C or lower.

4.3. Acidic Digestion using HNO_3

Many studies that employ chemical digestion techniques use HNO_3 owing to its strong acidic and oxidizing power that accelerates the decomposition of the organic material.[11,20,21,23] Despite its popularity, the physical and chemical data suggests this reagent can also have a dramatic effect on the plastics, and hence HNO_3 should be used with caution. Microscopic analysis indicated a change in the physical properties (yellowing and cracking) of the MP particles exposed for 12 h at 30 °C, which was

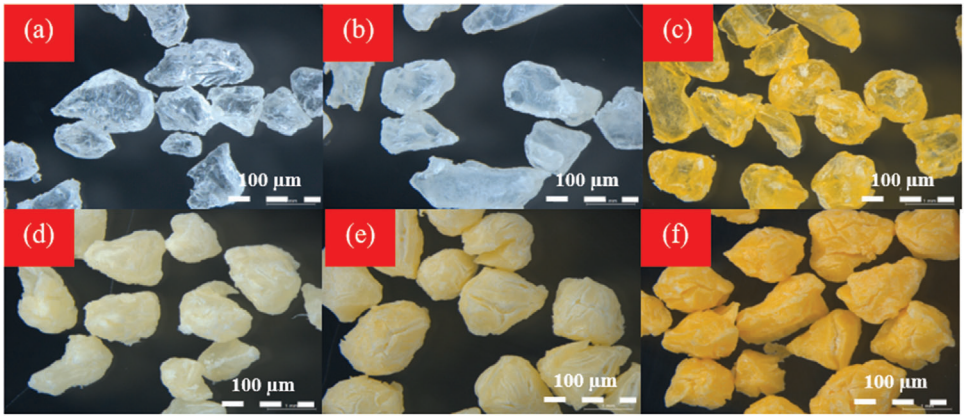

Figure 6. Microscopic analysis of PS treated with 15.8 M nitric acid at 30 °C for a) 1, b) 2, and c) 12 h, and at 60 °C for d) 1, e) 2, and f) 12 h.

Table 3. Suitability of potassium hydroxide (KOH, 1.8 M), sodium hydroxide (NaOH, 10 M), hydrogen peroxide (H_2O_2, 9.8 M), and nitric acid (HNO_3 15.8 M) as digestive reagents for the nondestructive recovery of polystyrene microplastics at 30, 60, and 90 °C.

	KOH	NaOH	H_2O_2	HNO_3
30 °C	**Good** No polymer reactivity or fragment deterioration.	**Good** No polymer reactivity or fragment deterioration.	**Good** No polymer reactivity or fragment deterioration.	**Fair** Significant polymer and reagent reactivity (i.e., aromatic nitration and polymer degradation), decreased infrared spectral similarity and mechanical damage after extended treatment – particularly of small MPs.
60 °C	**Good** No polymer reactivity or fragment deterioration.	**Good** No polymer reactivity or fragment deterioration.	**Good** No polymer reactivity, however, reagent degradation was linked to the introduction of –OH bands in the FT-IR spectra. No fragment deterioration.	**Bad** Significant polymer and reagent reactivity (i.e., aromatic nitration and degradation), infrared spectral dissimilarity. Fragment morphology was significantly deteriorated (i.e., surface cracking) and discolored.
90 °C	**Fair** No polymer reactivity, but significant morphological changes (i.e., swelling and pitting)	**Fair** No polymer reactivity, but significant morphological changes (i.e., swelling and pitting)	**Fair** No polymer reactivity, however, reagent degradation was linked to changes in FT-IR spectra. Significant morphological changes (i.e., swelling and pitting).	-

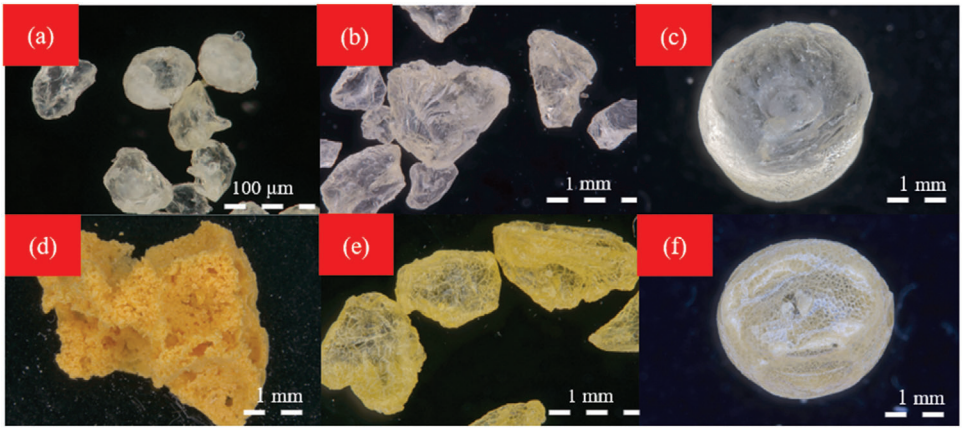

Figure 7. Microscope analysis of small (200–300 µm), medium (400 µm – 1 mm), and large (5 mm) polystyrene (PS) microplastics (MPs) treated with 15.8 M nitric acid for 24-h at a–c) 30 °C and d–f) 60 °C.

exacerbated by increasing the temperature to 60 °C (Figure 6). Heating of PS in the presence of HNO_3 has been associated with the production of the colorless gas, dinitrogen tetroxide (N_2O_4), and/or the formation of C–N bonds due to electrophilic aromatic substitution.[40] The change in the chemical structure of the polymer is evidenced by the appearance of new bands in the infrared spectra, as well as a downfield shift of protons in the ^{1}H-NMR spectra. The GPC analysis found a significant decrease in M_w, meaning that the polymer's hydrodynamic radii had changed due to the new bond formation and/or the breaking of the polymer chains. The PL intensity changed slightly, however, these changes were not as obvious even though there was a significant change in the polymer's structure after 1 hour exposure at 60 °C. The decrease in the PL intensity of the NR-stained MPs is likely due to the nitration of the PS or the NR itself.[41,42] Overall, while HNO_3 is considered the reagent of choice for digesting biological materials, it should be used with caution when attempting to recover MPs. Furthermore, prolonged use of this reagent at ambient temperature will degrade plastic polymers, and this degradation is more prevalent in smaller sized MPs (**Figure 7**; Figure S10, Supporting Information).

5. Conclusions

This work applies a molecular approach to analyze the effects of chemical digestion conditions on MPs. PL spectroscopy was found suitable for identifying polymer degradation in NR-stained PS MPs, however, due to the differences of the fluorophore reactivity and the polymer reactivity, this technique may be less reliable as a chemometric method of polymer degradation. As this study investigates the polymer on the molecular level, it can direct researchers towards a chemical digestion protocol which will nondestructively recover MPs from their biological

sample matrices. We recommend using caustic reagents (NaOH and KOH) and oxidative conditions (H_2O_2) in the recovery of MPs from biological materials as they have been found to be nondestructive (chemically and physically) up to 60 °C. At 90 °C, these reagents can be used with minimal degradation; however, blowing can occur in porous particles, thus giving a false indication of particle size. HNO_3 can degrade plastics even after 1 h exposure at 60 °C. If this reagent is to be used, we suggest minimizing the time the plastics are exposed to the HNO_3 at high temperatures as they are likely to experience some level of degradation that may impact their characterization. Finally, these digestion properties can be polymer specific,[43] therefore we recommend further investigation of polymer types specific to each study.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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