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 μm) 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.

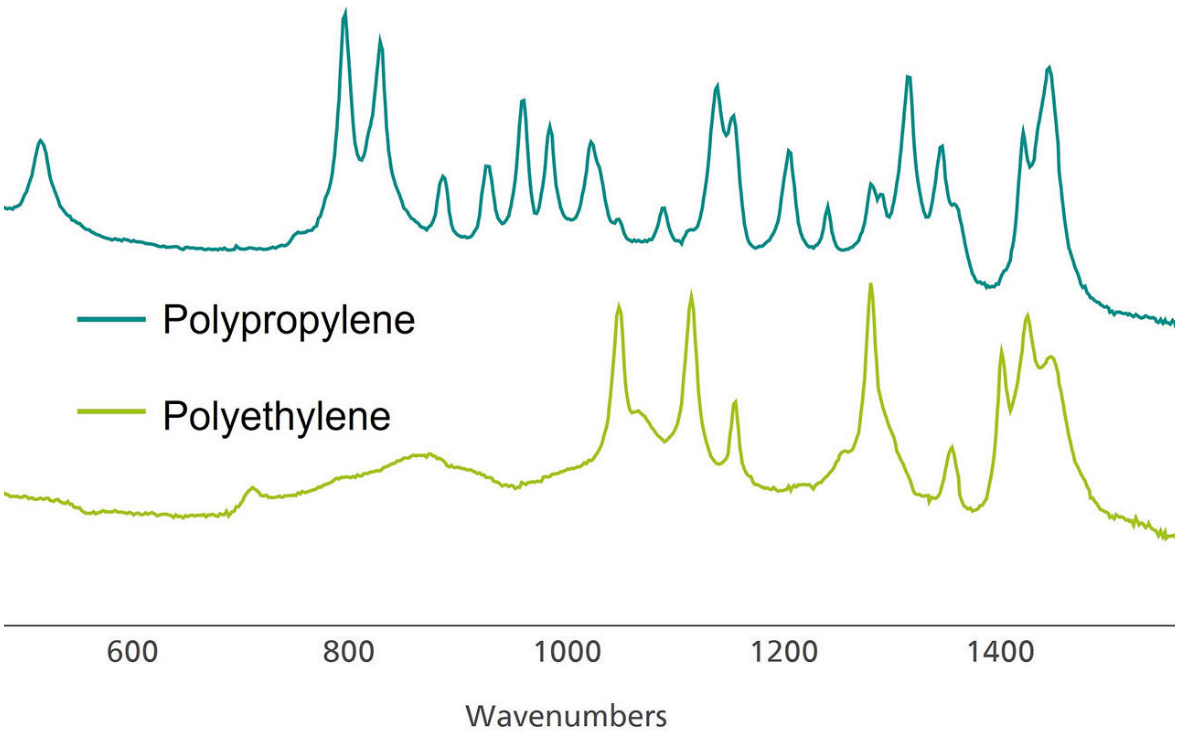

Figure 1. Raman spectra of polypropylene (top) and polyethylene (bottom). Spectra are manually offset for visual clarification.

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-fractioned 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.

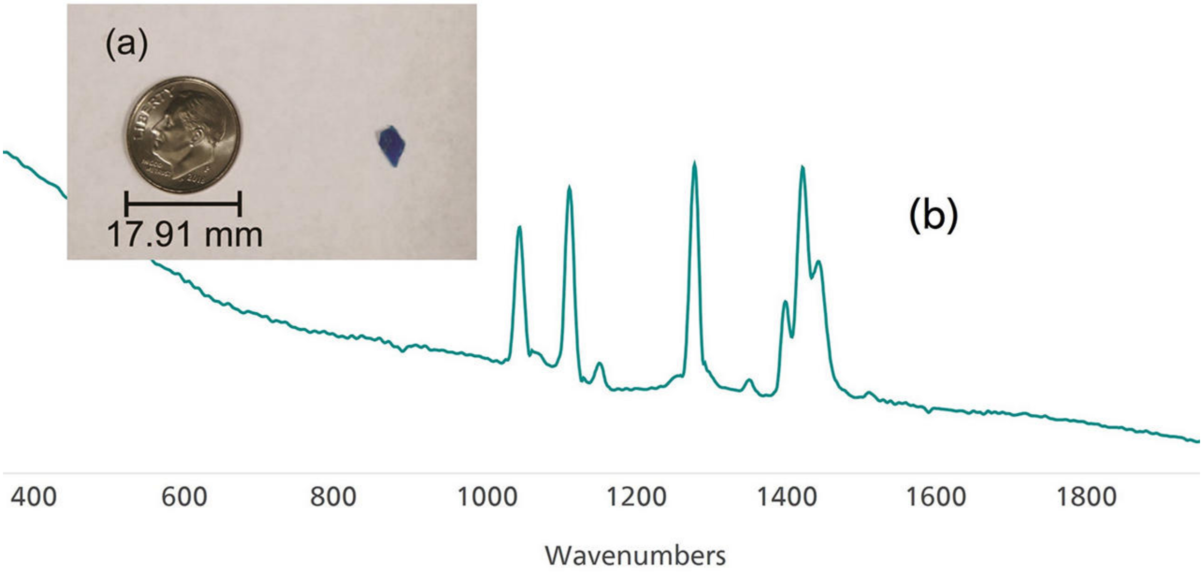

Figure 2. (a) Small blue plastic fragment (with American dime for comparison) and (b) Raman spectrum acquired from the sample.

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.

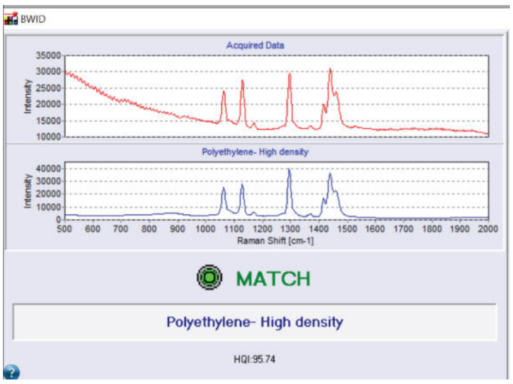

Figure 3. BWID match for polyethylene.

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.

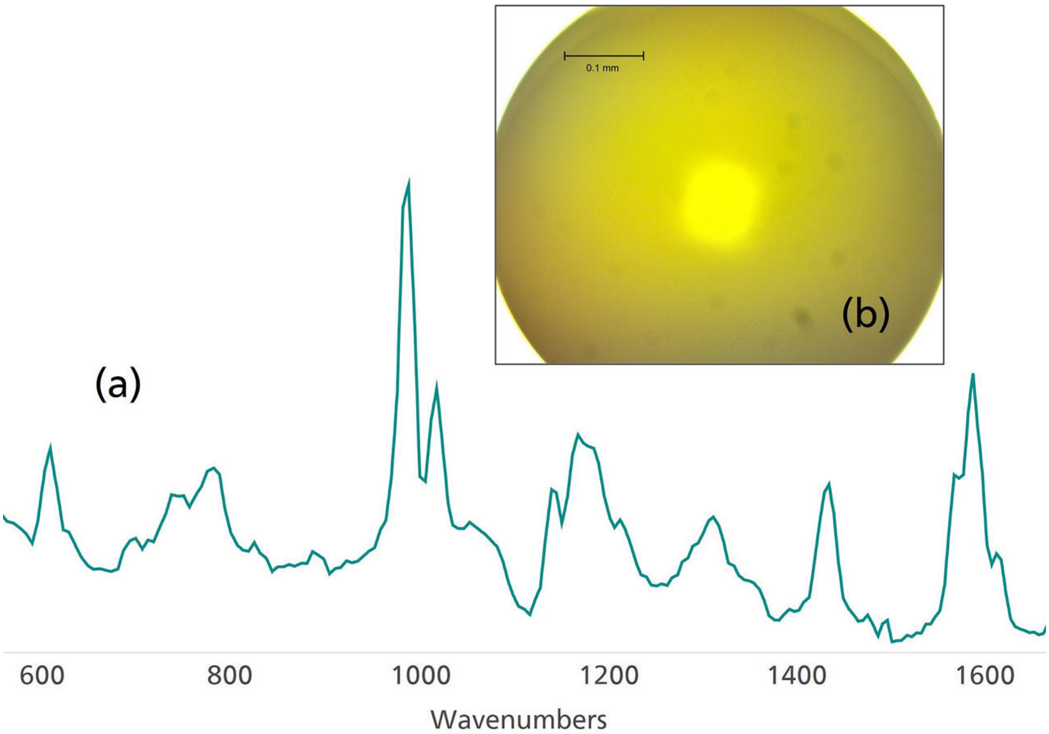

Figure 4. (a) Raman spectrum of polystyrene collected from (b) a polystyrene bead.

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.

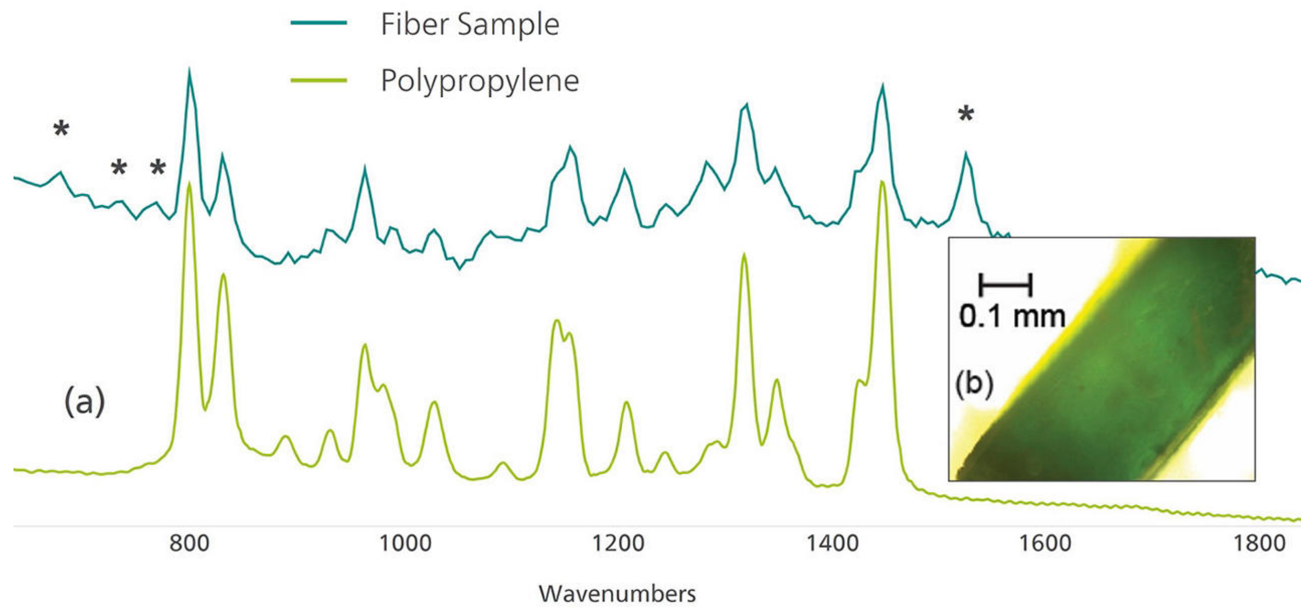

Figure 5. (a) Raman spectra of a colored fiber (top) compared to a reference spectrum of polypropylene (bottom) and (b) microscope image of the colored fiber. The asterisks denote peaks that can be attributed to the colorant used in the plastic.

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 670–790 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 (~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.

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.

ACKNOWLEDGEMENTS

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CONFIGURATION

i-Raman EX

i-Raman® EX は、数々の受賞歴を誇る i-Raman 携帯型スヘクトロメーターシリースの一つてあり、特許取得済みの 1.064 nm 励起の CleanLaze® レーサーを搭載したスヘクトロメーターてす。高感度 InGaAs アレイ検出器と TE 深冷、高タイナミックレンシ、高スルーフット分光器設計により、自家蛍光を発生させすに高い S/N 比を実現し、天然物、生体サンフル(細胞培養なと)、着色サンフルなとを幅広く測定てきる携帯型ラマンスヘクトロメーターてす。
i-Raman EX は、100 cm^{-1} から 2.500 cm^{-1} までの範囲のスヘクトルをカハーしており、指紋の全領域を測定することか可能てす。システムの設置面積か小さく、軽量設計、低消費電力て場所を選はす、研究レヘルのラマン分析か可能てす。 また、解析機能を拡張するために、当社独自の Vision ソフトウェアや多変量解析ソフトウェア BWIQ®、識別ソフトウェア BWID® と組み合わせて使用することかてきます。i-Raman EX により、蛍光を伴わない品質分析および定量分析のための高精度のラマンソリューションを常に使用することかてきます。
BWS485III

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マイクロスコーフ対物レンス、無限補正、50倍拡大、作業距離 (mm) = 9.15、焦点距離 (mm) = 4、開口数 (NA) = 0.55。
RML150A