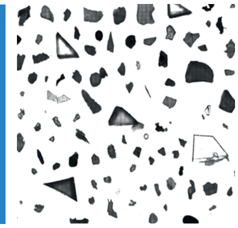
Raman Spectroscopy

Morphological and chemical characterizations of microplastic particles using ParticleFinder™ and Raman techniques



Application Note

Environment RA71

Laura Frère[1], Emmanuel Rinnert[2], Catalina David[3] and Sébastien Vergnole[3].
[1]Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539. CNRS/UBO/IRD/Ifremer, Institut Universitaire Européen de la Mer, F-29280 Plouzané, France / [2]Ifremer, Laboratoire Détection, Capteurs et Mesures, CS 10070, F-29280 Plouzané, France / [3]HORIBA FRANCE SAS, F-59650 Villeneuve Dascq, France.

Abstract : The assessment of microplastics in a marine environment is a multi-step process (sampling, extraction, detection and quantification of microplastics), in which each step is time consuming. Analyzing the chemical composition and morphology of microplastics represents a real challenge for answering crucial questions about the sources and fate of microplastics in aquatic environments. In this application note, we present a reproducible and time-effective method for fast and thorough morphological and chemical characterization of microplastics using a semi-automated scanning of particles coupled to micro-Raman spectroscopy. The rapid analysis of large number of collected particles allows for an exhaustive assessment of both large sample sizes and small subsamples.

Introduction

The scientific community is increasingly interested in environmental contamination by plastics. Recently, this interest in the contamination of aquatic systems by plastics has shifted to smaller particles, microplastics. Microplastics are plastic particles smaller than 5mm which can be directly made of microparticles, or are the result of the fragmentation of macroplastics. It has been shown that microplastics are the dominant size class among the debris of 92% of the 5.25 billion plastic particles that contaminate the ocean surface.

These plastic particles may contain potentially toxic additives to organisms (phthalates, bisphenol A, brominated flame retardants, nonylphenols, and antioxidants) which are incorporated into the polymers during their design in order to extend their life, giving them a better resistance to heat, oxidation and degradation mechanisms.

Given their harmful effects in the medium and long term, it is relevant to question and understand how they are fragmented, transported and dispersed in the marine environment. For this, it is essential to be able to characterize them morphologically and chemically.

The classical methodologies applied on large environmental samples are mainly based on visual sorting and characterization and usually they are not sufficient.

In this application note, we propose an efficient methodology for molecular identification and morphological description of microplastic particles. The advantage of this semi-automatic method is the coupling between a Raman micro-spectrometer and image processing software, ParticleFinder™, within the same analysis in order to obtain physical (morphological) and chemical (molecular composition) information from particles.

Several hundred particles may be analyzed while minimizing the intervention of the operator (suitable for potential contamination or loss of sample) as well as agent time.

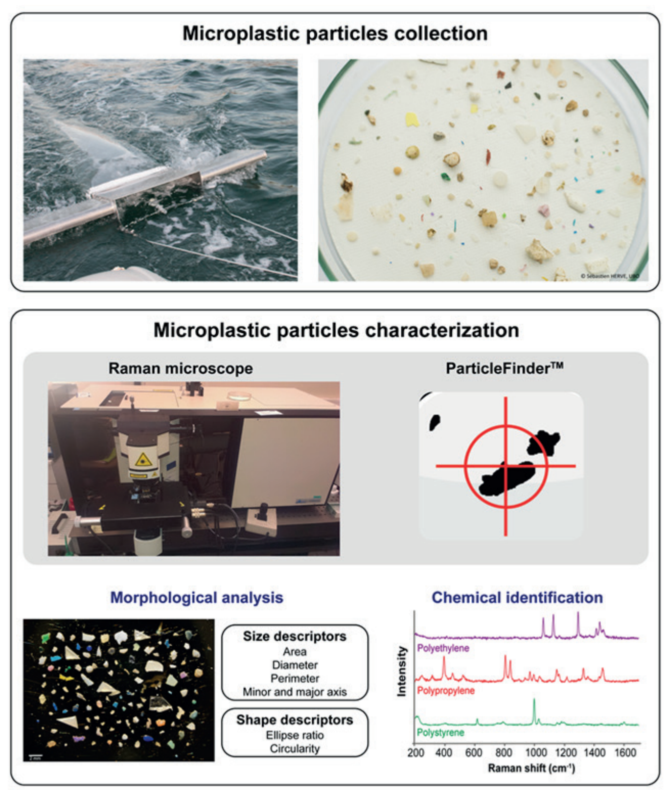


Figure 1. Typical workflow for the semi-automatic Raman micro-spectroscopy method for morphological and chemical characterizations of microplastic litter

Methodology

For the validation of the method, the study reveals the analysis of 103 particles randomly selected from floating micro-particles collected in the Bay of Brest (Brittany, France) in September 2014 using a Manta trawl. Particles of seven additive-free reference polymers were used as references and were positioned among 103 collected particles: polyethylene (PE), polypropylene (PP), polystyrene (PS), unplasticized polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyamide-6 (PA-6) and polyurethane (PUR).

Raman analysis

Localization, counting, 2D morphological characterization and Raman measurements of the 110 (103 + 7) particles were carried out using a LabRAM™ HR800 Raman micro-spectrometer equipped with ParticleFinder™ application for LabSpec6. The particles were placed on gold coated microscope slide, in order to avoid the Raman signals generated by borosilicate glass or "unclean" non-gold microscope slides. The image of a large area on the sample (25 x 21mm^2) was captured and then used in ParticleFinder™ for further analysis (Figure 2).

Statistics of key 2D morphological features (minor and major size, particle area, diameter, and perimeter) and 2D shape descriptors (ellipse ratio and circularity) were calculated by the software for each particle.

The next step consisted performing the automated Raman. The ParticleFinder™ software application allowed automatic motorized stage positioning and analysis of particles. One spectrum was collected on the center of each particle. Raman measurements were performed using 10-fold magnification objective (Olympus) and 785nm laser as the excitation line. The chemical identification of almost all particles was realized using commercial Raman libraries (KnowItAll™ Informatics Systems, Bio-Rad®, Raman ID Expert). The measurements were repeated 3 times in 3 different runs under the same conditions, except for particle spatial location.

Figure 2. a) Image of the montage

Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Brightness	Image	Raman
Use filtering														
Filter limits(min)			-11980.6	-8262.1	15648.9	141.2	489.3	155.6	105.9	0.12	0.37	24.8		
Filter limits(max)			12778.1	10842.1	4936294.5	2507.0	13123.1	3320.4	2028.0	1.00	0.95	212.3		
		29(88)	-7321.5	4509.8	2522183.5	1792.0	9326.5	2911.9	1333.1	0.46	0.60	50.3		
		30(20)	-7320.3	-5241.6	2288222.3	1706.9	7087.5	2411.3	1396.7	0.58	0.76	129.7		
		31(10)	-7111.5	-7630.6	405841.6	718.8	2850.6	907.5	603.7	0.67	0.79	53.1		
		32(78)	-6512.9	2581.2	696888.3	942.0	3415.0	1171.3	783.0	0.67	0.87	31.3		
		33(56)	-6237.0	-404.2	201745.3	506.8	2593.4	732.1	384.1	0.52	0.61	83.9		
		34(15)	-6234.0	-6306.5	353605.3	671.0	2857.9	952.2	498.7	0.52	0.74	40.5		
		35(38)	-6202.8	-3283.7	543265.0	831.7	3351.7	966.2	735.6	0.76	0.78	53.7		
		36(110)	-6056.4	7054.3	1078374.9	1171.8	5463.1	1819.6	892.5	0.49	0.67	41.8		
		37(65)	-5830.3	868.9	572028.0	853.4	4559.9	1437.5	562.0	0.39	0.59	57.7		
		38(28)	-5009.5	-5152.7	592121.8	868.3	3377.9	1141.0	682.0	0.60	0.81	47.9		
Mean			-703.5	203.2	592436.5	767.9	3223.7	1030.9	628.0	0.64	0.77	71.2		
Stdev			6881.0	5088.3	697645.1	405.7	1897.3	595.5	335.6	0.18	0.11	33.7		

Figure 2. b) Morphological analysis of microplastic particles of the 110 particles analyzed for method validation: Statistic of the 2D descriptors for each particle.

Once validated, the previously presented method was applied to a larger environmental sample (n=962 particles). Out of the 962 particles collected in surface water, 75% were chemically characterized. Microplastics (PE 48%, PP 12%, PS 11%) represented 71% of the whole sample (Figure 3). The 4% of identified particles remaining are quartz (2%) and carbonates (2%). The non-identified particles (25%) exhibited only PB15 dye spectrum (3%), spectra with no correspondence with databases (6%), or a signal that was either absent or saturated (16%).

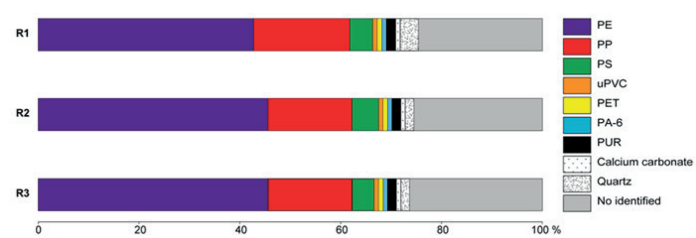


Figure 3. Proportion of each polymer family for 3 independent measurements

Morphological analysis was based on descriptors as minor and major axis, perimeter, diameter, area, ratio ellipse and circularity. Significant differences were observed in the distribution of quartz, PE, PP and PS particles (Figure 4).

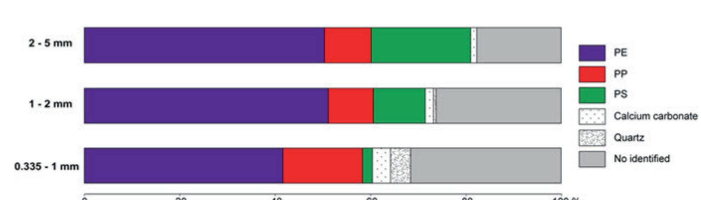


Figure 4. Proportion of particles identified, and not identified, in seawater samples (n=962 collected particles) depending on size class (0.333-1/ 1-2/ 2-5mm)

Conclusion

Every step of microplastic analysis (collection, extraction and characterization) is time consuming, representing an obstacle to the implementation of large scale monitoring. In this application note, we describe a semi-automated Raman micro-spectroscopy method, coupled to static image analysis, that allows the screening of a large quantity of microplastic in a time-effective way with minimal machine operator intervention. With this method, the complete analysis (Raman measurements and morphology descriptors) of a hundred particles takes around three hours for machine time and around 1 hour for machine operator intervention (without spectra identification).

The ParticleFinder™ module offers the possibility to accurately analyze the morphological features of all micro-particles (size, shape, density). Coupling it with powerful Raman capabilities for chemical identification, we provide a very efficient tool for environmental microplastic characterization.

Acknowledgements

HORIBA sincerely acknowledges and thanks Laura Frère and Emmanuel Rinnert for their kindness in sharing with us the results of their study. For detailed analysis and results of this study, please refer to their scientific publication:

Frère Laura, Paul-Pont Ika, Moreau, Jonathan, Soudant Philippe, Lambert Christophe, Huvet Arnaud, Rinnert Emmanuel, 2016. A semi-automated Raman micro-spectroscopy method for morphological and chemical characterizations of microplastic litter. Marine Pollution Bulletin. Volume 113, Issues 1–2, pages 461-468.

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LabRAM HR

info.sci@horiba.com www.horiba.com/scientific

USA: +1 732 494 8660
UK: +44 (0)1604 542 500
China: +86 (0)21 6289 6060
Other: +33 (0)1 69 74 72 00
France: +33 (0)1 69 74 72 00
Italy: +39 06 51 59 22 1
Taiwan: +886 3 5600606
Germany: +49 (0) 6251 8475 0
Japan: +81(75)313-8121
Brazil: +55 (0)11 2923 5400

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