

Identification of Microplastics with Portable Raman Microscopy

Introduction

Over the past few decades, plastic litter has emerged as a pollutant of concern in the oceans globally. Microplastic, defined as plastic litter less than 5 mm in size, is considered the most abundant form of marine debris.^{1,2} Microplastics include both small manufactured items such as fibers and beads (primary microplastics), as well as items that result from fragmentation of larger plastic items by a combination of physical, chemical and biological processes and include fragments, films and foams (secondary microplastics). Plastic marine debris commonly begins as land-derived waste and subsequently enters estuaries and the coastal ocean.³ After entering the marine environment, microplastics distribute throughout the water with the help of tides, turbulence, and ocean currents, dispersing them across the oceans and throughout the water column.⁴ The rich ecosystems and high biological productivity found in coastal environments suggest more frequent biological interactions with microplastics in the coastal region than in the open ocean.⁵ Although the likelihood of plastic accumulation is higher in estuarine and coastal waters, few studies have focused on microplastics in these regions.⁶ With the growth of microplastics research in recent years, it has become crucial to expand the capabilities of research laboratories to routinely analyze the chemical composition of candidate microplastics from environmental samples. Often visual inspection is initially used to isolate microplastics from field-collected samples, but this can lead to the misclassification of microplastics, as well as accidental exclusion of plastic pieces. Spectroscopic techniques are critical as they can confirm manual microplastic designation through polymer identification. Such approaches can aid in determining where material may have originated, and may identify

Table 1. i-Raman® EX Specifications

Laser wavelength	1064 nm
Maximum laser power at probe	330 mW
Spectral range	100-2500 cm ⁻¹
Detector type	InGaAs TE-cooled to -15 °C

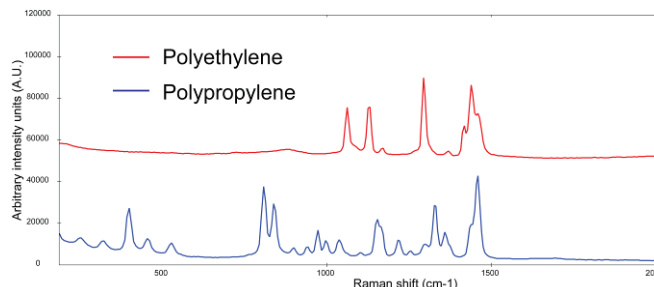


Figure 1 Raman spectra of polypropylene (blue) and polyethylene (red, spectra manually offset for clarification)

additives that themselves could pose negative biological impacts.

Generally polymers, and by extension plastics, generate strong Raman signal. Figure 1 shows the Raman spectra of bulk polyethylene and polypropylene materials measured with 1064 nm excitation. The plastics can be clearly distinguished based on their spectral features. A reference library can also be built for easy identification of unknown microplastic material (commercial libraries are also available). Although Fourier transform infrared (FTIR) spectroscopy is another technique that is frequently used for the identification of microplastics, Raman offers easier sampling of small (<100 μm) particles than FTIR. Raman spectrometers also lend themselves to portability better than FTIR systems, so portable Raman analysis may be used at the location of sample preparation.

In this application note, we'll explore the use of portable Raman microscopy for the identification of microplastics recovered from surface estuary waters.



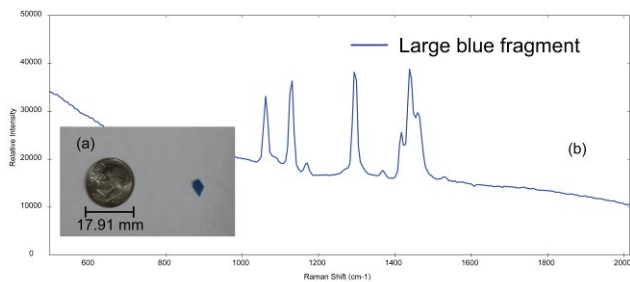


Figure 2 (a) Small blue plastic fragment (dime for comparison) and (b) Raman spectrum acquired from the sample

Experiment

Water samples were collected from the surface water of the Delaware Bay (USA) by conducting 5 min tows using a ring plankton net (1 m diameter, 200 µm nitex mesh) fitted with a flow meter.⁷ Samples were then transferred to glass jars and fixed with 4% formaldehyde. The total sample was size-fractionated on stainless steel sieves (5,000; 1,000; and 300 µm). After drying the samples overnight at 90 °C, wet peroxide oxidation and density separation processes were used to isolate microplastic from digested organic material in the two smaller size-fractionated samples.⁸ Microplastics were then collected onto 200 µm nitex mesh and folded in aluminum foil to dry. Microplastics from these samples were enumerated by manual examination under a stereomicroscope in a laminar flow hood and each piece was assigned a plastic type (i.e., fragment, fiber, bead, film, foam, rubber). Enumeration and categorization was followed by plastic identification using Raman spectroscopy. An i-Raman[®] EX portable Raman system with a 1064 nm laser excitation was used for all measurements (see Table 1 for specifications). The 1064 nm laser excitation is required to mitigate the fluorescence that is typically generated from colored microplastic samples upon excitation with a 785 nm laser. A portable video microscope setup with an objective lens of 50× magnification (9.15 mm working distance, 42 µm spot size) was used to image the microplastics. BWSpec[®] software was used for the data collection. Integration times ranged from 30s-3 minutes, and laser power was kept below 50% of the maximum laser power (<165 mW) in order to avoid sample burning. The spectra were intensity corrected against a NIST 2244 Raman intensity correction standard, but otherwise no other preprocessing was applied to the spectra. BWID[®] software was used to make the identification of the microplastics against a reference library of plastics spectra.

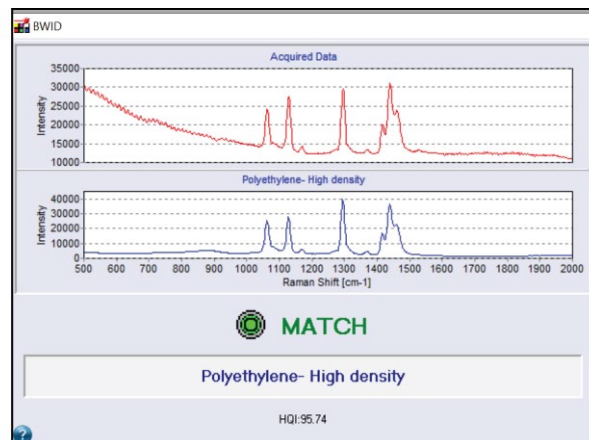


Figure 3 BWID match result for polyethylene. Red spectrum is the acquired sample spectrum from Figure 2. Blue spectrum is the reference spectrum of polyethylene.

Results

Several microplastics samples of various types and sizes were analyzed using the i-Raman EX system. Figure 2a shows the photo of a large blue microplastic fragment. The diameter of this fragment is ~4.5 mm, which is on the higher end of the size range for a particle to be designated as a microplastic. Due to the irregular shape of the particle, the sample is likely a secondary microplastic. Figure 2b is the Raman spectrum collected from the blue fragment. BWID software compares the acquired spectrum of the unknown sample to a library of reference materials using a calculated hit quality index (HQI). The HQI is a correlation coefficient that measures how similar the sample spectrum is to the reference spectrum. Spectral library search results are ranked from an HQI of 100 (best match) to an HQI of 0 (worst match). A first derivative is applied to the spectra for the calculation. There are a variety of spectral libraries available for use with BWID, and the software also supports custom library building.

BWID matched the blue fragment in Figure 2a to a reference spectrum of polyethylene (PE) with a

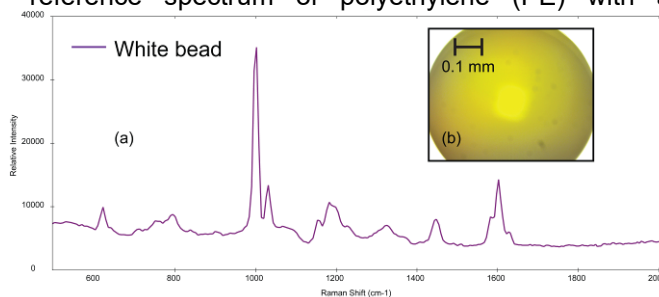


Figure 4 (a) Raman spectrum of polystyrene collected from (b) polystyrene bead (image not true color)

calculated HQI of 95.7 (Figure 3), indicating a strong match.

Other plastic materials such as polystyrene (PS) and polypropylene (PP) were also identified. Figure 4a shows the Raman spectrum acquired from a small, spherical bead (photomicrograph shown in Figure 4b). This bead is likely a primary microplastic. BWID matched the spectrum to a reference spectrum of polystyrene with an HQI of 98.2.

Fibers are an important subgroup of microplastic particles. They can be shed from synthetic garments and other plastic textiles during the production process, but also through wastewater during routine home maintenance. Synthetic fishing lines are also a major source of microfiber pollution.

Figure 5a shows the Raman spectrum collected from a thin, teal fiber (photomicrograph shown in Figure 5b). BWID matches the Raman spectrum of the sample to a reference spectrum of polypropylene, with a calculated HQI of 74.9. Although this value may seem low, upon further observation there are some additional peaks in the sample spectrum that cannot be attributed to polypropylene. Upon further investigation, the peak at $\sim 1537\text{ cm}^{-1}$ and the set of weak peaks from $670\text{--}790\text{ cm}^{-1}$ in the sample spectrum are consistent with the Raman spectrum of chlorinated copper phthalocyanine green pigment.⁹ Although the colorant used in the plastic is not generally a crucial research question, this is useful information for determining the origin of a sample.

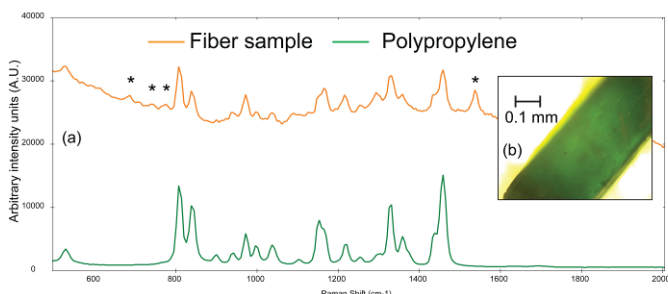


Figure 5 (a) Raman spectra of a teal fiber (orange) compared to a reference spectrum of polypropylene (green) and (b) photomicrograph of teal fiber. The asterisks denote peaks that can be attributed to the colorant used in the plastic.

Table 2 shows the summary of the microplastic analysis from the Raman spectra. All materials were identified as polyethylene, polypropylene, or polystyrene. Several samples had inconclusive results; the majority of these samples are black microplastic samples that absorb both the exciting radiation and scattered radiation, making their identification with Raman spectroscopy very difficult. Another limitation that was observed is the fragility of

the microfibers. The laser power applied to fibers should be kept low ($\sim 10\%$ of the maximum laser power), as higher laser powers may cause distortion and burning of the sample.

Table 2. Summary of BWID results

Match result	Number of samples identified
PE	11
PP	4
PS	2
inconclusive	5

Conclusions

Because the presence of microplastics in our marine environments represents a looming threat to our environment, their robust characterization will be an important research topic for years to come. Raman microscopy is an effective tool to unambiguously identify these microplastics. The use of near-infrared excitation is important to mitigate fluorescence from the dyes used in the plastics. Software correlation coefficient algorithms are useful for the simple identification of plastic material.

Authors

Jonathan H. Cohen*, *University of Delaware School of Marine Science and Policy*

Taylor Hoffman*, *University of Delaware School of Marine Science and Policy*

Kristen Frano, *B&W Tek*

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