Application Note

Environmental

Benefits of Agilent 8700 LDIR with Onboard ATR for Microplastics Characterization

Minimizing interferences and improving data accuracy using the 8700 LDIR chemical imaging system

Authors

Wesam Alwan
Agilent Technologies, Inc.

Subharthe Samandra and Bradley Clarke
Australian Laboratory for Emerging Contaminants, School of Chemistry, Faculty of Science, The University of Melbourne, Victoria, Australia

Abstract

Microplastics are considered as emerging contaminants that impact all aspects of the environment. To understand the ecotoxicological effects of microplastics on the environment and living organisms, accurate polymer-characterization techniques are required. Recent studies have shown that long carbon chain molecules, such as stearates, show similar infrared (IR) spectral features to microplastics such as polyethylene, leading to false positive identification of microplastics.[1] This study demonstrates how the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system can verify the identity of zinc stearate and distinguish it from microplastics, effectively overcoming interference from the long-chain compounds. Collecting attenuated total reflection (ATR) spectra using the 8700 LDIR-enabled external ATR-spectral libraries to be used for the identification of known and unknown particles.

Introduction

Microplastics are known to contain additives like plasticizers and ultraviolet (UV) stabilizers, as well as adsorbing contaminants from the environment. Some additives and contaminants present in the microplastics can interfere with the spectral matching. One of the major additive molecules reported in the literature that can interfere with the identification (ID) of polyethylene (PE) is zinc stearate ($(C_{17}H_{35}COO)_2Zn$).[2] Zinc stearate is a white powder that is widely used in plastics manufacturing. Its main uses include:

– **Release agent**: it prevents plastics from sticking to molding and extruding equipment.

– **Lubricant**: it reduces friction between plastics and machinery, improving the flowability of the plastic.

– **Stabilizer**: it increases the durability of plastics by preventing degradation from exposure to UV light and heat.

Typically, the main IR spectral region of interest for PE relates to the C-H bending at 1,480 to 1,440 cm^{-1}. Both PE and zinc stearate exhibit this absorbance band, leading to false positive ID of PE.

This study demonstrates the benefits of the onboard micro-Attenuated Total Reflection (µATR) within the Agilent 8700 LDIR (Figure 1) for the verification and ID of known and unknown materials using external spectral libraries. The Agilent Cary 630 FTIR fitted with ATR (Figure 2) was used as an independent confirmation tool to verify the ID of the known zinc stearate sample.

This study also shows the capability of the 8700 LDIR automated Particle Analysis workflow facilitated by the Agilent Clarity instrument control software for accurate microplastic characterization. The 8700 successively distinguished between zinc stearate and PE, eliminating false positive results for PE.

Figure 1. The Agilent 8700 LDIR chemical imaging system allows the high-speed routine analysis of microplastics, including reporting the number of particles present in a sample, size of particles, and chemical composition (identity) of particles.

Figure 2. The Agilent Cary 630 FTIR spectrometer coupled with a diamond ATR module and controlled using Agilent MicroLab software is widely used for the accurate and reliable ID of materials.

Experimental

To examine the capabilities of the 8700 LDIR chemical imaging system for the verification and ID of known and unknown materials, as well as distinguishing microplastics from non-microplastics, the following experiments were performed in sequence:

1. **Verification of identity of known material**: commercial zinc stearate (Riedel-de Haën, 26423, CAS Number: 557-05-1) was identified using the Cary 630 FTIR spectrometer equipped with a diamond-ATR module and 8700 LDIR chemical imaging system with on-board µATR. The identity of the known sample spectra that were collected on each instrument were compared against IR spectral libraries within Wiley's KnowItAll Analytical Edition software. The Cary 630 FTIR and the 8700 LDIR both feature powerful built-in library matching algorithms for microplastics. However, in this study, an external, system-agnostic (platform-independent) software was employed for ID verification.
2. **Confirmation of identity of unknown material**: unidentified particles previously detected in an infant formula sample were investigated using an 8700 LDIR chemical imaging system µATR method and Wiley's KnowItAll Analytical Edition software that contains various ATR-IR spectral libraries.
3. Distinguishing between zinc stearate and clear low density PE microspheres (Cospheric LLC, CPMS-0.96, 38 to 45 µm, 5 g) using the 8700 LDIR automated Particle Analysis workflow and the Agilent Microplastics Starter 2.0 library with user-added spectra. The Microplastics Starter 2.0 library is included in the Clarity software.

Results and discussion

Verification of identity of known material – zinc stearate

A small quantity of the zinc stearate sample was placed onto the ATR crystal of the Cary 630 FTIR, and contact was ensured using the ATR swivel press. The IR measurement was taken using the parameters presented in Table 1. After completion, the data was exported for material identity verification using an external IR spectral library.

Table 1. Parameters used for the Agilent Cary 630 FTIR-ATR method analysis of zinc stearate and polyethylene.

Parameter	Settings
Method	Data collect
Library Used	Wiley's KnowItAll Analytical Edition Software with IR Spectral Library
Spectral Range	4,000 to 650 cm^{-1}
Background Scans	64
Sample Scans	64
Spectral Resolution	4
Background Collection	Air

For the µATR analysis using the 8700 LDIR, approximately 1 mg of zinc stearate was suspended in absolute ethanol (50 mL) and filtered using vacuum filtration glassware until dry. The gold-coated membrane filter was then transferred to the filter holder for analysis. The following 8700 LDIR-µATR workflow was used to perform the analysis on zinc stearate particles:

1. Clean the germanium (Ge) crystal of the ATR by wetting a lint-free soft lab tissue or cotton swab with isopropanol or acetone. Optics grade cleaning supplies and solutions are recommended.
2. Focus the IR laser on the sample and adjust the ATR position (80 × 80 µm window). An ATR background will be collected automatically.
3. Adjust the force to increase or decrease ATR contact with the sample.
4. Acquire a single point ATR spectrum by double clicking on a point of interest (Figure 3).
5. Collected spectra can be exported from Clarity software into Wiley's KnowItAll Analytical Edition Software with one click, followed directly by the library search.
6. Clean the Ge crystal after each sample has been analyzed by ATR to avoid cross-contamination and to help obtain the best possible results.

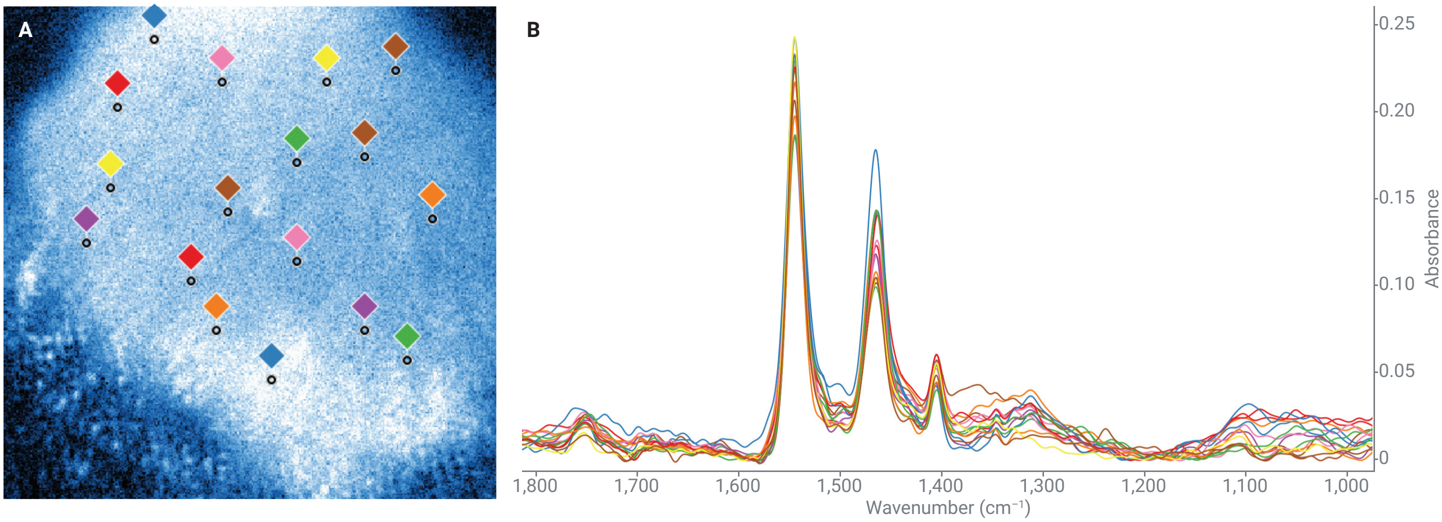
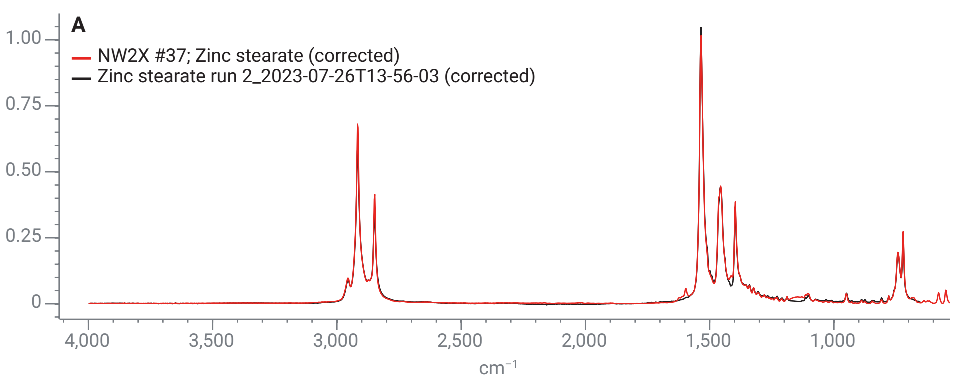


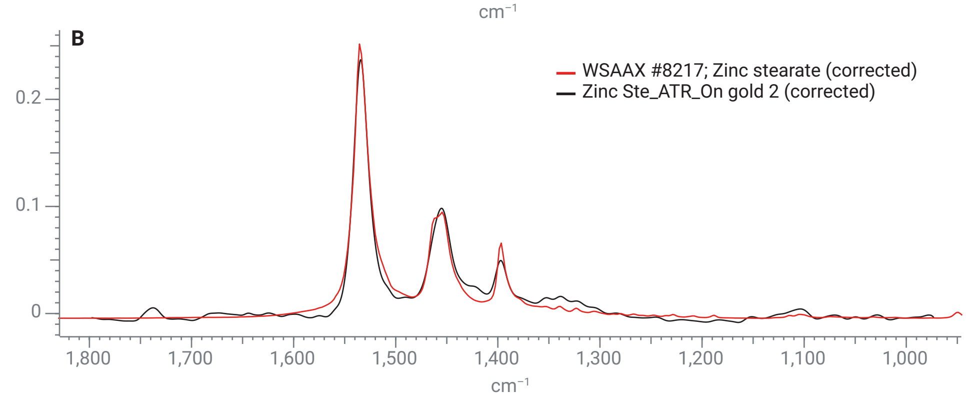
Figure 3. (A) Zinc stearate particle image (80 × 80 µm) with colored points representing the location of each ATR spectrum acquired by the Agilent 8700 LDIR. (B) ATR spectra acquired from zinc stearate particles. The color of each spectrum corresponds to the location of the particle.

Using the KnowItAll software, the material was correctly identified as zinc stearate by the Cary 630 FTIR and 8700 LDIR with a hit quality index (HQI) of 94.68 and 85.86, respectively. The results are shown in Figure 4.

Although the 8700 LDIR chemical imaging system exhibits a reduced range (975 to 1,800 cm^{-1}) compared to the Cary 630 FTIR (650 to 4,000 cm^{-1}), accurate ID of the known material was still achieved.



Name	Value
Resulting HQI	94.68
Database Abbreviation	NW2X
Database Title	ATR -IR - Sadtler Organometallics 2 - Wiley
Record ID	37
Name	Zinc stearate



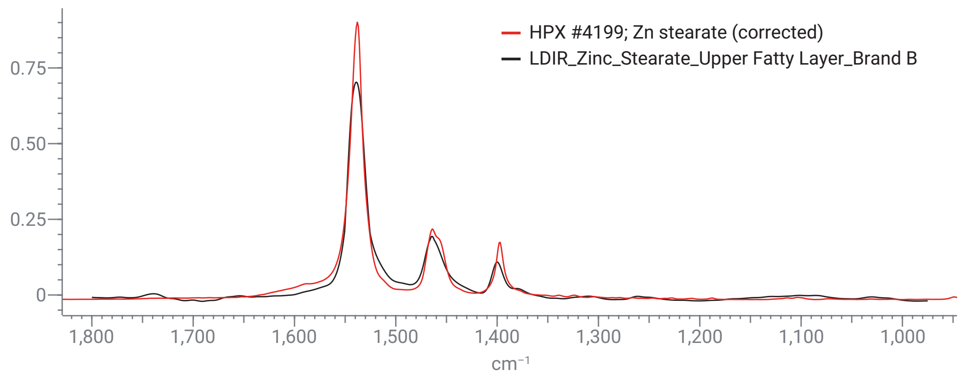
Name	Value
Resulting HQI	85.86
Database Abbreviation	WSAAX
Database Title	ATR-IR - Sigma-Aldrich Library of TR-IR Spectra - Wiley
Record ID	8217
Name	Zinc stearate

Figure 4. Identification analysis of zinc stearate known sample (black traces) and library hit (red traces) using spectral libraries within Wiley's KnowItAll Analytical Edition software. (A) Spectrum obtained by Agilent Cary 630 FTIR-ATR spectrometer; (B) spectrum obtained by Agilent 8700 LDIR-µATR.

Confirmation of identity of unknown material – unidentified particles in infant formula sample

In a previous study, microplastics were extracted from infant formula samples.[3] The automated Particle Analysis workflow of the 8700 LDIR revealed several unknown particles present in the infant formula sample during that study. To confirm the material identity of the unidentified particles, the µATR of the 8700 LDIR was used to acquire ATR-spectra, as described in the previous section.

As shown in Figure 5, some unknown particles in infant formula were identified as zinc stearate with an HQI of 85.12 using Wiley's KnowItAll Analytical Edition software. This example shows the advantage of the on-board ATR, which allows the analyst to further interrogate unknown particles using the 8700 LDIR.



Name	Value
Resulting HQI	85.12
Database Abbreviation	HPX
Database Title	IR - Polymers, Hummel Industrial - Wiley
Record ID	4199
Name	Zinc stearate

Figure 5. Unknown particles identification using the 8700 LDIR-µATR and Wiley's KnowItAll Analytical Edition software.

Distinguishing between polyethylene and zinc stearate

To determine the ability of the 8700 LDIR to distinguish PE from zinc stearate, samples of PE and zinc stearate were assessed separately using the automated Particle Analysis workflow and the parameters listed in Table 2.

Examples of each particle type are shown in Figure 6. Each sample was uniquely shaped and sized, which made them easily distinguishable from each other and from other contaminants.

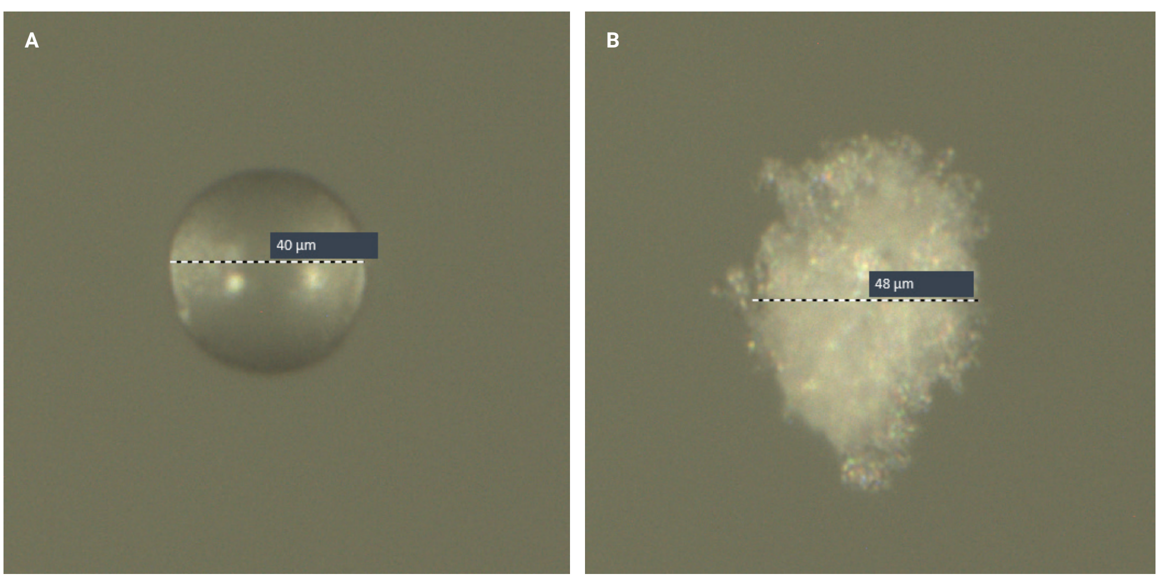


Figure 6. (A) Clear polyethylene microsphere. (B) Zinc stearate. Both images were obtained using the high-magnification visual camera of the Agilent 8700 LDIR chemical imaging system.

Table 2. Parameters used for the Agilent 8700 LDIR chemical imaging system automated method analysis for the characterization 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)	20–100 100–200 200–300 > 300
Scan Speed	Default (8)
Sweep Speed	Default (3, high speed)
Focus Offset	0
Polarization (Degree)	Default (0)
Attenuation (%)	Default (0)/Auto

Polyethylene microspheres

The first step was to evaluate the spectra obtained for each PE microsphere sample against the spectral library provided within the Clarity software (Microplastics Starter 2.0). In a scanned area (9.05 × 8.60 mm) containing a total of 262 particles, 258 (98.5%) particles were identified as PE with 256 particles (99.2%) having a HQI > 0.85, using the automated particle workflow (Figure 7).

Zinc stearate

Like PE particles, the collected zinc stearate spectra obtained using the 8700 LDIR were assessed against the same spectral library provided with the Clarity software (Microplastics Starter 2.0). A total of 419 particles were detected in a scanned area of 3.19 × 4.07 mm. Since a zinc stearate reference spectrum was not present in the Microplastics Starter 2.0 library, 367 (87.6%) particles were identified as PE with low to medium quality (HQI: 0.65 to 0.82). The rest of the particles were identified mainly as polyamide or polyurethane (40 particles; 9.5%) following the same quality trend as PE (HQI: 0.68 to 0.78).

To examine the capability of the 8700 LDIR to distinguish between PE and zinc stearate, a spectrum of zinc stearate was added to the Microplastics Starter 2.0 library. The Clarity software allows users to easily manage libraries and spectra. After collecting LDIR data on samples or standards, the spectrum of interest can then be added to the library, as described in a previous application note.[4]

Both PE and zinc stearate runs were reanalyzed using the Microplastics Starter library with the added zinc stearate spectrum. Reprocessing can be performed automatically within the Clarity software by selecting the updated library. The newly generated statistical data can then be checked.

As summarized in Table 3, 415 (99%) of particles in the zinc stearate sample were identified correctly (408 with an HQI > 0.8) and no false-reporting of PE was observed. Also, the updated library improved the PE scan results by identifying two particles as zinc stearate rather than PE (Figure 7).

Table 3. Identification results of polyethylene and zinc stearate using a spectral library with and without the inclusion of a reference spectrum for zinc stearate.

Material	Number of Particles	Identification Based on Microplastics Starter 2.0	
		Without Zn Stearate	With Zn Stearate
Polyethylene	262	– **Polyethylene (258)** – Polyamide (1) – Polyurethane (1) – Cellulosic (2)	– **Polyethylene (256)** – Polyamide (1) – Polyurethane (1) – Cellulosic (2) – **Zinc stearate (2)**
Zinc Stearate	419	– **Polyethylene (367)** – Polyamide (28) – Polyurethane (12) – Polyamide (naturally occurring) (2) – Chitin (2) – Carbonate (1) – Rubber (4) – Mg stearate (3)	– **Zinc stearate (415)** – Polyamide (naturally occurring) (1) – Chitin (1) – Carbonate (1) – Mg stearate (1)

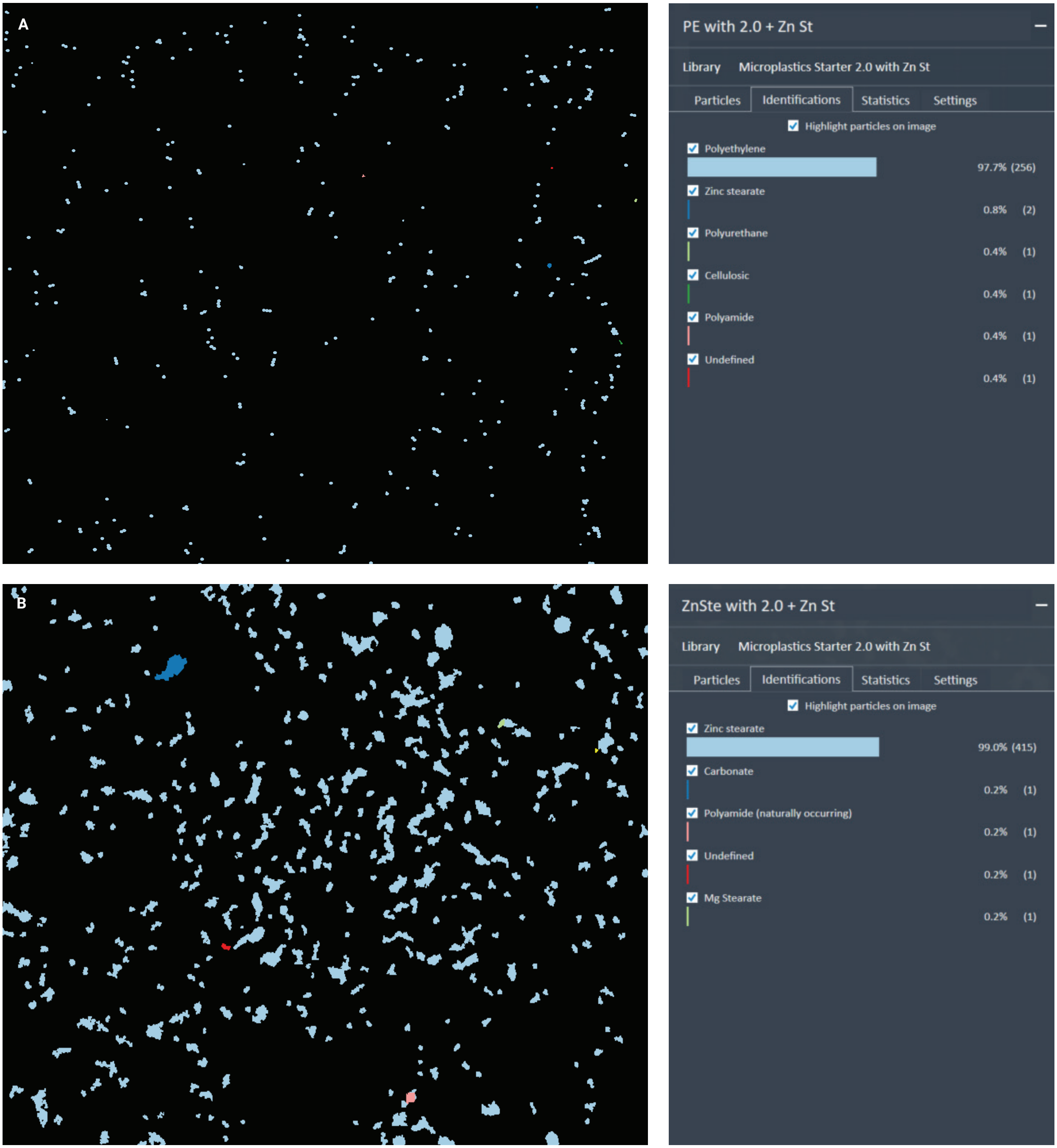


Figure 7. Automated particle analysis workflow for (A) polyethylene, and (B) zinc stearate analyzed using Microplastics Starter 2.0 with added zinc stearate spectrum.

The typical spectral region of interest for PE is the C-H bending at 1,480 to 1,440 cm^{-1}. Both PE and zinc stearate showed this absorbance band, as shown in Figure 8. However, zinc stearate shows another characteristic – a strong signal in the region of 1,500 to 1,660 cm^{-1}. Since the Clarity software uses first derivative spectral treatment as a matching algorithm, the addition of the zinc stearate spectrum with absorbance bands at 1,560 to 1,510 cm^{-1} and 1,410 to 1,390 cm^{-1} helped to accurately identify zinc stearate, with clear differentiation from PE (Figure 8).

This example illustrates the distinguishing capabilities of the 8700 LDIR between PE and zinc stearate, improving the accuracy of microplastic characterization studies.

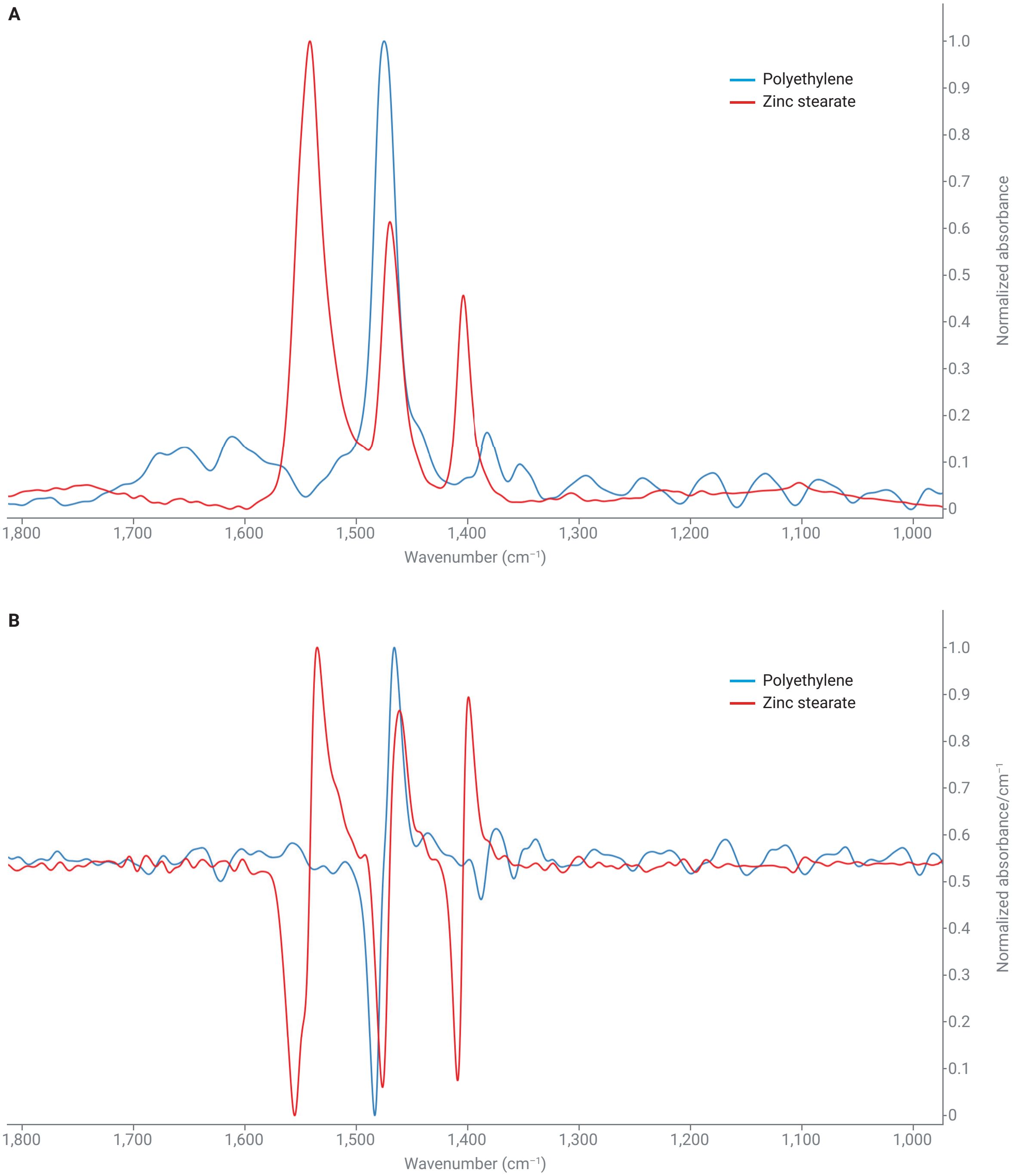


Figure 8. An overlay of polyethylene spectrum (blue) with zinc stearate (red). (A) Normalized absorbance; (B) first derivative.

Conclusion

The bottleneck in the accurate characterization of microplastics in environmental matrices is caused by contaminants or non-microplastics that are present in the sample or remain after sample digestion. These unknown materials create a challenge in providing accurate microplastic characterization as they may interfere with polymers, leading to false positive results.

This study has shown that ATR-spectra generated by both the Agilent 8700 LDIR and the Agilent Cary 630 FTIR provided accurate material verification of zinc stearate.

Also demonstrated was how the on-board micro-ATR of the 8700 LDIR chemical imaging system allowed for further interrogation of unknown particles in a sample. Generating ATR-spectra of unidentified particles in an infant formula sample meant that the data could be searched against different spectral libraries. Agilent Clarity software can be integrated with Wiley KnowItAll software, enabling LDIR-ATR generated spectra to be easily exported and matched with a single click.

In the third part of the study, the 8700 LDIR accurately differentiated between polyethylene and other interferences (zinc stearate) using the automated Particle Analysis workflow. The fully automated particle analysis method within the Agilent Clarity software is an efficient way for users to obtain visual images of particles, as well as information on particle size, distribution, and identification of microplastics. The accuracy of particle identification of polyethylene was improved significantly by adding a spectrum of zinc stearate to the Agilent Microplastics Starter 2.0 library.

References

1. Witzig, C. S.; Földi, C.; Wörle, K.; Habermehl, P.; Pittroff, M.; Müller, Y. K.; Lauschke, T.; Fiener, P.; Dierkes, G.; Freier, K. P., *et al.* When Good Intentions Go Bad – False Positive Microplastic Detection Caused by Disposable Gloves. *Environ. Sci. Technol.* **2020**; *54(19)*, 12164–12172. doi: 10.1021/acs.est.0c03742.
2. 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
3. Samandra, S. *et al.* Accurate Microplastic Characterization in Infant Formula. *Agilent Technologies application note*, publication number 5994-5928EN, **2023**.
4. Alwan, W. *et al.* Characterization of Microplastics in Environmental Samples by Laser Direct Infrared Imaging and User-Generated Libraries. *Agilent Technologies application note*, publication number 5994-4822EN, **2022**.

Further information

- Agilent 8700 LDIR Chemical Imaging System
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water
- Agilent Cary 630 FTIR Spectrometer
- MicroLab FTIR Software
- FTIR Analysis and Applications Guide
- FTIR Spectroscopy Basics – FAQs
- ATR-FTIR Spectroscopy Overview

www.agilent.com

DE3303400

Printed in the USA, November 8, 2023
5994-6843EN

Agilent
Trusted Answers