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Raman

Microplastics analysis in food: assessing human exposure through Raman micro-spectroscopy

Application Note

Microplastics RA94

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Abstract: Sugar, a widely consumed dietary ingredient, is present in numerous food products, including desserts and beverages. Its production involves several stages—extraction, purification, crystallization, and drying—each presenting potential contamination risks from plastic-based equipment and packaging. In this work, we demonstrate the typical workflow for microplastics analysis using Raman microspectroscopy.

Keywords: Raman Microscopy, ParticleFinder™, IDFinder™, Microplastics, Sugar, Food

Introduction

In 2022 the World Health Organization (WHO) published a report highlighting growing concerns about human exposure to microplastics through dietary intake and inhalation. While current data on exposure and potential health effects remain limited, WHO emphasized the urgent need for continued research, particularly focused on the smallest microplastic particles (<10 µm), and called for the development of standardized methods to generate reliable data on human exposure.

Among the available analytical techniques, Raman micro-spectroscopy stands out as the only reference method capable of providing comprehensive information on microplastic particles in this critical size range [1]. Raman analysis enables determination of particle count, chemical composition, and size distribution, making it an indispensable tool for advancing microplastics research and risk assessment.

In this application note, we demonstrate how Raman spectroscopy can be effectively used to detect and characterize microplastics in food matrices. Using sugar as a model sample, we present a step-by-step workflow for sample preparation, Raman analysis, and data interpretation to highlight the potential of this technique for routine monitoring of microplastics in food products.

In this application, you will learn:

- How to prepare samples for microplastics analysis using HORIBA's filtration kit, optimized for maximizing recovery of small particles.
- How to analyze thousands of particles in a fully automated workflow using the latest generation Raman microscope, LabRAM Soleil™, combined with the powerful ParticleFinder™ software for particle detection and spectral acquisition.
- How to rapidly and reliably identify thousands of Raman spectra using the integrated IDFinder™ module, part of the LabSpec 6 software suite, which streamlines the particles classification based on dedicated spectral database.
- How to compile, interpret, and report results with confidence — quantifying the presence of microplastics in a sample and distinguishing between microplastics originating from the sample and those introduced during the sample preparation process.

This comprehensive workflow provides a robust approach to detect and characterize microplastics in food matrices, supporting the development of harmonized protocols for future routine monitoring and regulatory studies.

Instrument and methods

Sample preparation

For this study, 5 grams of white sugar obtained from a local supermarket were dissolved in 500 mL of hot Milli-Q water to ensure complete dissolution of the sugar crystals. The resulting solution was then filtered through a silicon (Si) filter with a pore size of 5 µm using the HORIBA filtration kit

(Figure 1). This filtration step concentrated any non-soluble particles present in the sugar sample onto the filter surface, facilitating subsequent Raman analysis.

To ensure data accuracy and account for potential contamination during sample preparation, analytical blanks were prepared by filtering 500 mL of hot Milli-Q water through the same type of 5 µm Si filters. These blanks served as controls to detect any environmental contamination introduced during the sample preparation process.

The choice of Si filters is a key element of this analytical workflow. Their flat reflective surface facilitates automated particle detection and imaging during Raman analysis (see section Raman Analysis). In addition, silicon generates minimal and well-defined Raman signals, reducing background interference and avoiding overlap with the characteristic Raman fingerprints of polymer particles. This property significantly enhances the accuracy of microplastic identification, even in complex food matrices.

Figure 1. Filtration kit: glass funnel, glass support base, silicone stopper, glass flask, vacuum pump and Si filters (5 µm porosity).

Raman analysis

The Raman analysis was performed using the LabRAM Soleil™ Raman microscope (Figure 2). For particle analysis, including automated Raman spectra acquisition, particle counting, and size characterization, ParticleFinder™ software, a module in LabSpec 6, was used.

Dark-field illumination was employed to enhance the optical contrast of the particles on the Si filter, facilitating their automatic localization with ParticleFinder. Once the particles were located in the image, the Raman spectrum of each particle was recorded using a 532 nm laser.

The filter was analyzed in a so-called "Dynamic mode," meaning that the following analytical sequence was automatically repeated for small zones of the filter, including several fields of view of the optical objective:

1. Image acquisition
2. Automatic particle localization
3. Raman spectra recording

This approach is well-suited for large filters, minimizing the risk of particle displacement during the analysis due to external factors, and ensuring high precision in localization and particle size characterization.

Figure 2. LabRAM Soleil Raman microscope

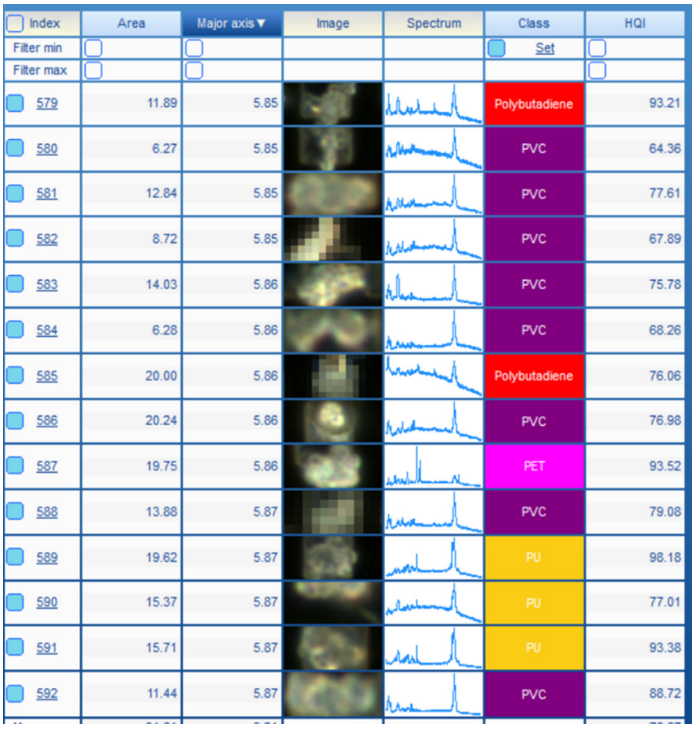
Data treatment

Apart from baseline correction, no additional processing was applied to the spectra. The spectra identification was performed using IDFinder™ software (part of the LabSpec 6 suite). The spectra were compared with a dedicated spectral library, and a matching score called the Hit Quality Index (HQI) was assigned to each spectrum. Pearson's correlation was used to calculate the HQI (Figure 3). The compound with the highest HQI was considered the chemical identity of the particle. The minimum acceptable HQI score for automatic particle identification was set at 60%.

The spectral library was customized to include spectra of the 10 most abundant polymers (listed in Table 1) and common non-plastic materials (such as Si, amorphous carbon, cellulose, proteins, $CaCO_3$, TiO_2, etc.). It should be noted that the above-mentioned list forms the basic content of the spectral library adapted for microplastics analysis, which can be supplemented with other organic and inorganic compounds expected to be present in the samples.

Table 1. List of most abundant polymers included in basic spectral library for microplastics analysis.

Polymer	Abbreviation
Polyethylene	PE
Polypropylene	PP
Polyethylene terephthalate	PET
Polycarbonate	PC
Polystyrene	PS
Polytetrafluoroethylene	PTFE
Polyvinyl chloride	PVC
Polyamide	PA
Polymethyl methacrylate	PMMA
Polyurethane	PU



Index	Area	Major axis	Image	Spectrum	Class	HQI
Filter min					Set	
Filter max						
579	11.89	5.85			Polybutadiene	93.21
580	6.27	5.85			PVC	64.36
581	12.84	5.85			PVC	77.61
582	8.72	5.85			PVC	67.89
583	14.03	5.86			PVC	75.78
584	6.28	5.86			PVC	68.26
585	20.00	5.86			Polybutadiene	76.06
586	20.24	5.86			PVC	76.98
587	19.75	5.86			PET	93.52
588	13.88	5.87			PVC	79.08
589	19.62	5.87			PU	98.18
590	15.37	5.87			PU	77.01
591	15.71	5.87			PU	93.38
592	11.44	5.87			PVC	88.72

Figure 3. Extract of the results table: each spectrum is compared with dedicated spectral library, the component with the highest matching score (Hit Quality Index, HQI) is mentioned in a column "Class".

Results and Discussion

Filters for Raman analysis

Figure 4 presents optical images of the filters corresponding to the sugar sample and the analytical blanks. A clear visual difference can be observed: significantly more particles were detected in the sugar sample compared to the blank, indicating the presence of insoluble residues not attributable to environmental contamination during sample preparation. It should be noted that although sugar is soluble in water, matrix residues can still remain on the filter. Therefore, for successful analysis of individual particles using Raman spectroscopy, the sampling mass should be adjusted to avoid particle overlap while maintaining a representative quantity of the sample for analysis. For more complex food matrices that are not water-soluble, additional sample preparation steps—such as chemical digestion and/or density separation—may be required prior to filtration on a silicon (Si) filter (see examples of sample preparation here [2] and in HORIBA microplastics e-book).

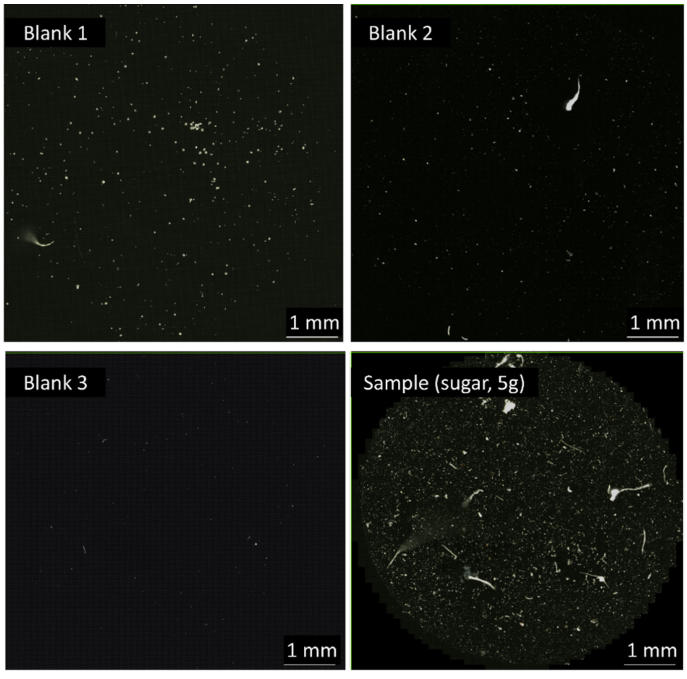


Figure 4. Optical images of analyzed filters.

Particles identification

The results of particle identification and counting are summarized in Figure 5 (a). For clarity, particles identified as cellulose, minerals, proteins, fatty acids, or amorphous carbon were grouped into a single category labeled "non-plastics." Most of the outlier spectra—defined as those with a hit quality index (HQI) below 60%—either lacked a clear Raman signal due to fluorescence interference or displayed only the characteristic Raman peak of the silicon (Si) filter. Figure 5 (b) shows the size distribution of the detected

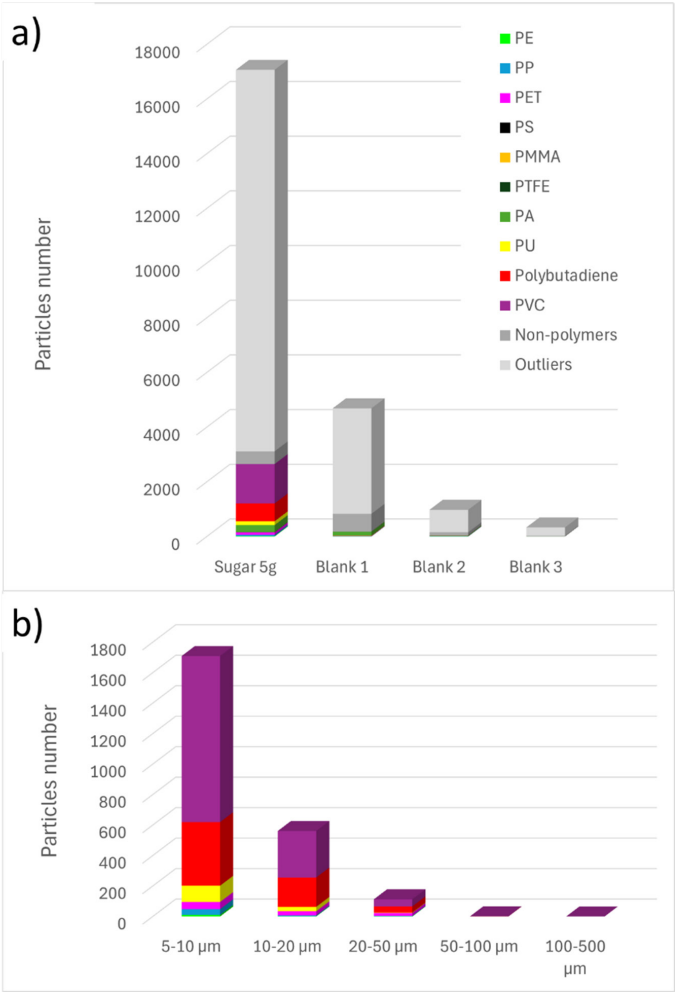


Figure 5. Results of Raman analysis for the particles with Feret diameter > 5 µm: a) Particles number in sugar sample and in the blanks; b) Microplastics size distribution in Sugar sample. Values > LOD are considered in this figure.

microplastic particles. The data reveal that the majority of microplastics detected in the sugar sample are smaller than 20 µm, highlighting the importance of using Raman spectroscopy for the detection of small-sized particles that are typically below the detection limit of other techniques.

Besides the standard spectra included in the microplastics analysis library, an additional spectrum was detected in the sugar sample. This spectrum was identified as a mixture of acrylonitrile-butadiene copolymer and titanium dioxide (TiO_2) using the ST Japan and HORIBA spectral libraries (Figure 6). To facilitate automated identification and counting of similar particles, this spectrum was added to the reference library and labeled as "Polybutadiene" for simplicity in further analysis.

Results reporting and blanks management

To evaluate the significance of the detected microplastics, a limit of detection (LOD) was calculated for each polymer using the following formula:

$$LOD\ (polymer) = Average\ (blanks) + 3 \cdot Standard\ Deviation\ (blanks)$$

Where Average (blanks) is the mean number of microparticles of a specific polymer detected in the blank samples, and Standard Deviation (blanks) is the variability for that polymer across blank measurements.

To confirm the presence of a given polymer in the sugar sample, the number of detected particles was compared to the LOD for that polymer. If the number of particles exceeded the LOD, the polymer was considered present in the

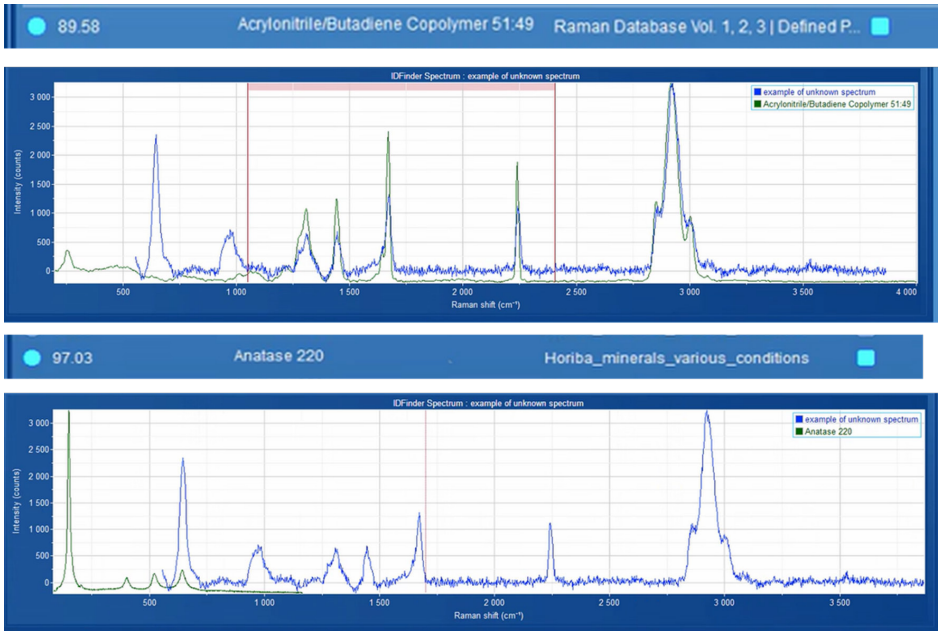


Figure 6. Identification of an unknown spectrum using the ID Finder™ software. The recorded unknown spectrum (blue) was compared against entries from the ST Japan and HORIBA spectral libraries (green). The best match was identified as a combination of acrylonitrile-butadiene copolymer and titanium dioxide (anatase).

sample at a statistically significant level, above background contamination. Table 2 presents the calculated LOD values for each polymer and the corresponding particle counts in the sugar sample. Based on this comparison, the presence of PE (polyethylene), PP (polypropylene), PET (polyethylene terephthalate), PVC (polyvinyl chloride), PU (polyurethane), and Polybutadiene is confirmed. Among these, PVC, PU, and Polybutadiene were the most abundant. The presence of PA (polyamide) remains uncertain due to similar levels being observed in the blank, suggesting possible contamination during handling or filtration.

Table 2. Limit of detection (LOD) of microplastics particles and results of sugar sample. LOD was calculated per type of polymer using formula LOD = Average of blanks + 3 St Dev of blanks. Values in red are above the limit of detection.

Polymer	LOD Particles number	Sugar Particles number
Polyethylene	2	**14**
Polypropylene	14	**45**
Polyethylene terephthalate	20	**93**
Polystyrene	4	2
Polyamide	289	**262**
Polyvinyl chloride	6	**1442**
Polyurethane	2	**139**
Polytetrafluoroethylene	1	1
Polymethyl methacrylate	1	0
Polycarbonate	1	0
Polybutadiene	2	**651**

Conclusions

This application note illustrates the capabilities of Raman micro-spectroscopy for the detection, identification, and quantification of microplastics in food products, using white sugar as a test case. The applied workflow—combining HORIBA's filtration kit, LabRAM Soleil™ Raman microscope, and automated analysis with ParticleFinder™ and IDFinder™—proved effective for characterizing microplastics down to the <20 µm range.

Several polymer types were identified in the sugar sample, including PVC, PU, and Polybutadiene, with the majority of particles measuring below 20 µm. The consistent presence of these polymers raises important questions regarding their origin. Potential sources may include food processing equipment (e.g., conveyor belts, seals, or packaging), environmental contamination during production or transport, or additives and processing aids used in sugar refining.
These findings underscore the need for continued research into the pathways through which microplastics enter the food chain. In particular, the identification of sub-20 µm particles, which are of growing concern due to potential human health impacts, aligns with WHO's 2022 recommendations to improve data on human exposure and to develop standardized analytical methods.

We encourage researchers to expand studies on various food matrices and explore the toxicological relevance of different polymer types and sizes. At the same time, food manufacturers and suppliers are urged to begin routine monitoring of their raw materials and production environments to ensure the highest quality standards and anticipate future regulatory requirements.

The methodology presented here offers a robust and scalable solution to support these efforts and contribute to a better understanding of microplastics exposure through diet.

References

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