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APPLICATION NOTE

No. AN53167

Straightforward identification of microplastic particles in bottled water

Using FTIR & Raman Spectroscopy

Plastics can be formed into an endless variety of shapes with different physical characteristics and that makes them attractive materials for a wide assortment of products. Large and diverse usage results in a significant amount of plastic materials eventually ending up as refuse. The size of the plastic waste can range from large, easily visible, easily handled, and easily detectable macroplastics down to microplastic particles that may be too small to detect with normal vision. The European Chemical Agency (ECHA) defines microplastic particles as particles with dimensions from 5 mm to 1 nm. The source of these microplastics is from primary sources where the plastics have been produced as small particles (such as in some cosmetic products and cleaners) or as secondary products that arise from the mechanical, photochemical, or thermal break down of larger plastic products. Many studies on microplastics have centered on contamination in marine environments and related areas (sea salt, seafood, and aquatic animals) they also appear in tap water and even in commercial products such as bottled water and other beverages. The detection and identification of microplastic particles are important for determining the possible sources of these contaminants and for evaluating the environmental impact and health risks associated with the different types of materials. There are ongoing efforts to understand the full impact of these microplastics.

One of the biggest challenges with the analysis of microplastics involves distinguishing microplastic particles from other types of materials and making sure that misidentification does not lead to over-estimating the amount of microplastics in a sample. While there can be all types of matrices that contain microplastic particles this report will focus on microplastics in a dilute water suspension. The particles are isolated by filtration. After filtration the sample consists of a collection of particles on a filter and at that point the analysis requires a facile way of distinguishing and identifying microplastic particles within a field of other particles.

FTIR and Raman spectroscopy (two different forms of vibrational spectroscopy) are widely used for the unambiguous identification of different types of polymeric materials. The molecular information reflected in the vibrational spectra of polymer materials provides a molecular fingerprint that can be used with spectral databases to positively identify different types of polymer materials. The spectral information supplied by infrared and Raman spectroscopy is similar but not exactly the same. Infrared spectroscopy depends on changes in dipole moments and is particularly useful for detecting polar functional groups found in many different types of polymer materials (hydroxyl, amines, amides, carbonyls, etc.). Raman spectra depend on a change in polarizability and thus are good for looking at polymer backbone structures as well as where there are delocalized electrons such as alkenes and aromatics. Raman spectroscopy can be more sensitive to differences in molecular structures. Infrared spectroscopy is more useful for identifying some types of polymers and Raman spectroscopy is better for other types of polymers but when used in conjunction they deliver complementary data that is more useful than either technique alone. Both FTIR and Raman spectroscopy can be used to analyze microscopic-sized samples. This makes vibrational spectroscopy an indispensable tool for the identification and analysis of microplastic particles.

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The analysis of microplastic particles described here can be called a targeted discrete point analysis approach. In this approach a visible image is obtained from multiple fields of view through the microscope stitched together to form a visual mosaic. This visual mosaic is used to automatically select possible particles for analysis as well as to provide the information on the particle size and shape. Once all the potential particles have been targeted, vibrational spectra are collected from these particles and used to determine which particles are truly microplastics. The keys to this approach are a good visual image of the particles and a filter material that has minimal spectral interferences. This approach has an advantage over just collecting a Raman image of the whole filter in that imaging such a large area at a reasonable resolution requires an incredible amount of spectral data. If the distribution of particles on the filter is relatively dilute then a majority of the spectral data shows only the filter and no particles.

Experimental

The sample used in this investigation was a commercially available 500 ml bottle of drinking water. The drinking water was filtered using the Thermo Scientific™ Microparticle Sample Preparation Kit. The kit utilizes a 10 x 10 mm silicon filter that can be used for transmission FTIR analysis, reflection FTIR analysis, or Raman analysis of the isolated particles.

The silicon filters were then analyzed using both a Thermo Scientific™ Nicolet™ iN10 MX FTIR Imaging Microscope and a Thermo Scientific™ DXR2 Raman Microscope. Both of these instruments utilized software options specifically designed for automated particle analysis. The Nicolet iN10 MX FTIR Imaging Microscope uses the Particle Wizard option in the Thermo Scientific™ OMNIC™ Picta™ Software and the DXR2 Raman Microscope uses the Particle Analysis option that is part of the Thermo Scientific™ Omnic™ Atlus Software and the Thermo Scientific™ Omnic™ for Dispersive Raman Software.

The analysis using the iN10 MX FTIR Imaging Microscope was done in transmission mode using an MCT A detector and 64 scans per spectrum. The background position was selected as an area clear of particles near the center of the silicon filter. The apertures were automatically selected as part of the Particle Wizard routine and are based on the particle size. The number of potential particles was determined from an image analysis of the visible mosaic image and refined through adjustments made to the sensitivity and particle size parameters.

The analysis using the DXR2 Raman Microscope was done using a 10X objective and a 532 nm laser. Each spectrum was collected using a 2s exposure and 10 exposures. The number of potential particles were selected using the Particle Analysis routine and like the iN10 MX FTIR Imaging Microscope analysis this is based on an image analysis of the visual mosaic along with sensitivity and size parameters.

Results and discussion

The workflows using both the iN10 MX FTIR Imaging Microscope and the DXR2 Raman Microscope are very similar. In both cases the analysis starts with defining and collecting a visual mosaic of the area on the silicon filter containing the particles filtered from the drinking water. Then an image analysis of the visible mosaic is used to select possible particles. This is based on the contrast in the image and the sensitivity can be adjusted to determine what visual features are selected as particles. There is also a sieve based on size that can be used to adjust what sizes of particles are chosen for analysis. These parameters help determine the number of potential particles selected for spectroscopic analysis. Figure 1 shows the process of particle selection using the Particle Wizard software in Picta Software with an iN10 MX FTIR Imaging Microscope and a silicon filter with particles filtered from bottled drinking water.

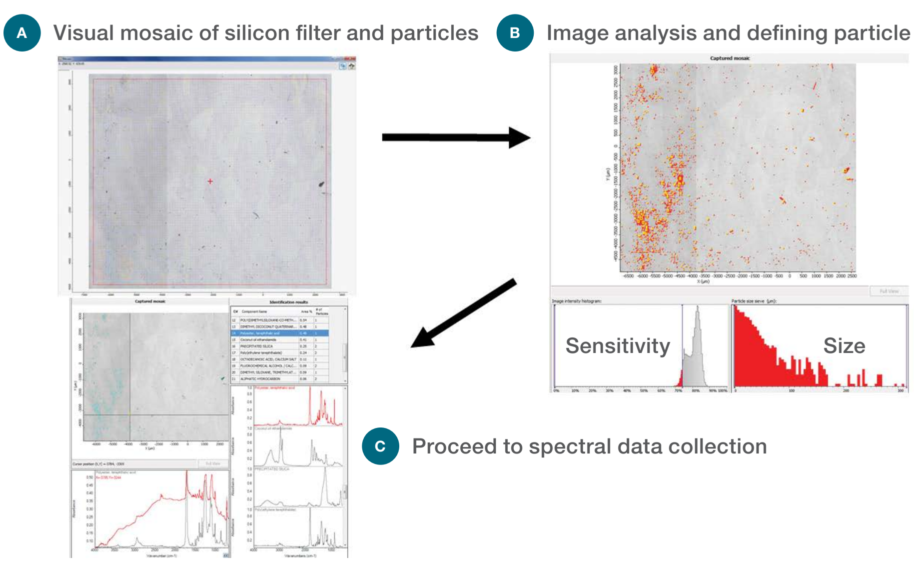

Figure 1. Workflow for particle analysis: (a) Visual Image, (b) Selecting potential particles, (c) Collect spectral data and report results

Once the potential particles have been selected, the next step is to collect spectral data to identify the microplastic particles. Not all the features visually identified as particles are actually particles. They could arise from things like scratches or discoloration of the filter as well as other visual differences that are not actually different materials. It is also possible to have particles that are biological or mineral based. These types of particles while interesting are not the goal of this analysis. Some particle materials such as metals would not be either Raman or infrared active and thus would not provide a vibrational spectrum. Actually a small portion of the features are microplastics and the goal of the analysis is to ferret out these particles.

After the spectral data is collected, the spectra are searched against spectral databases to find matches and identify the particles. A summary of the number of particles of each type is included as part of the analysis report along with the spectra from the individual particles and the dimensions of the particles. Analysis reports can be exported as text document that can be incorporated into other software programs or can be printed in hard copy or electronic format. Table 1 shows the summary of the identified microplastic particles from the silicon filter both for the FTIR and Raman analysis. The size of the particles was about 10–200 microns and length and width information is provided for each particle in the full report. An example of a particle from the Raman analysis is shown in Figure 2. This is a polypropylene fiber that is approximately 16 microns wide and 78 microns long.

Table 1. Summary of the microplastic particles identified on the silicon filter by both the FTIR analysis and Raman analysis

	FTIR (total particles – 801)	Raman (total particles – 1065)
PTFE	5	9
Polyester (PET)	3	3
Polystyrene	0	3
Polypropylene	3	3
Unspecific long chain aliphatic hydrocarbon containing materials	2	1
Polyethylene	2	1

While the FTIR and Raman analysis did not identify exactly the same number of microplastic particles the majority of the materials are the same. The differences can be explained by the differences in the selection rules, the way the spectral data is collected, and differences in the spatial resolution. Some materials are better suited to identification by each technique. A combination of the two is advantageous for optimizing the analysis of a wider range of particles.

As indicated previously, only a small number of the potential particles are actually microplastics. Particles of other materials such as minerals (talc, quartz, and titanium dioxide) and biological-based materials (cellulose, proteins, and beta-carotene) were also identified. A majority of the features selected by the image analysis routine were not identified. Many of these are simply visual features that did not represent real particles. Some are from materials that are not Raman or FTIR active. The spectra from these areas show no features beyond those associated with the filter itself. However, since the goal was to identify microplastics particles the fact that these other materials are not clearly defined does not matter for this application. While the collection of spectral data from particles that are not microplastics might be seen as slowing down the analysis, the fact that the data collection and most of the analysis is automated means that the arduous and involved task of separating microplastics from non-microplastic particles does not require manual subjective visual sorting and thus saves working hours.

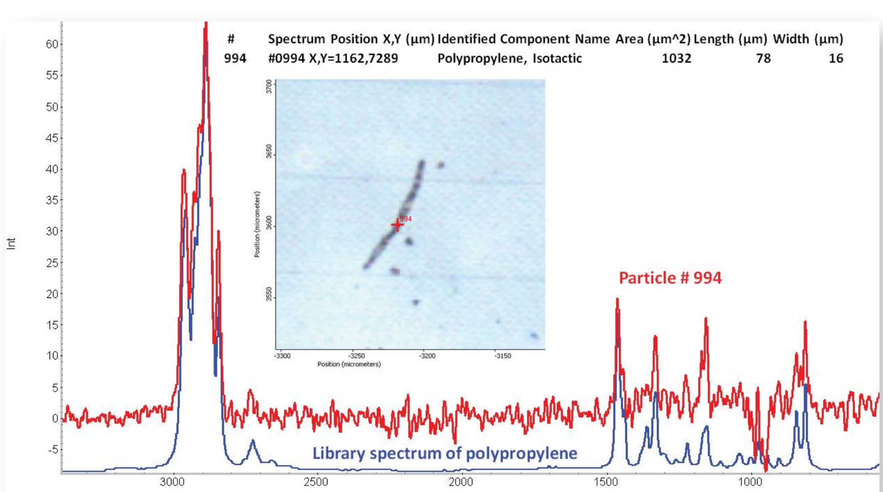

Figure 2. Example of a microplastic particle from the Raman particle analysis of the silicon filter. The particle is a small polypropylene fiber approximately 78 microns long and 16 microns wide. The Raman spectrum of the fiber matches a library spectrum of polypropylene.

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Summary

Microplastics have been discovered in a wide variety of locations ranging from sea life to food and beverages. While the majority of microplastics are likely to be biologically benign with the scope of the distributions and the amounts of the materials being as large as they are it is important to understand the total impact. To that goal, it is important to be able to isolate, detect, and identify microplastic particles. For spectroscopic analysis it is important to select an isolation technique that allows for subsequent spectroscopic analysis. We have found that silicon-based filters provide certain advantages for both FTIR and Raman analysis. Vibrational spectroscopy provides access to molecular information and can identify a wide variety of materials including polymeric materials. Micro-spectroscopy allows for analysis of microscopic particles and is ideal for the analysis of microplastic particles. While infrared and Raman spectroscopy both provide molecular structure information they do excel at identifying different aspects of polymer materials. Where one is weak the other is strong so that together they provide exceptional coverage for identifying a wide variety of different materials.

Find out more at **thermofisher.com/microplastics**

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