



Application Note 410000051-B

Identification of microplastics with Raman microscopy

Quick identification of environmental microplastic particles

Microplastics have become an environmental health and safety concern, though we do not completely understand their long-term impacts. Microplastic, defined as plastic litter less than 5 mm in size, is the most abundant form of marine debris [1,2]. Microplastics are categorized as primary or secondary. Primary microplastics include small, manufactured items such as fibers and beads [3]. Secondary microplastics include fragments formed by a combination of physical, chemical, and biological processes [3].

Research laboratories *must* expand their capabilities

to routinely analyze candidate microplastics from environmental samples. Spectroscopic techniques are well suited to polymer identification. This aids the determination of origin and helps predict biological impacts. Laboratory Raman spectroscopy is an alternative to confocal Raman microscopes and Fourier transform infrared (FTIR) microscopes for quick identification of polymer materials. However, very small samples are poor candidates for traditional Raman analysis. Raman microscopy was used to identify very small microplastic particles in this Application Note.

INTRODUCTION

Raman spectroscopy has many benefits and adaptations for different applications. Raman microscopy allows easier sampling of small particles ($<100\text{ }\mu\text{m}$) than FTIR, another technique frequently used for microplastics identification. Raman systems tend to be much more portable than most other techniques, so testing can occur directly on site.

Aside from some interference from dyes, polymers and plastics are good candidates for Raman analysis.

Figure 1 shows the Raman spectra of bulk polyethylene and polypropylene materials measured with 1064 nm excitation. The plastics can be clearly distinguished by their spectral features.

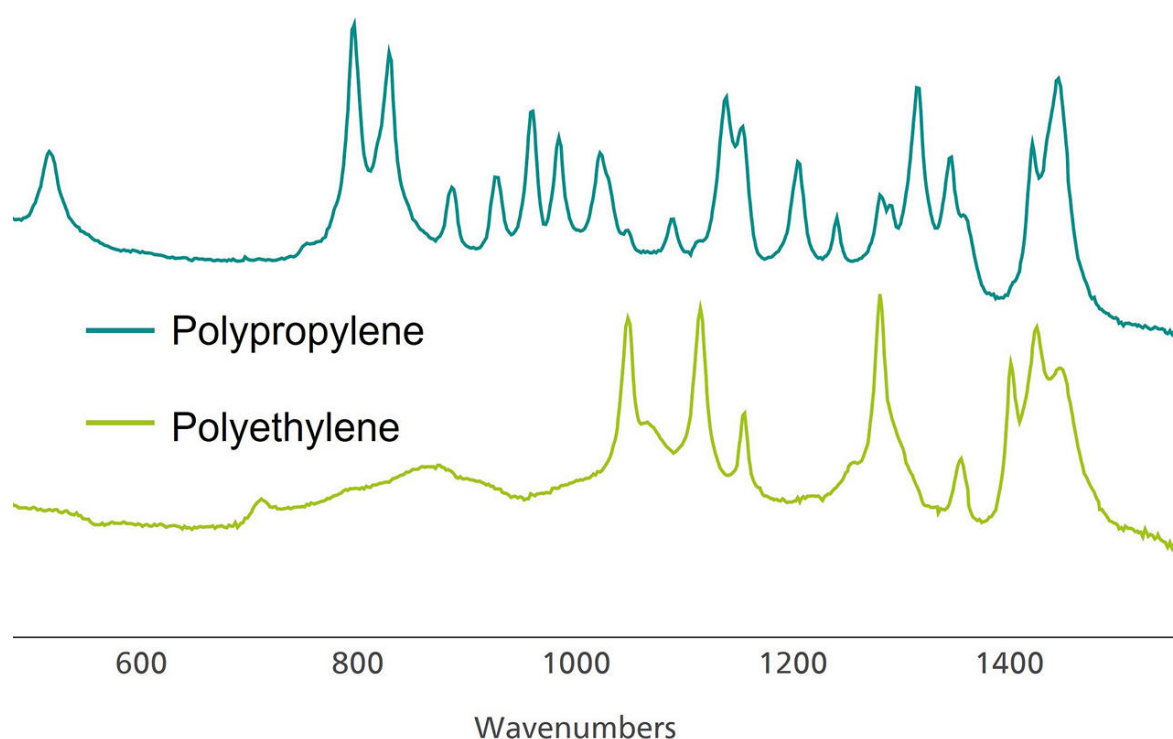


Figure 1. Raman spectra of polypropylene (top) and polyethylene (bottom). Spectra are manually offset for visual clarification.

This Application Note explores the use of portable Raman microscopy for the identification of

microplastics recovered from surface estuary waters.

EXPERIMENT

Water samples were collected from the surface water of the Delaware Bay (USA). They were then transferred to glass jars and fixed with 4% formaldehyde. The total sample was size-fractionated on stainless steel sieves (5000, 1000, and 300 μm).

The 300 and 1000 μm samples were dried overnight at 90 °C. After drying, wet peroxide oxidation and density separation processes isolated microplastics from digested organic material [4].

Microplastics were collected onto 200 μm nitex mesh

and dried. These samples were examined under a stereomicroscope and each piece was assigned a plastic type (i.e., fragment, fiber, bead, film, foam,

rubber). This was followed by plastic identification with Raman spectroscopy.

Table 1. Experimental parameters.

Equipment	Acquisition settings	
i-Raman EX	Laser Power	<165 mW
BAC151 video microscope	Int. time	30 s–3 min
BWID software	Average	1

An i-Raman® EX portable Raman system with 1064 nm laser excitation was used for all measurements (see **Table 1** for specifications). 1064 nm laser excitation mitigates the spectral fluorescence resulting from 785 nm laser excitation of colored microplastic samples.
A BAC151C video microscope with an objective lens

of 50× magnification (9.15 mm working distance, 42 µm spot size) was used to image the microplastics. Laser power was kept below 50% of the maximum (<165 mW) to avoid sample burning. BWID® software was used for identification of the microplastics against a reference library of plastics spectra.

RESULTS

Secondary microplastics

Several microplastic samples were analyzed. **Figure 2a** shows a blue microplastic fragment at the larger end of the microplastic size range (diameter

approximately 4.5 mm). The irregular shape of this particle indicates that it is likely a secondary microplastic. **Figure 2b** is the Raman spectrum collected from the blue plastic fragment.

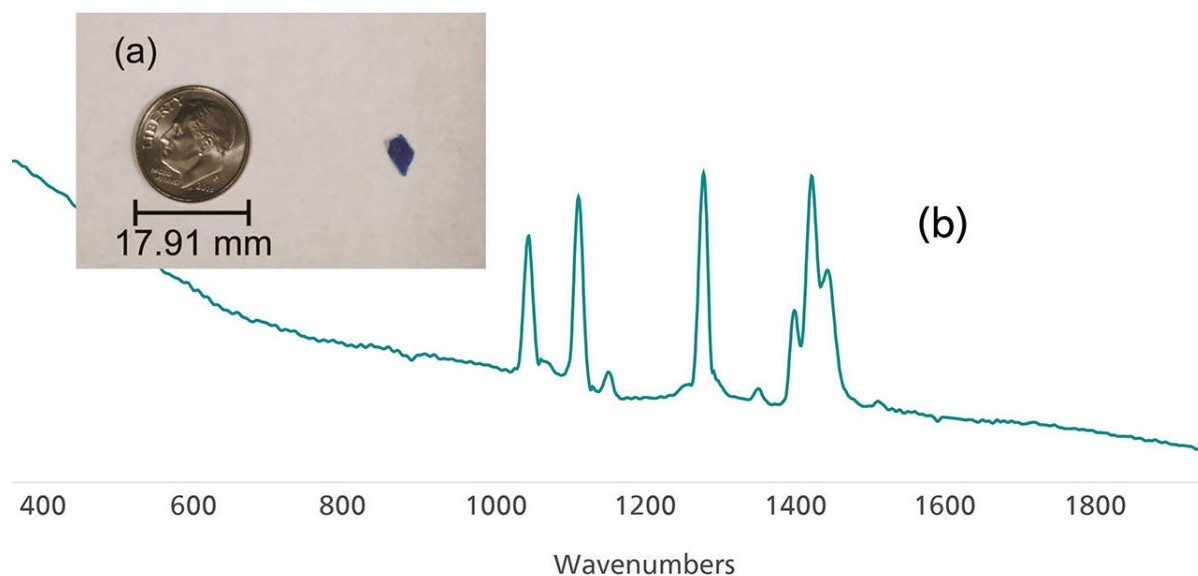


Figure 2. (a) Small blue plastic fragment (with American dime for comparison) and (b) Raman spectrum acquired from the sample.

BWID software compares the acquired spectrum of an unknown to a library of reference materials to generate a hit quality index (HQI), a correlation coefficient. A first derivative is applied to the spectrum for the calculation. Spectral library search results are ranked from an HQI of 100 to 0 (best to worst match). BWID can be used with a variety of commercial

spectral libraries, and it supports custom library building.

BWID matched the blue fragment in **Figure 2a** to a reference spectrum of polyethylene (PE) with a calculated HQI of 95.7 (**Figure 3**), indicating a strong spectral correlation.



Figure 3. BWID match for polyethylene.

Primary microplastics

Figure 4a shows the Raman spectrum acquired from a small, spherical bead (Figure 4b). This bead is likely a

primary microplastic. BWID matched the sample spectrum to a reference spectrum of polystyrene with an HQI of 98.2.

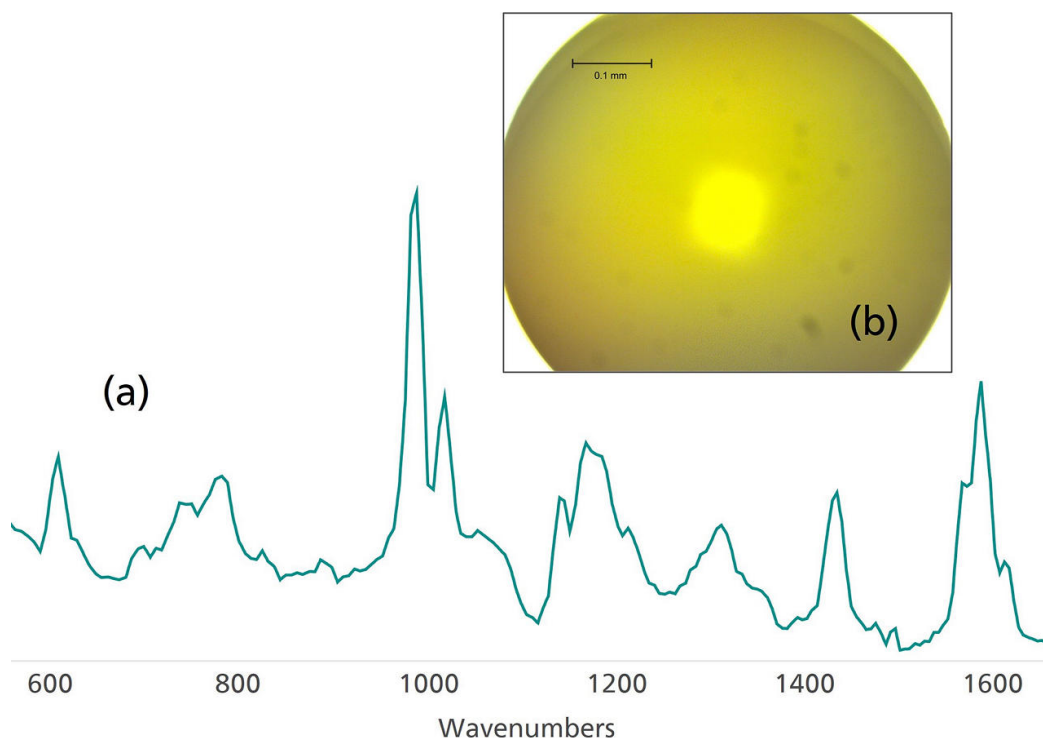


Figure 4. (a) Raman spectrum of polystyrene collected from (b) a polystyrene bead.

Fibers are an important and common subgroup of microplastic particles. Figure 5a shows the Raman spectrum collected from a thin colored fiber (Figure

5b). BWID matched the Raman spectrum of the sample to a reference spectrum of polypropylene, with a calculated HQI of 74.9.



Figure 5. (a) Raman spectra of a colored fiber (top) compared to a reference spectrum of polypropylene (bottom) and (b) microscope image of the colored fiber. The asterisks denote peaks that can be attributed to the colorant used in the plastic.

This relatively low value prompted further investigation into peaks in the sample spectrum that cannot be attributed to polypropylene. The peak at approximately 1537 cm^{-1} and the set of weak peaks from $670\text{--}790\text{ cm}^{-1}$ are consistent with the Raman spectrum of chlorinated copper phthalocyanine green pigment [5]. This is useful information for determining the origin of a sample.

Microplastics summary

A summary of the microplastics measured in this

study indicates that the samples were mainly composed of polyethylene, polypropylene, or polystyrene (Table 2). Inconclusive results tend to come from black microplastics, which are a historically challenging material for Raman.

Sample degradation is another observed limitation. Low laser powers should be used ($\sim 10\%$ of maximum) to prevent distortion and burning of the sample.

Table 2. Summary of identification results.

Match Result	Number of samples
Polyethylene	11
Polypropylene	4
Polystyrene	2
Inconclusive	5

CONCLUSION

Microplastics represent a potential threat to human health and our environment. Their robust characterization will be an important research topic in the near future. Raman microscopy is an effective tool to unambiguously identify these microplastics.

ACKNOWLEDGEMENTS

Thanks to Jonathan H. Cohen and Taylor Hoffman from the University of Delaware School of Marine

1064 nm excitation mitigates fluorescence from the dyes used in the plastics. Software correlation coefficient algorithms are useful for the simple identification of plastic material.

Science and Policy for co-authoring this Application Note and providing the microplastic samples.

REFERENCES

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CONTACT

Metrohm Brasil
Rua Minerva, 161
05007-030 São Paulo

metrohm@metrohm.com.br

CONFIGURATION

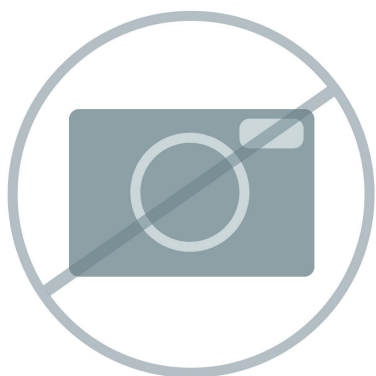


i-Raman EX Portable Raman Spectrometer

The i-Raman® EX is part of our award-winning series of i-Raman portable Raman spectrometers with our patented CleanLaze® laser with 1,064 nm laser excitation. Using a high-sensitivity InGaAs array detector with deep TE cooling, high dynamic range, and a high throughput spectrograph design, this portable Raman spectrometer delivers a high signal-to-noise ratio without inducing autofluorescence, making it possible to measure a wide range of natural products, biological samples (such as cell cultures), and colored samples.

The i-Raman EX provides a spectral coverage range from 100 cm^{-1} to $2,500\text{ cm}^{-1}$, enabling you to measure across the entire fingerprint region. The system's small footprint, lightweight design, and low power consumption ensure research-grade Raman analysis capabilities at any location. For expanded analysis capabilities, it can be used with our proprietary Vision software as well as BWIQ® multivariate analysis software and BWID® identification software. With the i-Raman EX, you always have a high precision Raman solution for qualitative and quantitative analysis without fluorescence.

BWS485III



Video Microscope Objective, 50x (RML150A)

Microscope objective, infinity-corrected, 50x, working distance (mm) = 9.15, focal length (mm) = 4, numerical aperture (NA) = 0.55.

RML150A