Application Note

Food

Accurate Microplastic Characterization in Infant Formula

Using the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System

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Abstract

Reports of the omnipresence of microplastics have mainly focused on their presence in the environment, but there is a growing interest in investigating the health impacts of microplastics.[1] Many people would assume that infants' exposure to microplastics would be limited. However, infant formula was found to be a possible exposure pathway.[2] Extracting and isolating microplastics from infant formula can be difficult due to the range of formulations, ingredients, and components, such as fats, proteins, minerals, vitamins, and sugars.[3] This application note demonstrates the importance of quality control in microplastics analysis. The study also shows how the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System can accurately identify and quantify microplastics in infant formula.

Introduction

Microplastics are defined as small plastic particles between 1 µm and 5 mm in size.[4] They have been detected in seafood, drinking water (bottled and tap water), fruits, vegetables, and everyday condiments and beverages (honey, sugar, milk, and soft drinks).[5, 6] Microplastics have also been detected in infant formula.[2, 7] In a study published in 2022, researchers detected 17.3 microplastics/gram of infant formula (median), with polyurethane and polyamide accounting for 67% of the total number of microplastics detected.[8] Typically, infant formula is packaged in an aluminum tin with a plastic lid. A plastic scoop is often provided inside the container for measuring out the infant formula.

Recent studies have described several microplastic extraction methods from infant formula and other milk products using various digestion reagents.[7–9]

In this study, the automated microplastic analysis workflow within the Agilent Clarity software and the 8700 LDIR Chemical Imaging System (Figure 1) was used to characterize microplastics present in two commercial brands of infant formula. This study also highlights the challenges of isolating microplastics from infant formula using a newly developed extraction method.

Figure 1. The Agilent 8700 LDIR Chemical Imaging System allows the high-speed routine analysis of microplastics, including the number of particles present in the sample, their size, and their chemical composition.

Experimental

Samples

Two commercially available infant formula brands (brand A and B) were bought from a local supermarket in Melbourne, Australia.

Sample preparation

Initially, 5 g of infant formula was dissolved in 30 mL of saturated sodium chloride (sat. NaCl). This mixture was then shaken and centrifuged at 3,000 rpm for 30 minutes. This resulted in the separation of the white cream upper layer from the aqueous lower layer.

During the development of the method and to understand how microplastic particles behave in this mixture, 25 µL of green polyethylene beads were added. It was observed that the beads mostly imbedded in the upper layer, with few beads in the lower layer. Therefore, both layers of infant formula were prepared for analysis by LDIR.

The two layers were separated into two clean beakers by decanting the lower layer. To digest the upper layer, 100 mL of 0.1 M sodium hydroxide (NaOH) was added and heated to approximately 50 to 60 °C for 20 minutes.[9] This resulted in a slight color change from a white cream to a yellowish liquid. While the liquid was still hot, the mixture was filtered through a 47 mm, 14 µm polycarbonate filter. The pore size (14 µm) was selected to achieve easy filtration without blocking the filter. Once dried, absolute ethanol (EtOH) was used to wash the particles into a 50 mL clean tube.

The lower layer was directly filtered through a 47 mm, 14 µm polycarbonate filter and once dried, EtOH was used to wash the particles into a 50 mL clean tube.

Finally, for both layers, the EtOH suspension was then filtered through Sterlitech polyester (PETG) gold-coated membrane filters, 0.8 µm, 100/0 nm coating, 25 mm and analyzed directly using LDIR (Figure 2).

Instrumentation

An 8700 LDIR chemical imaging system controlled using Clarity software was used in this study. The polyester (PETG) gold-coated membrane filters loaded with microplastic particles from each sample layer were analyzed by the LDIR using the fully automated Particle Analysis method in the Clarity software. The method setup parameters used for data acquisition are shown in Table 1. Instrument parameters were set to the instrument default settings.

The Clarity software Particle Analysis method used both LDIR scan and sweep modes. Scan mode was first used to rapidly scan the sample selected area at a single wavenumber (1,442 cm^{-1}) to both locate particles and determine each particle's boundary. Once particles were located, the LDIR then rapidly and automatically moved to each particle and acquired a full spectrum over the wavelength range (sweep mode through the mid-infrared fingerprint region). The spectra were then compared to the Microplastics Starter 2.0 spectral library in real time.[10, 11] The best fit match for the spectrum was determined and reported for each particle.

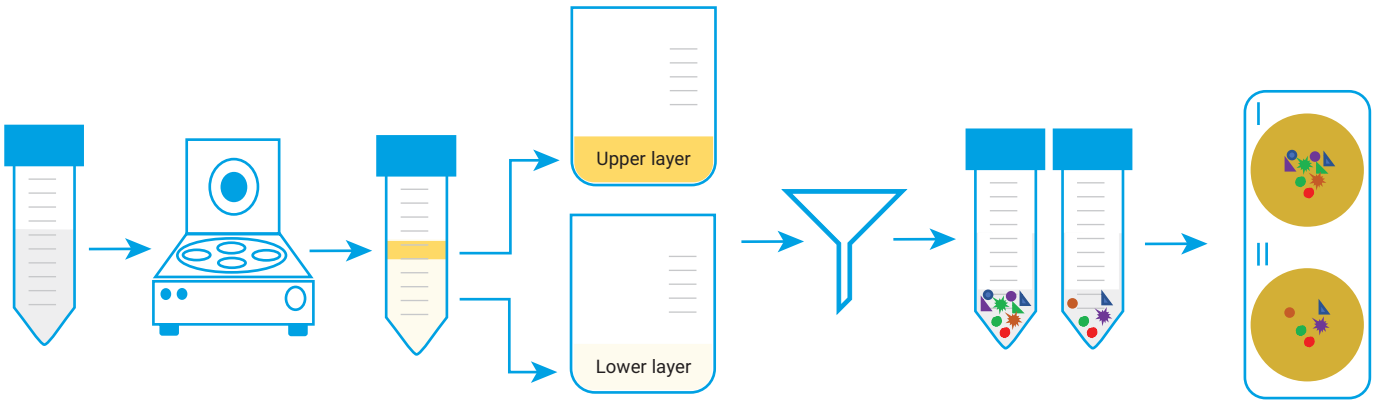


Figure 2. Microplastics isolation workflow from infant formula.

Table 1. Parameters used for the Agilent 8700 LDIR chemical imaging system automated method analysis of microplastics.

Parameter	Setting
Method	Particle Analysis
Library Used	Microplastics Starter 2.0
Auto Scan	On
Collect Visible Images	Yes
Particle Sensitivity	Automatic
Hit Quality Index Ranges	Hit quality describes how closely the spectrum of the sample matches that in the reference library. For this experiment, classification ranges (i.e., the characterization of spectral match quality by "high," "medium," and "low") were set to: – Low confidence (0.65 to 0.75) – Medium confidence (0.75 to 0.85) – High confidence (0.85 to 0.99) Any particles falling outside this range (i.e., < 0.65) were classified as "undefined"
Size Classification Range (µm)	20 to 100 100 to 200 200 to 300 >300
Scan Speed	Default (8)
Sweep Speed	Default (3, high speed)
Focus Offset	0
Polarization (Degree)	Default (0)
Attenuation (%)	Default (0)/Auto

Results and discussion

Reporting

For the reporting of the microplastic data, all non-microplastic particles were excluded (e.g., natural polyamide, stearates, cellulosic materials, carbonates, etc.). All other major microplastic types were reported based on selected hit quality index (HQI) criteria (>0.8). The microplastics included: acrylonitrile butadiene styrene (ABS), polyamide (PA), polycarbonate (PC), polyethylene (PE), polyethylene terephthalate (PET), polyoxymethylene (POM), polypropylene (PP), polystyrene (PS), polyurethane (PU), and polyvinyl chloride (PVC).

Quality control (QC) of microplastics in reagents

To minimize contamination of the infant formula samples during sample preparation, all reagents (Milli-Q ultrapure water, EtOH, sat. NaCl) were checked for the presence of microplastics using LDIR. Based on the results, the reagents were filtered several times before use to remove any microplastics. All glassware and centrifuge tubes were washed with filtered ultrapure water and covered in aluminum foil before use.

To assess the microplastic content of ultrapure water, 500 mL of water was filtered directly on-gold-coated filter and analyzed by LDIR. A total of 184 particles were detected. However, only six particles were quantified as microplastics (PET and PU: both n = 3) within the 20 to 100 µm range (Figure 3, top filter). The library supplied with the Clarity software helped to identify microplastics, including PET, and non-microplastics, such as naturally occurring polyamide, in the ultrapure water, as shown in Figure 3C (top filter). The water was then filtered several times before reanalysis by LDIR. The final QC showed that no microplastics remained in the water.

Likewise, the sat. NaCl solution was checked for microplastic contamination by directly filtering 500 mL into a gold-coated filter (Figure 3, bottom filter) for analysis by LDIR. A total of 392 particles were detected. 81 of the particles were microplastics (ABS: n = 2; PE: n = 42; PP: n = 27; PET: n = 5; PS: n = 3; PC and PU: n = 1) within the 20 to 100 µm range (examples are shown in Figure 3C). Therefore, the sat. NaCl solution was filtered another eight times before a final check showed minimal microplastic contamination.

The results for ultrapure water and sat. NaCl solution show the importance of checking reagents for microplastics before using them in sample extractions. EtOH was also quality assured before use. QC testing of reagents ensures that any microplastics being reported originate from the infant formula samples.

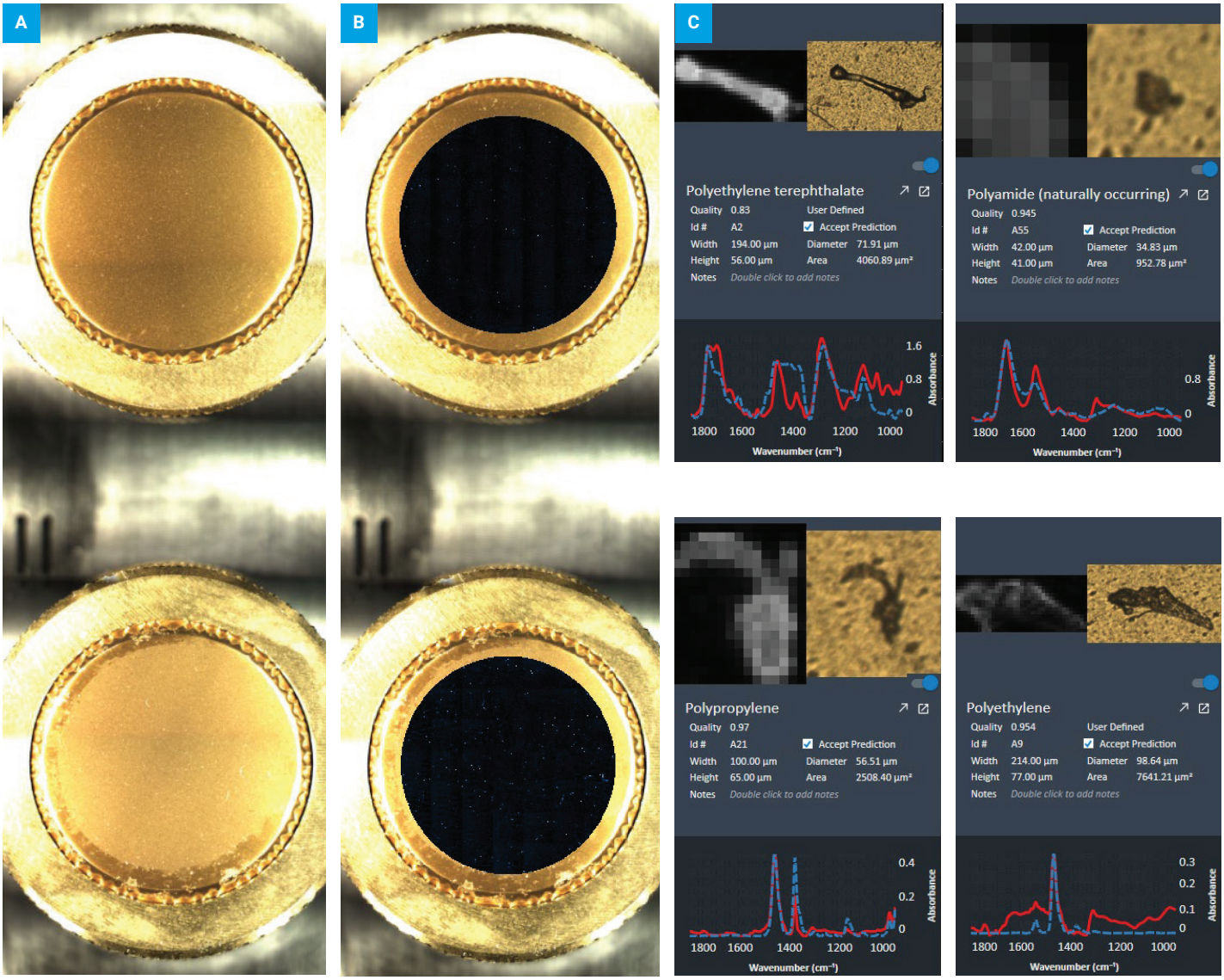


Figure 3. Identification and classification data of microplastics in Milli-Q ultrapure water (top) and sat. NaCl (bottom), analyzed directly on gold-coated polyester membrane filters using an Agilent 8700 LDIR. (A) Visible image of both filters. (B) IR image scanned at 1,442 cm^{-1} of both filters. (C) Examples of microplastics and non-microplastics identified in each filter.

Microplastics in infant formula

Each of the two-layer samples for the infant formula brands were analyzed directly on-filter using the 8700 LDIR. With two sample positions on the specially designed filter holder, the samples could be automatically characterized sequentially and results for each sample reported separately. However, if desired, results for the two positions could also be reported as one sample.

In the Clarity software, the user can define the area of interest as either a circular or rectangular shape. In this study, a circular area of approximately 16 mm in diameter for each filter was used, allowing the entire area of the filter containing particles to be included in the analysis. Due to the fast and automated capabilities of the 8700 LDIR, the analysis of each filter can be completed quickly, without further operator intervention.

Compared to other sample preparation techniques, the direct on-filter analysis method simplifies the workflow by eliminating the need for water/solvent evaporation before analysis. It also helps to reduce sources of sample contamination.

For both brands of infant formula, the analysis and reporting of the microplastics data was conducted as described in the QC section for the analysis of reagents.

For brand A of infant formula, many particles were detected in both layers (a total of 4,472 particles), with most particles identified as naturally occurring polyamides. This finding can be explained by proteins present in the infant formula that have not been fully digested in the extraction method.

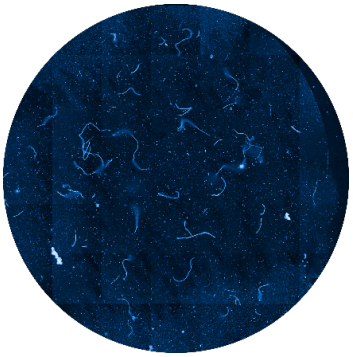
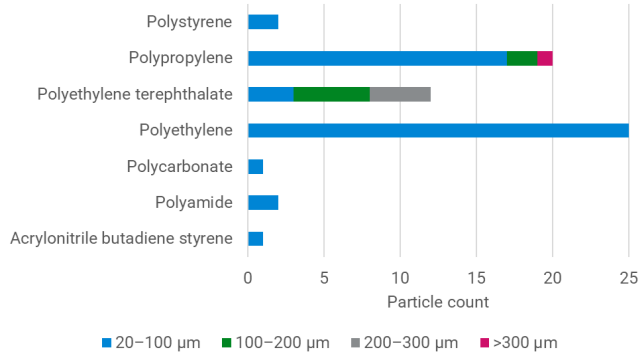
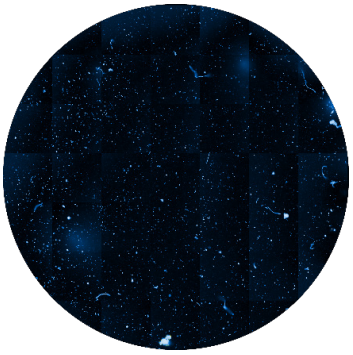
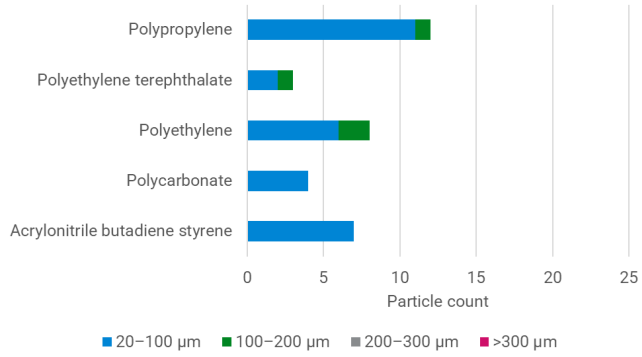
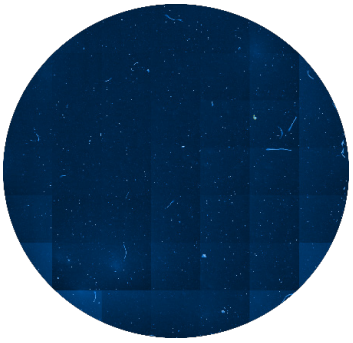
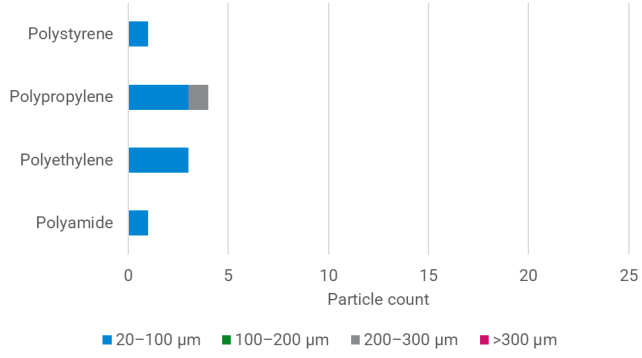
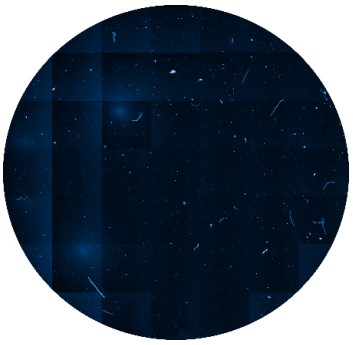
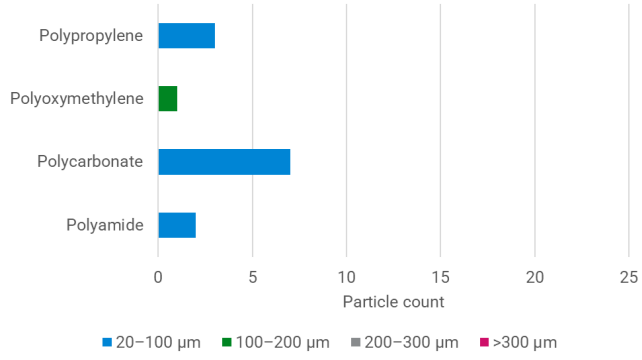
A total of 97 particles were identified as microplastics (63 and 34 in the upper and lower layers, respectively) with most microplastics in the size range of 20 to 100 µm. The main polymer types found in both layers were PE and PP (Table 2).

A lower number of particles were detected in both layers of infant formula brand B (a total of 1,078 particles). For the upper layer, a total of 712 particles were detected and only nine particles were identified as microplastics (HQI >0.8). In the lower layer, 366 particles were detected with 13 particles identified as microplastics (HQI >0.8). Microplastics identified in brand B were mainly PC, PE, and PP; all classified in the 20 to 100 µm size range (Table 2).

The remaining particles in both brands of infant formula were non-microplastics, such as naturally occurring polyamides and cellulosic materials or unidentified materials with HQI <0.8, such as infant formula components that were not present in the library.

The sample preparation described for the two infant formula samples resulted in effective extraction of microplastics. However, further optimization of the extraction method might be required based on the analytical needs. For example, additional matrix cleanup using enzymatic digestion or prolonged heating could reduce the number of non-microplastics present in both layers. Reducing the number of non-microplastics on the filter would lead to shorter analysis times.

Table 2. Microplastic characterization results summary for both infant formulas brand A and B.

Brand	Layer	Infrared Image	Total Number of Particles	Microplastics with >0.8 HQI	Size Distribution and Identification of Microplastics
A	Upper		1,979	63	
	Lower		2,493	34	
B	Upper		712	9	
	Lower		366	13	

The 8700 LDIR chemical imaging system combined with Clarity software automated workflow provided accurate and quick characterization of microplastics in infant formula in both brands. LDIR identification capabilities for non-microplastics helped in differentiating between synthetic polymers and natural materials, such as cellulose and naturally occurring polyamides, as shown in Figures 4 and 5.

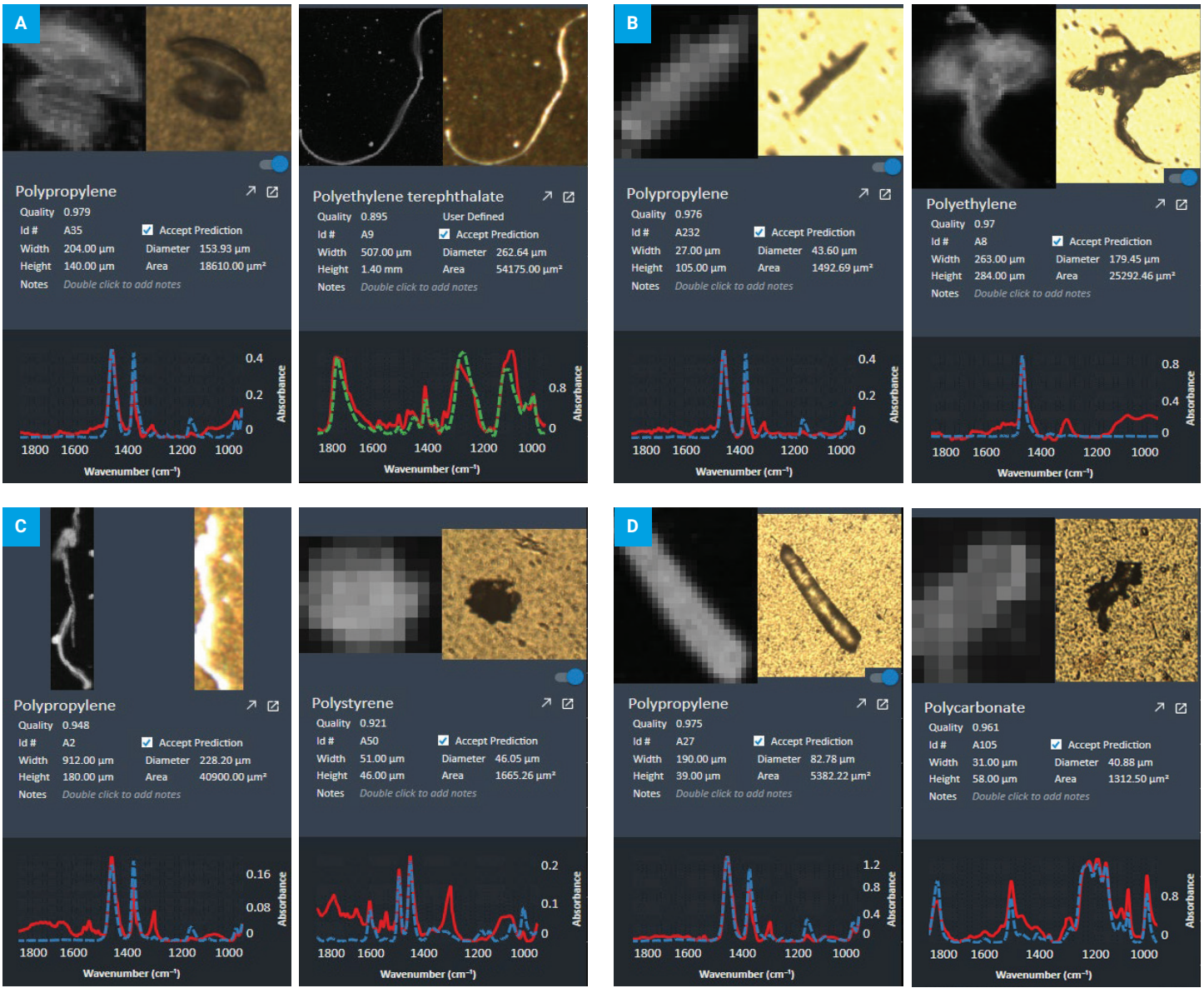


Figure 4. Examples of microplastics identified in both brands of infant formula. (A) Brand A upper layer. (B) Brand A lower layer. (C) Brand B upper layer. (D) Brand B lower layer. For each particle detected, LDIR provides IR and visible images of particle, material identification, size information, overlap of spectrum (red line), and matched library spectrum (blue or green dashed line).

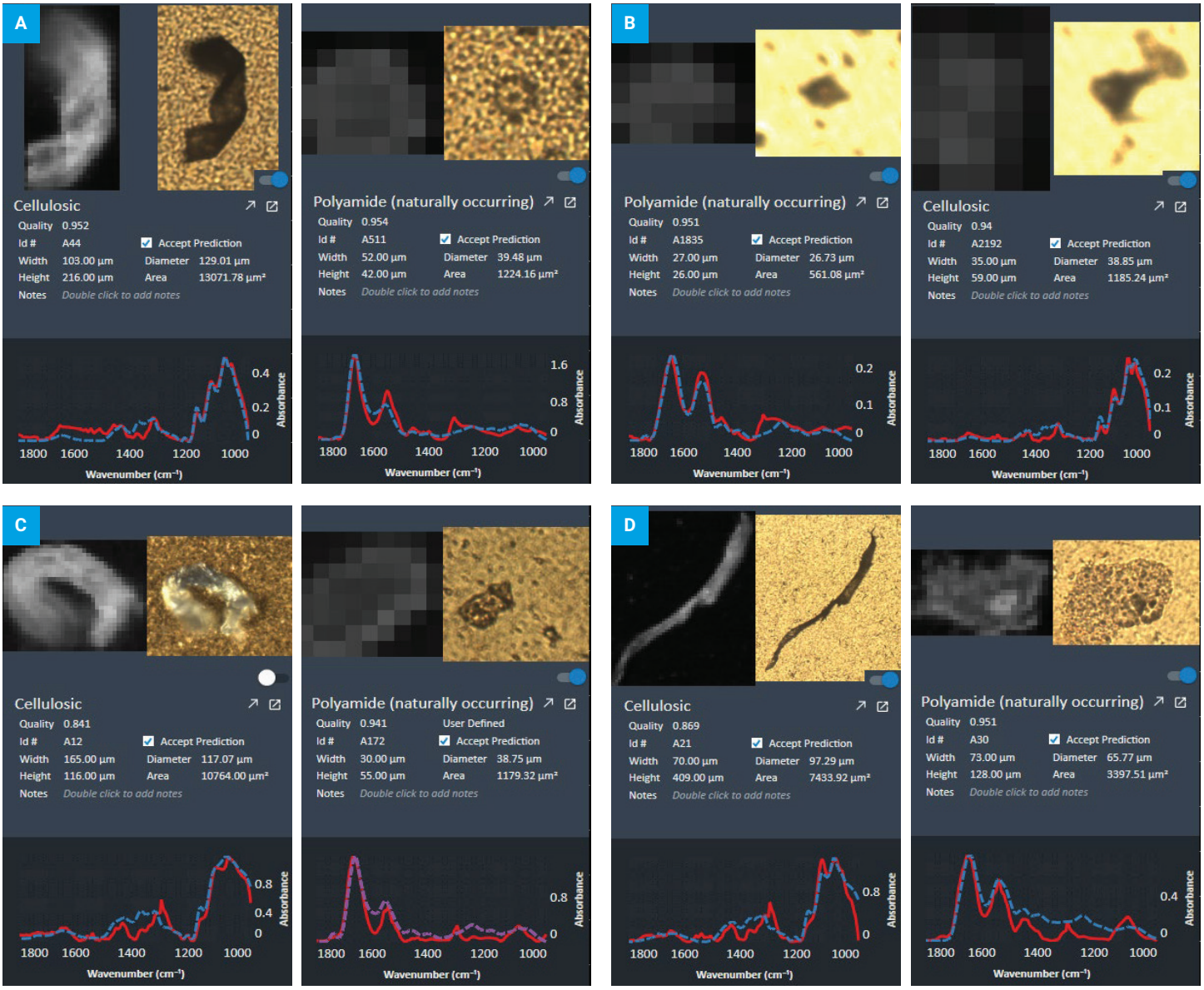


Figure 5. Examples of non-microplastics identified in both brands. (A) Brand A upper layer. (B) Brand A lower layer. (C) Brand B upper layer. (D) Brand B lower layer. For each particle detected, LDIR provides IR and visible images of particle, material identification, size information, overlap of spectrum (red line) and matched library spectrum (blue or purple dashed line).

Few fiber-shaped particles were detected in the upper layer of infant formula brand A (Table 2, IR image). Lab coats worn by analysts during the extraction of microplastics could potentially release synthetic textile fibers into the sample.[12] LDIR successfully identified these fiber-shaped particles as cellulosic materials with an HQI >0.8, as shown in Figure 6.

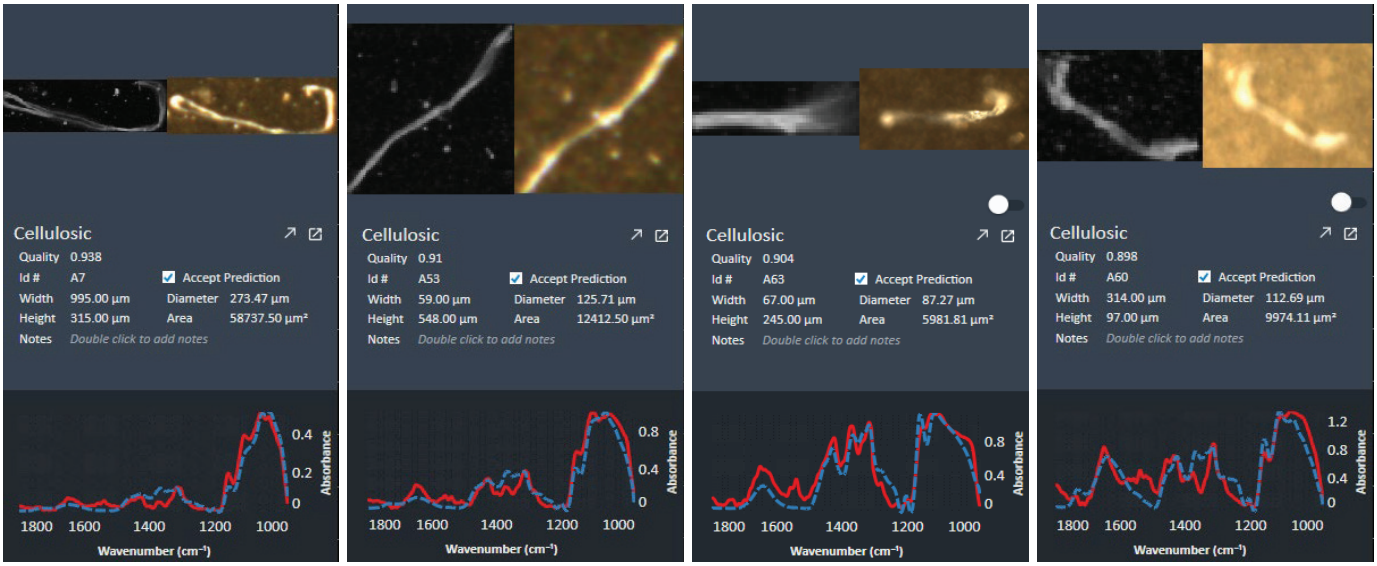


Figure 6. Examples of fiber-shaped particles identified as cellulosic materials in the upper layer of infant formula brand A.

Conclusion

Microplastic particles were detected in two brands of infant formula using the on-filter analysis capability of the Agilent 8700 LDIR Chemical Imaging System. A total of 97 and 22 microplastic particles were detected with a hit quality of greater than 0.80 in brands A and B, respectively. PC, PE, and PP were the most frequently detected polymers in both brands. High levels of identification accuracy and confidence were achieved for microplastic and non-microplastic particles on the gold-coated filters.

This work demonstrated the key components of microplastics analysis and the importance of quality control testing of reagents before use. All reagents were found to contain microplastics, so each was filtered and rechecked for contaminants before use. Also, all glassware was washed with ultrapure water that was free of microplastics.

Isolating microplastics from complex matrices such as infant formula can be challenging. However, the 8700 LDIR combined with Clarity software provided fast and accurate microplastic characterization.

Compared to other techniques, the direct-filter LDIR method requires less sample handling, reducing the potential for sample contamination and improving sample throughput. The vacuum filter sample preparation procedures and LDIR methods provide efficient microplastic analysis and significant time saving.

The automated workflow of the 8700 LDIR is ideal for the accurate characterization of microplastics in different matrices. It can provide fast sample throughput of high numbers of samples, making it suitable for routine applications or for large monitoring studies.

References

1. Campanale, C. *et al.* A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. *Int. J. Environ. Res. Public Health* **2020**, *13*, *17(4)*, 1212. doi: 10.3390/ijerph17041212
2. Li, D. *et al.* Microplastic Release from the Degradation of Polypropylene Feeding Bottles During Infant Formula Preparation. *Nat. Food 1* **2020**, 746–754. https://doi.org/10.1038/s43016-020-00171-y
3. Diaz-Basantes, M. F.; Conesa, J. A.; Fullana, A. Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants. *Sustainability* **2020**, *12*, 5514. https://doi.org/10.3390/su12145514
4. SAPEA. Science Advice for Policy by European Academies A Scientific Perspective on Microplastics in Nature and Society (SAPEA, **2019**).
5. Sewwandi, M. *et al.* Microplastics and Plastics-Associated Contaminants in Food And Beverages; Global Trends, Concentrations, and Human Exposure. *Environ. Pollut.* **2022**, *317*, 120747. 10.1016/j.envpol.2022.120747
6. Samandra, S. *et al.* Assessing Exposure of the Australian Population to Microplastics through Bottled Water Consumption. *Sci. Total Environ.* **2022**, *1(837)*, 155329. doi: 10.1016/j.scitotenv.2022.155329
7. Zhang, Q. *et al.* Microplastics in Infant Milk Powder. *Environ. Pollut.* **2023**, *15(323)*, 121225. doi: 10.1016/j.envpol.2023.121225
8. Liu, S. *et al.* Detection of Various Microplastics in Placentas, Meconium, Infant Feces, Breastmilk and Infant Formula: A Pilot Prospective Study. *Sci. Total Environ.* **2022** *13(854)*, 158699. doi: 10.1016/j.scitotenv.2022.158699
9. Kutralam-Muniasamy, G. *et al.* Branded Milks - Are They Immune From Microplastics Contamination? *Sci. Total Environ.* **2020**, *20(714)*, 136823. doi: 10.1016/j.scitotenv.2020.136823
10. Primpke, S. *et al.* Reference Database Design for the Automated Analysis of Microplastic Samples Based on Fourier Transform Infrared (FTIR) Spectroscopy. *Anal. Bioanal. Chem.* **2018**, *410*, 5131–5141. doi: 10.1007/s00216-018-1156-x
11. De Frond, H.; Rubinovitz, R.; Rochman, C. M. µATR FTIR Spectral Libraries of Plastic Particles (FLOPP and FLOPP-e) for the Analysis of Microplastics. *Anal. Chem.*, **2021** *93(48)*, 15878–15885. doi: 10.1021/acs.analchem.1c02549
12. Schymanski, D. *et al.* Analysis of Microplastics in Drinking Water and Other Clean Water Samples with Micro-Raman and Micro-Infrared Spectroscopy: Minimum Requirements and Best Practice Guidelines. *Anal. Bioanal. Chem.* **2021**, *413*, 5969–5994. https://doi.org/10.1007/s00216-021-03498-y

Further information

- Agilent 8700 LDIR Chemical Imaging System
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water

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