Raman Applications on Microplastics

Eunah LEE
Bridget O’DONNELL
Andrew WHITLEY

Microplastics (MPs) is an emerging and rapidly growing research field, the potential for which the scientific impact and business opportunities are abundant. HORIBA is working with several key opinion leaders (KOLs) help develop and drive the critical thinking to establish analytical standards and regulation guidelines. The availability of harmonized methods and key instrumental and software capabilities will determine how well we can monitor and control MP pollution and health risks in the future. This paper will summarize ongoing collaboration projects with KOLs being performed by the HORIBA US Raman team, and what we’ve learned from this so far.

Introduction

It has been reported that less than 10% of manufactured plastics are recycled. With approx. 30% in use, this leaves 60% of plastics to be discarded into environment, or being incinerated. Discarded plastics take tens (e.g. plastic bags and foam cups) to hundreds (e.g. plastic bottles and disposable diapers) of years to decompose, accumulating on land, including in landfill sites, coastlines, in Arctic sea ice, and on the sea surface and floor.[1] During these long years, plastics fragment into small particulates, forming microplastics (MPs) and nanoplastics (NPs). They are not only hazardous for wildlife, but also pose health risks for humans[2] through air pollution (e.g. nanotoxicity due to enhaling NPs), water (e.g. MPs in drinking water) and food sources.

Governing bodies around the world have recognized the detrimental impact of MPs, and started implementing new regulations. Scientific guidelines are critical for effective regulation, and key opinion leaders (KOLs) are actively working to develop standard methods for sample collection, preparation, analysis, and evaluation. HORIBA has been working with several KOLs to understand analytical requirements for MPs, including software and hardware optimization and automation. These efforts have been successful so far, resulting in various publications and presentations[3]. There is however still a long way to go.

It has been demonstrated[4] that using multiple technologies, including Raman microscopy, is essential to identifying MPs without ambiguity. Raman microscopy is growing in its importance due to its high spatial resolution (necessary for particles on the order of ~ 10 µm and smaller), high tolerance toward wet samples (advantageous for field analysis), sensitivity to additives (useful for brand and source identification) and polymers (useful for chemical identification), and specificity for polymer type (for positive identification) and minerals (for exclusive identification). HORIBA Scientific’s XploRA PLUS confocal Raman microscope is well equipped to handle MP analysis, and has been installed at multiple KOL laboratories, successfully proving its performance and usefulness. As

Figure 1 HORIBA Raman instruments. (a) XploRA PLUS, (b) MacroRAM and (c) XploRA Nano

research progresses, the demand for Raman analysis (and instruments) will expand to field analysis instruments (e.g. compact Raman spectrometer such as HORIBA's MacroRAM coupled with optical fiber probes), NPs analysis (e.g. AFM-Raman hybrid system) aided by nano-tags (e.g. nanoGPS), and multi-modal imaging and microscopy (e.g. epi-fluorescence and hyperspectral imaging microscopy). HORIBA is well placed to explore the business potential of MPs with its versatile collection of products.

Importance of collaboration

MP analysis is just emerging and rapidly growing as a research field; exponentially increasing in volume and importance as an application area and at critical timing as a business opportunity. Analytical requirements are not well determined, yet. Standards and regulations are yet to be defined. It is critical to work with KOLs to accumulate and exchange knowledge, learn requirements specific for MP analysis, establish Raman as a standard analysis technology for MPs, and establish and brand HORIBA as a go-to vendor for Raman analysis for MPs.

Collaboration with Prof. Chelsea Rochman, University of Toronto[5]

Professor Rochman is a faculty member in the Department of Ecology & Evolutionary Biology at the University of Toronto. The ultimate goal of this collaboration is to develop an automated technique for handling small-size MPs (< 100 µm).

Automating MP analysis must be proceeded by establishing standard operating procedures (SOPs). SOPs are critical in MP analysis because MPs migrate over a long distance (e.g. from river to ocean to shore), and require collective characterization by multiple research groups. Unless every research group follows the same SOP, it will be impossible to combine/compare data, follow the plastics lifecycle as they migrate through the environment, or establish effective guidelines to minimize MPs in the environment.

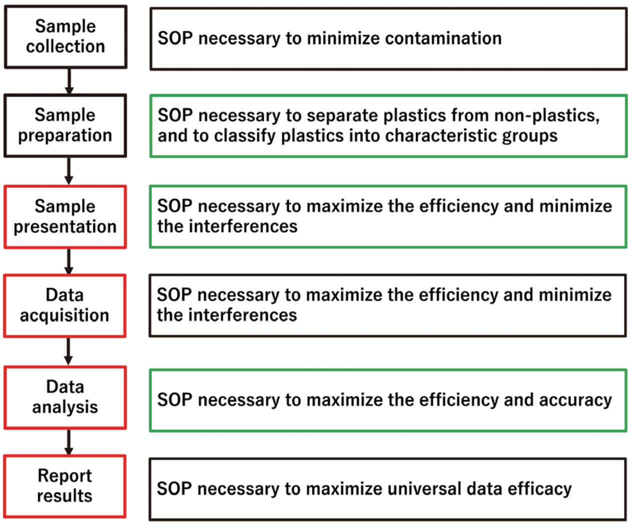

Figure 2 A flowchart of MP analysis. Items highlighted in red mark processes of high demand for automation. Items in green mark Rochman group research focuses at this time.

Dr. Rochman's group has developed SOPs for sample preparation that include:

0. MPs are collected from various matrices (water, sediment, biota)
1. Acid, base, or enzymatic digestion is carried out to remove organic material
2. MPs are size fractioned using sieves down to 100 µm in size
3. Remaining small MPs are filtered into size groups down to 1 µm

When collected from the environment, MP samples contain inorganic materials (e.g. sand), organic materials (e.g. biofilm), and non-synthetic materials (e.g. natural fibers) along with MPs. SOPs developed by Dr. Rochman's group separate MPs from non-plastic materials (step 1 of SOP), and divide MPs into size groups (steps 2 and 3 of SOP).
Step 2 divides MPs into multiple size categories using sieves, the smallest size category being 100 µm in diameter. Particles smaller than 100 µm are divided into multiple size categories using filters in step 3, the smallest size threshold being 1 µm in diameter. It is not an accident that size thresholds are set at 100 µm and 1 µm. 100 µm represents the smallest size a researcher with a reasonable training can handle manually (e.g. picking up with tweezers). In other words, step 2, with the smallest sieve, separates MPs that can be manually sorted from those too small for that, and designates them for further sample preparation in step 3. 1 µm represents the smallest size an optical microscope can detect, resolve, count, and measure. In other words, step 3, with the finest filter, separates MPs that can be analyzed with an optical microscope i.e. 1 µm or greater, from those too small for that, i.e. < 1 µm, and designates those < 1 µm for nanomaterial analysis by, for example, an atomic force microscope (AFM).

Dr. Rochman's group recognized the advantage of minimizing the number of sample transfers, and utilized filters as a substrate to present the sample to Raman microscopes. This opened the door to further research to optimize the filter material for step 3. Currently multiple filters, commercial and custom developed in the lab, are tested for their efficiency in terms of interference, cost, mass manufacturing, etc.

As the Rochman group's SOPs are being finessed for practicality, the volume of samples they analyze increases, and so do their data accumulation challenges. They have started compiling accumulated data into Raman spectral libraries dedicated for MPs research called SLoPP (spectral library of plastic particles) and SLoPP-E (spectral library of plastic particles aged in environment)[6]. SLoPP is made of pure and pristine MPs spectra, and SLoPP-E spectra of weathered MPs. These libraries are being continuously expanded.

Collaboration with Dr. Ashok Deshpande, NOAA and Dr. Jennifer Lynch, NIST

Raman is a relatively new technology for MP analysis, gaining recognition in recent years. It is important to evaluate a new technology with respect to existing technologies. For MP analysis, the two methods used are mainly pyrolysis gas chromatography-mass spectrometer (pyro-GC-MS) and Fourier transform infrared (FT-IR) spectroscopy. MP samples from Hawaii were analyzed by three technologies for comparison, each at a different laboratory using a different technology. The results proved not the superiority of a single technology but the importance of multi-technology analysis for accurate identification.

Dr. Jennifer Lynch of the Biochemical and Exposure Science Group of National Institute of Standards and Technology (NIST)[8] is a Research Biologist at and co-director of Center for Marine Debris Research (Hawaii Pacific University). She provided samples in this collaboration, and her student Kayla Brignac performed the analysis using FT-IR.

Dr. Ashok Deshpande of National Oceanic and Atmospheric Administration (NOAA)[9] is a Research Chemist at Northeast Fisheries Science Center (James J. Howard Marine Sciences Laboratory at Sandy Hook). He performed the analysis with pyro-GC-MS.

Dr. Bridget O'Donnell of HORIBA is Manager of Raman Applications at HORIBA Scientific (Piscataway, New Jersey). She coordinated the collaboration, and performed analysis using Raman.

- Identification was 'perfect' for approximately 70% of samples, all three technologies making the same identification, and boosting the confidence in the results by cross-validating each other.
- Identification was 'good' for approximately 10%

Figure 3 Photo of Hawaiian marine debris items, Windward Oahu beach. Kahuku Transect 1[7]

Table 1 Comparison of pyro-GC-MS, FT-IR and Raman

	Raman	ATR-FT-IR	Pyro-GC-MS
Technology	Scattering technique	Absorption technique	Chromatographic technique
Sample	Little to no sample preparation	Sample mounted on diamond crystal and compressed	Sample is completely pyrolyzed (destroyed)
Advantages	Sensitive to molecular vibration based on change in polarizability Sensitive to polymer backbone structure Spatial resolution < 1 μm	Sensitive to molecular vibration based on change in dipole moment Sensitive to polymer side chains Extensive databases available	Sensitive to molecular structure based on breakdown into fragments Quantitative results
Disadvantages	Susceptible to background fluorescence	Susceptible to water absorption	Long measurement time Large sample volume
Analysis by	Bridget O'Donnell, HORIBA	Jennifer Lynch, NIST	Ashok Deshpande, NOAA

of samples, two technologies making the same identification. The third technology identifies these as a variety of or similar structure to the accurate identification.

- Identification was 'poor' for approximately 10% of samples, each technology making different identification.
- The remaining 10% of samples were identified by only one technology, the other two technologies failing to yield usable data.

Given the extreme heterogeneity of plastic products with diversity in manufacturing process, this is not surprising. It would be possible to improve these results with further analysis or additional analytical technologies, and calls for adding even more technologies for the MPs' analytical tool box.

Collaboration with Southern California Coastal Water Research Project (SCCWRP)

Southern California Coastal Water Research Project (SCCWRP)[10] is a public Research and Development agency that is leading the project to establish the guideline for MP analysis, which will become the standard method for California State Government regulations. Dr. Steve Weisberg (Executive Director) and Dr. Charles Wong (Department Head, Chemistry Department) initiated and organized a study plan to evaluate MP measurement methods. This is the first step in establishing standard measurement methods that every MP research group will share and follow. In this study plan, they invited researchers around the world, including HORIBA, to participate in drafting measurement methods. SCCWRP has completed drafting a proposal for the method, and is in the process of producing and distributing standard samples in clean water, dirty water, sediment, and biological tissue. Each of the participating laboratories will analyze standard samples with the proposed method, evaluate its practicality, and help finesse improvements. HORIBA contributes expertise on Raman spectroscopy by proposing an augmentation to the study plan, and will evaluate methods for automating MP analysis and for potential field analysis using optical probes.

In preparation for and as a part of the study plan, SCCWRP organized three events in 2019: a workshop and round table discussion in April, sample extraction and preparation training in October, and sample analysis training in November. HORIBA participated in all three events, playing a key role in the sample analysis training.

Many environmental researchers spend limited time in a chemical laboratory, and thus require training operating analytical instruments. The training session at SCCWRP for sample analysis in November 2019 was dedicated to Raman microscopy (provided by HORIBA Scientific) and FT-IR microscopy (provided by Thermo-Fisher). Researchers from key regulatory (e.g. Environmental Protection Agency) and academic (e.g. California State Universities) institutes were present, and trained on Raman using HORIBA Scientific's XploRA PLUS. Researchers will travel back to SCCWRP to perform Raman analysis as part of the study plan, when standard samples are distributed, using the XploRA PLUS.

Instrument requirements of Raman analysis for MP analysis

One of the biggest complexities of MPs (and NPs) analysis is the extremely wide size distribution, ranging from 5 mm to 1 µm (and smaller). As mentioned above, one of the first steps of sample preparation SOPs are classifying MPs into size groups.

MacroRAM with BallProbe[11] (by MarqMetrix) would be suitable to analyze large MPs (in the order of millimeters), especially for field screening. Handheld Raman may be an intuitive candidate for field screening, but is typically designed for bulk analysis. Its measurement spot may be too large for MPs. The focus point of BallProbe is small (in the order of 200-400 µm), making it a good match for large MPs in size. The MacroRAM is small, light and rugged, and suitable to transport between field basecamps. There are no moving parts, so installation is minimal and alignment is not required: plug it in, and turn it on.

One of the important aspects when selecting a field unit is its compatibility to a lab unit. MPs are sent back to a lab for further analysis if their analysis results are unsatisfactory for various reasons (e.g. too small even for the BallProbe, inconclusive identification, possibility of toxicity, etc.). It simplifies data comparison when the field unit and the lab unit use the same software. HORIBA's benchtop MacroRAM, just like all Raman instruments from HORIBA, uses the LabSpec 6 Spectroscopy Suite (LS6) software, making it easy and simple to compare data to those from a lab unit such as the XploRA PLUS.

XploRA PLUS confocal Raman microscope is well equipped to handle small MP analysis (typically smaller than 1 mm down to less than 1 µm) with a fully featured optical microscope, multiple lasers, confocal spectrometer, motorized stage, and advanced software.

The first step of any analysis is to observe MPs to gauge its color, size, shape and texture. For small MPs (typically smaller than 1 mm), the observation requires an optical microscope. Optical microscopy technologies are dedicated to improve the contrast and the image authenticity. It may sound simple, but it is not trivial to improve contrast or image authenticity. To improve the contrast, one must improve the signal-to-noise ratio (SNR), achieve the best possible focus, and be able to differentiate subtle differences in optical properties (e.g. color, refractive index, birefringence, etc.). To improve image authenticity, one must minimize interference such as 'shadows' from non-uniform illumination, artifacts and distortions from optics themselves, and aberrations from polychromatic light. HORIBA's XploRA PLUS is compatible with all optical microscopy technologies such as darkfield (DF) imaging, polarized light microscopy (PLM), epi-fluorescence imaging, and hyperspectral imaging (HSI) microscopy, allowing the researcher to observe, analyze and classify MPs by their size, color, shape and texture. It is imperative to note that there is absolutely no sample transfer to utilize any or all of these multimodal imaging techniques, which improves the practicality, speed and reliability of correlated analysis immensely.

Chemical composition of synthetic plastics are complex with multiple polymers and additives[12] such as stabilizers, flame retardants, pigments, reinforcements, etc. The complexity increases as MPs undergo weathering in environments, which changes the physical and chemical characteristics. It has been reported[13] that the impact of weathering on MPs is significant, and that the accuracy improves a great deal when spectra from weathered MPs, as well as those from pristine plastics, are used in identification methods. Optimum experiment conditions, naturally, vary depending on MPs' characteristics, the first of which is selecting the 'right' laser to suppress any fluorescence baseline. The XploRA PLUS can house three lasers from blue to near-infrared (NIR), and switch between them easily with a single click, making laser selection a quick and intuitive operation.

One of the most neglected steps in MP analysis is sample presentation. It is necessary to place MPs on some kind of substrate to present it to a Raman microscope. The substrate may be a petri dish, a glass slide or a filter on a filter holder. It is possible to get interference from substrates. For example, the substrate may be Raman active, and its spectral features mix with MP spectra. Confocality of the spectrometer suppresses signals from substrates by suppressing out of focus signal. The XploRA PLUS employs a true confocal optical design for the Raman scattering beam path, making it ideal to acquire 'clean' Raman spectra by minimizing non-sample signals.

As Dr. Andrew Whitley's article in this issue of Readout mentioned, assessing MPs in environments for its quantity, migration and hazard requires statistical analysis of a massive amount MPs. Automated high throughput analysis is absolutely necessary, and a critical research goal. While research groups, with collaboration with HORIBA, are developing SOPs to become the template of automated analysis, a few elements are already identified as necessary.

MPs are often presented for analysis arrayed in a petri dish or retained on a filter. The size of a petri dish or a filter is much bigger than MPs, and navigation requires the stage travel a long distance while stopping at precise positions. A motorized stage with precision control makes navigation easier and more precise, making the experiment more efficient.

Another advantage of a motorized stage is software control and programmed movements. Coupling this ability to the microscopy functionalities of the XploRA PLUS, HORIBA developed an application module called ParticleFinder (PF)[14] on the LS6 platform. PF acquires a microgram, and analyzes the counts, sizes, shapes, and locations of MPs. PF then moves the stage to each of the MP's location, and acquires a Raman spectrum. The final results include total counts of all MPs, statistics of sizes and shapes, location and Raman spectrum of each MP, and spectral classifications.

As mentioned repeatedly, MPs are highly complex and heterogeneity targets for analysis. Data processing and analysis are proportionally complex[15] requiring sophisticated software. LS6 offers extensive functionalities for data processing (e.g. baseline correction, smoothing, substrate spectrum subtraction, etc.), data analysis (e.g. multivariate analysis, library search, etc.) and visualization (e.g. image rendering) both developed in house and working with business collaborators: Bio-Rad Laboratories, Inc. to incorporate KnowItAll® library search, and Eigenvector Research Inc. for multivariate analysis (MVA).

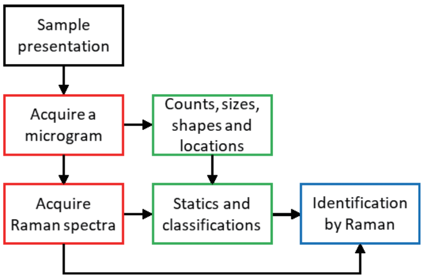

Figure 4 Workflow diagram of PF operation followed by identification by Raman library search. Red boxes highlights data acquisition steps in PF, green results generated in PF, and blue results coupled with library search

Table 2 Summary of HORIBA technologies useful for MPs analysis

Raman spectroscopy	Fluorescence spectroscopy	Particle size analysis	EDXRF† spectroscopy
Chemical identification & differentiation of: • Polymers • Additives • Dyes/Pigments • Natural particles	• Probe effect of MPs on DOM* • Determine concentration of NPs • Quantify MPs for toxicity studies • Measure DOM in control studies	• Measure particle size distribution of possible MPs • Viewsizer - Potential for nanoparticle tracking analysis for NP's	• Analyze metals accumulated on MPs[16]

* DOM stands for dissolved organic matter

† EDXRF stands for energy dispersive x-ray fluorescence

HORIBA technologies for MPs analysis

The complexity and heterogeneity of MP analysis requires, as demonstrated in the collaboration work with NOAA and NIST above, multimodal imaging and multi-technology approaches. It has, therefore, become a natural focus of interest how to combine, correlate and compare data and results from multiple sources. For now, we are still tackling data from one technology at a time. However, we are preparing for next steps, investigating future development possibilities such as data fusion, correlated microscopy and machine learning.

Conclusion

Microplastic (MP) is a mega trend that is growing even bigger presently. HORIBA is collaborating with KOLs of the field to determine (and influence) the market demand (and trend), and staying relevant to this newly emerging field. We have made an impression as 'the' Raman company for researchers who will provide guidelines to MPs regulations. The scientific and business opportunities are abundant, and we are making good progress.

* Editorial note: This content is based on HORIBA's investigation at the year of issue unless otherwise stated.

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Eunah LEE, Ph.D.

Raman Projects Manager
HORIBA Instruments Incorporated (HII)

Bridget O'DONNELL, Ph.D.

Manager of Raman Applications
HORIBA Instruments Incorporated (HII)

Andrew WHITLEY, Ph.D.

Vice President of Sales and Business Development
Global Director of Business Development
HORIBA Instruments Incorporated (HII)