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WHITE PAPER WP53077

Guide to the identification of microplastics by FTIR and Raman spectroscopy

Introduction

The presence of microplastics in the environment and our food-chain is of growing concern. This has led to increased testing for the presence of microplastics in a variety of samples including bottled, ocean and fresh water, which has brought about tougher legislation to limit the amount of plastics entering the ecosystem. Fourier Transform Infrared (FTIR) and Raman spectroscopies have long been used for the analysis of polymers, and it is, therefore, natural that they be the de facto techniques to identify microplastics. This note provides an overview of FTIR and Raman techniques as applied to the identification of microplastics.

Microplastics – common materials

A microplastic is a small piece of plastic that is <5 mm in size.[1] A list of common polymers found in microplastics is provided in Table 1.

Of these materials, polypropylene and polyethylene are particularly prevalent in the environment due to their production in vast quantities for consumer packaging applications. The humble plastic bag is made from polyethylene, while polypropylene is used for candy wrappers and bottle caps. These polymers float on both fresh and salt water, enabling them to travel long distances from the initial source of pollution.

Name	Abbreviation	Typical Density (g/cm^3)
Expanded Polystyrene	EPS	0.02
Polypropylene	PP	0.89
Polyethylene	PE	0.96
Acrylonitrile-butadiene-styrene	ABS	1.05
Polystyrene	PS	1.06
Polyamide (Nylon)	PA	1.14
Polymethyl methacrylate	PMMA	1.18
Polycarbonate	PC	1.21
Cellulose Acetate	CA	1.3
Polyvinyl chloride	PVC	1.39
Polyethylene terephthalate	PET	1.39
Polytetrafluoroethylene	PTFE	2.2

Table 1: Common polymers (densities derived from Teegarden[2])

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The infrared (IR) and Raman spectra of polyethylene and polypropylene are shown in Figures 1 and 2, respectively. Although both polyethylene and polypropylene are simple polyolefins, they can be readily identified and distinguished by both FTIR and Raman instruments, which are commonly used techniques throughout the polymer and plastics industries. The other polymers listed in Table 1 are also identifiable by their IR and Raman spectra.

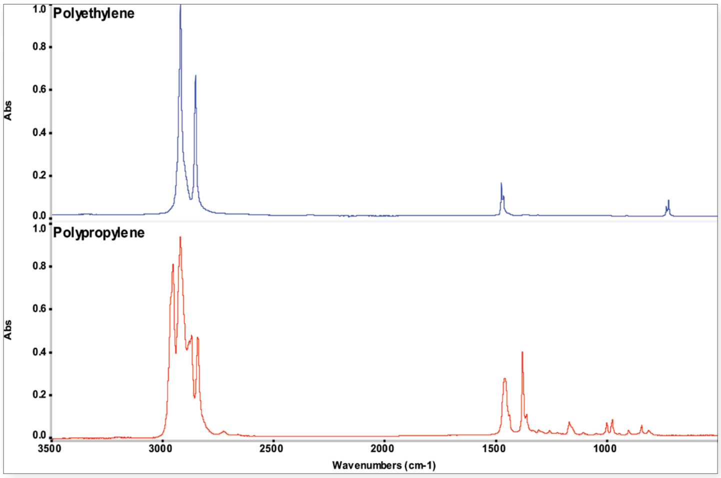


Figure 1: IR spectra of Polyethylene and Polypropylene.

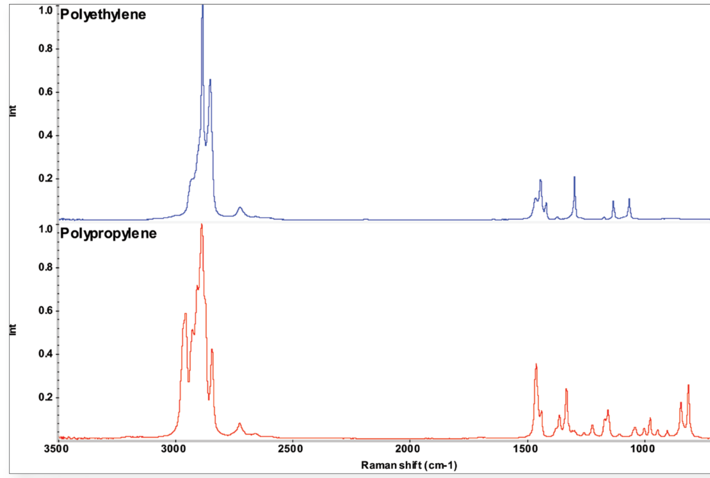


Figure 2: Raman spectra of polyethylene and polypropylene.

Microplastics – spanning a range of sizes

To be classified as a microplastic, the piece of plastic in question has to be small. How small? The National Oceanic and Atmospheric Administration (NOAA) defines a microplastic as being less than 5 mm long. Many particles of concern are smaller than this, typically between 100 µm and 1 µm. This is quite a range of sizes; from objects that are easily visible to the naked eye to small particles or fibers that can only be observed with a high-quality microscope.

Some microplastics are deliberately engineered to be small. These are termed “primary” microplastics. Primary microplastics are a target for legislative control. An example is the US ban on microbeads in personal care products enacted under the Microbead-Free Waters Act of 2015 (H.R.1321). Other microplastics start off as larger items that get broken down to smaller particles in the environment. These are designated as secondary microplastics. Both primary and secondary microplastics, spanning the range of particle sizes, are of concern in the environment due to their potential impact on marine life.

Concerns around the threat posed by microplastics to the health of organisms throughout the food chain include ingestion by marine organisms, e.g., zooplankton,[3] the presence of toxic materials used in the manufacture of the plastics, for example bisphenol-A, (BPA)[4] and the transport of persistent organic pollutants (POPs) by the microplastic particles.[5] While both FTIR and Raman can identify a long list of plastic materials, a number of instrument choices come into play when addressing the range of particle sizes. As the size of the particle decreases, the sophistication and cost of the equipment needed for its analysis increases. Therefore, the first consideration should be given to the size of microplastics to be studied when selecting the appropriate analytical platform. These considerations and more will be discussed throughout this paper, providing an overview and general guidance on spectroscopic instruments for microplastics analysis.

Analysis of particles from 5 mm to 100 microns

Particles in the size range of 5 mm to 100 microns are visible to the eye and can, with a steady hand, be manipulated with tweezers. As these are easy to see and handle, the spectroscopic system required for their analysis is relatively simple. By far the most common spectroscopic technique for the analysis of polymers is an FTIR spectrometer coupled with an Attenuated Total Reflection (ATR) accessory. The ATR allows the IR spectrum of a material to be obtained simply by pressing the sample against a transparent crystal, commonly diamond. The infrared light passes through the crystal into the sample where energy is absorbed by the sample, and the light is reflected back into the crystal to generate a spectrum. A Thermo Scientific™ Nicolet™ Summit FTIR Spectrometer equipped with a Thermo Scientific™ iD7 Diamond ATR is shown in Figure 3. The diamond is a couple of mm in diameter and does not need to be

Figure 3: Nicolet Summit FTIR Spectrometer with the iD7 ATR accessory in the sample compartment.

completely covered by the sample, making it ideal for the analysis of samples in this size range. One caveat with an ATR measurement is that it will detect materials that are on the surface of the sample. This is advantageous if a surface coating, such as an absorbed toxin, is of interest. However, if a sample has been weathered (irregular surface), this may interfere with its identification. If this occurs, the surface should be removed prior to analysis by slicing or polishing.

The ATR accessory pictured in Figure 3 does not allow the sample to be viewed after it has been sandwiched between the ATR arm and the diamond crystal. This presents no issue when dealing with samples in the 5 mm to 1 mm range. However, for samples smaller than this, it is preferable to be able to see the sample throughout its placement on the accessory and subsequent measurement. There are ATR accessories available that can provide viewing and magnification, facilitating analysis of samples in the 1 mm to 70 micron range. An example of such an accessory is the Czitek SurveyIR® Microspectroscopy Accessory as pictured in Figure 4.

Figure 4. Nicolet Summit FTIR Spectrometer with the SurveyIR® Accessory in the sample compartment.

An FTIR spectrometer with an ATR accessory is simple to use and relatively inexpensive. Further, the small form factor of the Nicolet Summit Spectrometer allows it to be moved close to where microplastics are to be collected and studied. This can be an advantage in environmental studies done outside the laboratory.

Analysis of particles from 100 microns to 1 micron

Once the particle size falls much below 100 microns, some magnification is required. There are two options here; IR microscopy and Raman microscopy (both techniques are also referred to as microspectroscopy). For particles less than 10 microns in size, Raman microscopy is the preferred choice.

Infrared Microscopy

Infrared (IR) microscopy enables the identification of particles down to 10 microns or less. There are several options available for IR microscopy in terms of both the **sampling technique** used and the **degree of automation** desired for the analysis.

Sampling techniques used in infrared microscopy include transmission, reflection and ATR. Transmission generally results in the best quality spectra, but often requires the sample to be pressed or otherwise processed to be less than 100 microns thick in order to allow the infrared light to pass through the sample. Reflection is, in principle, the easiest technique, as it requires no sample preparation or interaction between the microscope and the sample. However, it can result in distorted spectra, which may complicate the identification of polymer components. An ATR sample measurement works as described in the previous section. A downside of using ATR with microscopy is the potential of cross contamination between consecutive measurements because the ATR element comes into contact with the sample. This is not an issue with manual ATR systems where the crystal is easily cleaned between measurements. However, in automated microscope systems, in which the ATR comes into repeated contact with the sample without being cleaned between measurements, sample carry-over can present a problem. The choice of sampling technique is, therefore, largely dependent upon the nature of the sample.

The **degree of automation** available on an infrared microscope runs from simple point-and-shoot analysis of a single spot to fully-automated imaging covering a larger area of the sample and measuring multiple particles.

Figure 5 shows the Thermo Scientific™ Nicolet™ iN5 Infrared Microscope attached to the Nicolet iS20 FTIR Spectrometer. This is a point-and-shoot IR microscope designed for simplicity of operation. Example spectra

Figure 5. Nicolet iN5 IR Microscope attached to the Nicolet iS20 FTIR Spectrometer.

of microbeads, a primary microplastic in consumer products, were obtained using this IR microscope system shown in Figure 6.

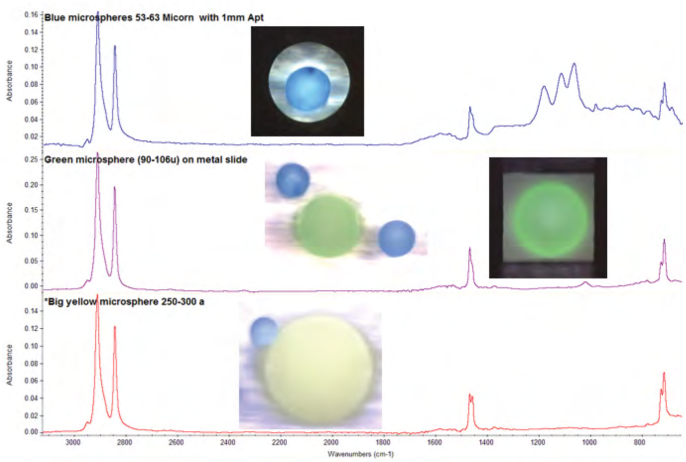


Figure 6. Spectra of microbeads obtained on a Nicolet iN5 IR Microscope. All spectra match polyethylene. The spectrum of the blue sphere also indicates the presence of barium sulfate.

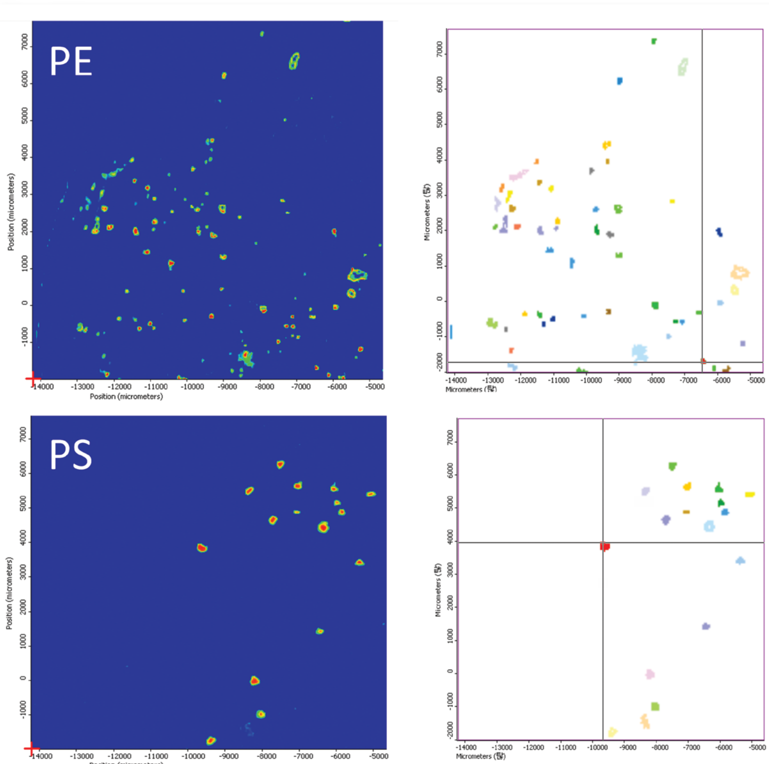
Point-and-shoot or single-point analysis is ideal for situations in which only a small number of particles are to be located and analyzed. The cost of this system is relatively low and, due to the few operator controls, it is easy to learn and use. However, if large numbers of particles are to be analyzed, some level of automation is desirable.

Filtration is frequently used as a final step in the isolation of microplastics from their matrix. Many particles may be captured on a filter surface where analyzing these filtered particles one by one is a laborious process. Thus, some level of automation is desirable when analyzing particles across the filter surface. Data collection and analysis can be automated through the use of a microscope equipped with a motorized stage and associated software. The Thermo Scientific™ Nicolet™ iN10 MX FTIR Imaging Microscope, pictured in Figure 7, provides this useful level of automation.

Figure 7: Nicolet iN10 MX FTIR Imaging Microscope with ATR accessories.

There are two main approaches to collecting data from particles distributed across a filter. The first is discrete particle analysis. In this approach, image analysis is performed on the video image of the filter to locate the positions of the particles. The system then automatically collects IR spectra from each location and then identifies each particle from its spectrum.

The second approach is imaging. In this case, an infrared image is collected from the entire region of interest, in which every pixel contains an infrared spectrum. This provides a chemical 'picture' of the filter. Automated analysis of this image by software can produce information about the identity, number and sizes of the individual particles. Such an analysis is shown in Figure 8. Here, two types of particles are identified from their IR spectra. Image analysis provides information about the number and dimensions of these particles. While, in principle, this is an elegant solution, there are some drawbacks compared to discrete particle analysis. The first is that the image may contain



Feature Sizing Results

Feature #	Area (µm²)	Length (µm)	Width (µm)	Aspect
1	29998	302.40	150.00	2.0
2	14999	75.00	100.00	0.8
3	27499	149.88	150.00	1.0
4	39998	187.18	150.00	1.2
5	19999	92.68	150.00	0.6
6	17499	92.68	100.00	0.9
7	182491	1055.18	399.99	2.6
8	22499	100.00	150.00	0.7
9	17499	85.35	150.00	0.6
10	14999	75.00	100.00	0.8
11	19999	278.07	150.00	1.9
12	14999	75.00	100.00	0.8
13	14999	75.00	100.00	0.8
14	17499	85.35	150.00	0.6
15	67497	331.50	299.99	1.1
16	19999	92.68	100.00	0.9
17	14999	75.00	100.00	0.8
18	17499	85.35	150.00	0.6
19	22499	108.13	100.00	1.1
20	19999	100.00	100.00	1.0
21	19999	110.35	150.00	0.7
22	37498	142.67	199.99	0.7
23	24999	110.35	150.00	0.7
24	59997	195.71	249.99	0.8
25	137493	495.07	449.99	1.1
26	34998	160.35	199.99	0.8
27	27499	150.00	150.00	1.0
28	27499	160.35	150.00	1.1
29	29998	133.93	199.99	0.7
30	29998	145.71	150.00	1.0
31	37498	153.03	199.99	0.8

Feature Sizing Results

Feature #	Area (µm²)	Length (µm)	Width (µm)	Aspect
1	57497	203.83	249.99	0.8
2	79996	520.11	299.99	1.7
3	59997	192.67	249.99	0.8
4	64997	211.16	249.99	0.8
5	39998	150.80	150.00	1.0
6	47498	175.69	249.99	0.7
7	64997	213.10	249.99	0.9
8	94995	263.38	349.99	0.8
9	59997	198.74	249.99	0.8
10	19999	100.00	100.00	1.0
11	39998	153.03	150.00	1.0
12	32498	142.56	150.00	1.0
13	42498	167.67	150.00	1.1
14	52497	178.03	199.99	0.9
15	47498	168.48	199.99	0.8
16	44998	195.71	249.99	0.8
17	49997	178.03	249.99	0.7
Total image area	94082712			
Total feature area	899955			
Feature area percentage	0.96%			

Figure 8. Chemical images of a filter (left) obtained on a Nicolet iN10 MX FTIR Imaging Microscope showing polyethylene (PE) and polystyrene (PS) particles, together with particle size statistics derived from the chemical images.

a substantial amount of redundant data. There may be only a small percentage of the image data set that contains information about the particles, the rest being the filter. As the image contains at least four dimensions of data (x-positon, y-position, spectral wavelength and absorption) the size of the 'data cube' containing many thousands of spectra can be excessive. The second factor is that the array detectors used for imaging are more expensive than the simple, single-point detectors used for discrete particle analysis. The approach chosen is situation dependent on 1.) how many particles are to be analyzed 2.) in what time period and 3.) over how large an area. Fortunately, the Nicolet iN10 MX IR Microscope provides options for all modes of sampling and automation.

Raman microscopy

Both Raman and FTIR spectroscopy are capable of identifying microplastics. However, Raman spectroscopy does have three distinct advantages when applied to microscopy. The first is that Raman spectroscopy uses sub-micron wavelength lasers as its light source and, as such, is capable of resolving particles down to 1 micron and less. FTIR microscopy uses mid-infrared light as its source, resulting in a wavelength range that loses the ability to identify particles much below 10 microns. The second is that, unlike IR systems, Raman microscopes are built around research-grade, white-light microscopes, which facilitate easy viewing of the particles. The third is the ease of sampling. There is no need to choose between transmission, reflection and ATR sampling techniques required for FTIR measurements. The Raman system laser is focused on the sample, and the spectrum is simply acquired by collecting the scattered light.

So, why is Raman microscopy not always the best choice for microplastics analysis? To balance the advantages, there are three key disadvantages to Raman microscopy. The first is the body of knowledge. FTIR spectroscopy has been around longer than Raman spectroscopy as a common analytical technique, and it evolved as the polymer industry grew. As such, there is a greater amount of historical data around IR spectroscopy than Raman spectroscopy for the analysis of polymers. However, this gap is decreasing as time goes on, and there are certainly enough reference spectra available to enable the identification of common microplastics. The second is cost. Typically, due to component costs, research-grade Raman systems are more expensive than their IR equivalents. The third drawback is fluorescence. Some samples exhibit fluorescence when irradiated by a laser. Fluorescence can obliterate the useful analytical Raman signal. This may be mitigated by the selection of appropriate laser wavelengths, but sample fluorescence is an issue in Raman microscopy that is not encountered in FTIR microscopy. With all this said, Raman microscopy is still the technique of choice for particles less than 10 microns in size due to the wavelength of the probing radiation.

As discussed above, sampling options for Raman microscopy are generally trivial. Unlike FTIR microscopy, where the quality of the spectrum is critically dependent upon the sampling technique, Raman microscopy simply measures the laser light scattered from the sample with no special sampling required. The key considerations for Raman microscopy are around the choice of laser, which affects signal strength and sample fluorescence.

As with IR microscopy, Raman microscopy offers a choice of automation options, from simple point-and-shoot for discrete particle analysis to high-speed imaging. The Thermo Scientific™ DXR3 Raman Microscope shown in Figure 9 is a fully automated Raman microscopy system. Just as with IR microscopy, the cost, complexity and sophistication of data analysis increases with the degree of automation required. The analysis of microplastics on a filter by Raman is shown in Figure 10.

Figure 9: DXR3 Raman Microscope for analysis of Microplastics.

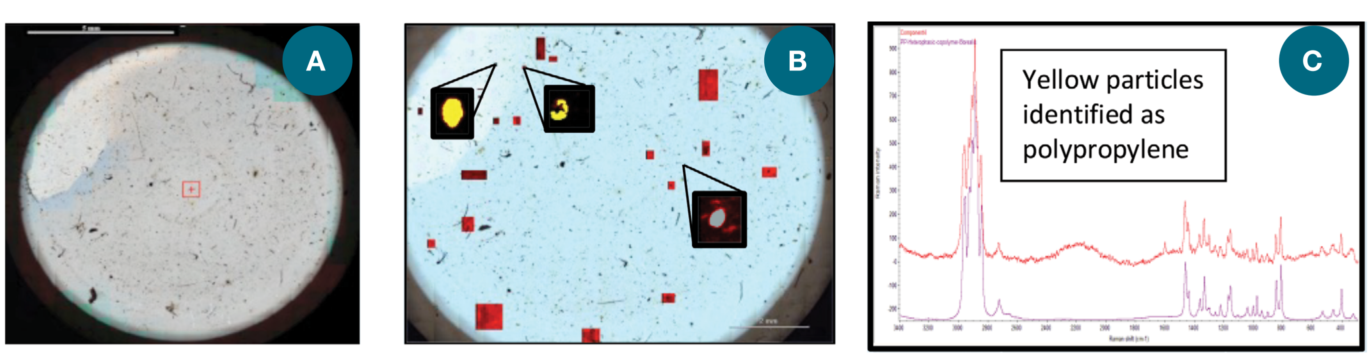


Figure 10: An example of microplastics analysis using the DXR3xi Raman Imaging Microscope. (A) Video image of the alumina filter with microplastic particles; (B) Chemical image of the filter with microplastic particles; and (C) Spectrum of one of the yellow particles compared to the library spectrum of polypropylene.

Conclusions

FTIR and Raman spectroscopy are powerful analytical tools to identify microplastics in the environment and bottled water. Many solutions are available, from simple point-and-shoot devices to sophisticated imaging systems. The choice of system depends upon the size of particles under investigation, where the analysis is to be performed, and the degree to which automation is required. This information is summarized in the instrument selection guide shown in Table 2.

	Microplastic size	FTIR + ATR	FTIR + Small Spot ATR	Point-and-Shoot FTIR Microscope	FTIR Imaging Microscope	Raman Microscope
Configuration		 Nicolet Summit FTIR Spectrometer and iD7 ATR Accessory	 Nicolet Summit FTIR Spectrometer and SurveyIR Accessory	 Nicolet iS20 FTIR Spectrometer and Nicolet iN5 IR Microscope	 Nicolet iN10 MX IR Imaging Microscope	 DXR3 Raman Microscope
Measureable Particle Size	5 mm	↑				
	1 mm	↕	↑			
	500 µm	↓	↕			
	100 µm		↓	↑	↑	
	10 µm			↓	↓	↑
	1 µm					↓
Manual Sample Placement Only		Yes	Yes	Yes	No	No
Automated Analysis of Filters		No	No	No	Yes	Yes
Immunity to Sample Fluorescence		Yes	Yes	Yes	Yes	No
Relative Cost		$	$$	$$$	$$$$	$$$$$

Table 2: Analytical instruments for microplastics analysis.

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