



Designation: D8489 – 23<sup>ε1</sup>

# Standard Test Method for Determination of Microplastics Particle and Fiber Size, Distribution, Shape, and Concentration in Waters with High to Low Suspended Solids Using a Dynamic Image Particle Size and Shape Analyzer<sup>1</sup>

This standard is issued under the fixed designation D8489; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reappraisal. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reappraisal.

<sup>ε1</sup> NOTE—Research report information was added to Section 14 editorially in May 2023.

## 1. Scope

1.1 This test method covers the determination of microplastic particle size distribution, shape characterization, and number concentration (particle counts) in sample extracts containing particles between 5  $\mu\text{m}$  and 100  $\mu\text{m}$ . Light is transmitted through a flow cell containing particles in liquid medium. The particles create shadows as they pass through the field of vision of a camera, producing a multitude of images. The images are then used to measure size, shape, and concentration.

1.2 This test method is used as a complementary technique for microplastic particle and fiber polymer identification methods infrared microscopy and gas chromatography/mass spectroscopy pyrolysis.

1.3 This test method requires that samples are collected according to Practice D8332 and prepared according to Practice D8333 prior to use.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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## 2. Referenced Documents

### 2.1 *ASTM Standards*:<sup>2</sup>

D883 Terminology Relating to Plastics

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

D8332 Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers

D8333 Practice for Preparation of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers Using Raman Spectroscopy, IR Spectroscopy, or Pyrolysis-GC/MS

E131 Terminology Relating to Molecular Spectroscopy

E1617 Practice for Reporting Particle Size Characterization Data

E2651 Guide for Powder Particle Size Analysis

## 3. Terminology

3.1 *Definitions*—For definitions of terms relating to this test method, refer to Terminologies D1129 and D883, and for definitions of terms relating to molecular spectroscopy, refer to Terminology E131.

### 3.2 *Definitions of Terms Specific to This Standard*:

3.2.1 *microplastic, n*—plastic particle less than 5 millimeters (mm) in size.

3.2.1.1 *Discussion*—The lower size of a plastic particle determined as a microplastic is usually classified as 1 micron ( $\mu\text{m}$ ); however, the smaller size is limited by sample collection, sample preparation, and analysis.

<sup>2</sup> For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

#### 4. Summary of Test Method

4.1 This test method has been validated for microplastic particle size distribution, shape, and particle counts in sample extracts containing particles between 5 μm and 100 μm.

4.2 This test method is applicable to microplastic particles in municipal wastewaters, including sewage, treated effluent, the rivers in which they are discharged, ambient waters, finished drinking water, and bottled water.

4.3 Samples are collected by Practice **D8332** and prepared for analysis using Practice **D8333**.

4.4 This test method is applicable to common plastics including polyethylene (high-density polyethylene (HDPE) and low-density polyethylene (LDPE)), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET), and polystyrene (PS). The preliminary sample preparation by Practice **D8333** removes most non-plastic material that would otherwise interfere.

4.5 The quantity and size distribution of microplastic particles present in a sample are counted and theoretical estimates can be inferred for the larger body of water.

#### 5. Significance and Use

5.1 Many microplastic particles enter the environment, including ambient waters and drinking water supplies, via wastewater sources resulting from both industrial processes and consumer products. The presence of high percentages of organic particles, including cellulose material originating from toilet paper and chitin-based materials originating from insect exoskeletons, makes visual identification and subsequent quantification of microplastic particles in wastewater difficult.

5.2 This test method, associated sampling Practice **D8332**, and preparation Practice **D8333** provide a standardized approach for the preparation of water and, particularly, wastewater samples. The isolation of microplastic particles from interfering contaminants by Practice **D8333** enables positive identification and, therefore, quantification of microplastic particles.

5.3 Using this test method, microplastic particles are characterized in terms of size, shape, and quantity, allowing for the enumeration of subsequent particle count for a given volume of sample. The method does not provide qualitative identification of plastic composition.

#### 6. Interferences

6.1 Because of the high percentage of organic matter, cellulose, chitin, skin fragments, and clothing fibers present in wastewater, the complete differentiation of microplastic particles is impractical, if not impossible. Samples shall be collected using Practice **D8332** and prepared using Practice **D8333** to facilitate identification of microplastic particles and to make imaging analysis feasible. Practice **D8333** digests all non-plastic material.

6.2 This test method is used for particle sizes of 5 μm to 100 μm in diameter that may be too small for accurate identification and quantitative analysis by optical microscopy

techniques. Use infrared (IR) or Raman to identify and quantitate microplastics greater than 100 μm.

6.3 The solvents used in this test method may contain particles within the measurement range (size) of the method. In addition, pipette tips may contain microparticles and require a preliminary rinse.

6.4 Other techniques (such as infrared microscopy and gas chromatography/mass spectroscopy pyrolysis) are complementary and provide additional information, such as qualitative identification of individual polymers. Each of these test methods uses samples collected and prepared by Practices **D8332** and **D8333**.

#### 7. Apparatus

7.1 Refer to Guide **E2651** for a detailed description of the Dynamic Digital Image Processing technique.

7.1.1 *Principle of Operation*—A sample of particulate material is dispersed in a liquid stream. As the particles pass through the measurement zone they are illuminated, either from front or back, to create two-dimensional projected images that are captured by a digital camera and then analyzed using image analysis software. Both size and shape information can be obtained. Use an analyzer capable of determination of size distribution, shape, and particle counting in sizes of about 5 μm to 100 μm. It is desirable that the cell width is smaller than the depth of field.

7.1.2 *Specific Requirements:*

7.1.2.1 The suspension must remain dispersed in the dilution solvent.

7.1.2.2 The rate of fall is slowed by the dilution solvent of high viscosity used in this test method.

#### 8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society,<sup>3</sup> where such specifications are available. Other grades may be used provided they are pure enough to be used without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification **D1193**.

8.3 *Glycerin (sp.gr. 1.26)*—Glycerin or glycerol 100 % (C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>).

8.4 *Methanol (sp.gr. 0.791)*—Methanol (CH<sub>3</sub>OH).

8.5 *Dilution Solvent*—(1 + 1) methanol and glycerin.

8.6 *Standard Beads*—Beads, 10 μm, 20 μm, 50 μm, and 100 μm, are available from commercial suppliers. These are available with defined size distribution and an approximate

<sup>3</sup> *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

count. Some certified reference materials (CRM) may include particle count also. Use the CRM as a primary standard to verify counts of other reference materials (RMs) even if of a different size, if necessary.

8.6.1 The aliquoting of standards required by this test method may result in a slight 10 % to 15 % negative bias.

8.6.2 Some RMs may contain contaminant particles of a size significantly smaller than labelled. For calibration verification using RMs, set the instrument to discard readings of particles that fall 20 % or more outside of the expected size of the particles in this test method.

## 9. Hazards

9.1 Some of the chemicals specified in this test method may be hazardous (see 1.5).

## 10. Procedure

10.1 Following sample preparation as described in Practices D8332 and D8333, a suitable sample may be prepared for subsequent analysis using this test method.

10.2 Pipet 4750 µL of dilution solvent into a 15 mL centrifuge tube.

10.3 Add 250 µL of Practice D8333 well-mixed sample extract (microplastic particles suspended in methanol) into the tube. During pipetting, be aware of potential segregation caused by the sedimentation of larger particles.

10.4 Mix using a 10 mL pipet by pulling and dispensing 4 mL at least 20 times.

10.5 Follow the manufacturer's instructions to introduce the sample into the instrument. Collect size distribution and concentration (count/mL). Particle images may be captured and saved for sample shape analysis.

## 11. Calculation or Interpretation of Results

11.1 Calculate concentration in the Practice D8333 extract as follows:

$$\text{Conc} = A \times 5000/B \quad (1)$$

where:

conc = concentration (counts/mL) of particles in the Practice D8333 extract,

A = concentration (counts/mL) of particles measured by the instrument (10.5), and

B = volume of sample in microlitres added to the dilution solvent (typically 250 µL).

11.2 Visually examine the images and classify the shapes as bead, fragment, or fiber. Other shapes may be identified depending on project needs.

## 12. Quality Assurance/Quality Control (QA/QC)

12.1 To be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following QC procedures shall be followed.

12.2 *Calibration and Calibration Verification*—Follow manufacturer's recommendation for calibration or calibration verification.

12.2.1 Collect images on microbeads or standard reference materials of known particle size and particle counts, if available, and verify a recovery and particle size distribution within 10 % of the theoretical value.

12.3 *Analyst Performance Check*—If a laboratory has not performed the test before or there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study shall be performed to demonstrate laboratory capability.

12.3.1 Analyze four replicates of 10 µm, 20 µm, 50 µm, and 100 µm. Particle sizes outside of 20 % of the known particle size of this test method may be excluded from this test.

12.3.2 Calculate the mean, standard deviation, and size distribution of these values and compare to the acceptable ranges of precision and bias that may be calculated by the user using the precision and bias relationships listed in Section 14. This study should be repeated until the single-operator precision and the mean recovery are within acceptable limits. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the *F*-test and *t*-test in evaluating the acceptability of the mean and standard deviation.

12.4 *Laboratory Control Sample (LCS)*—To ensure that the test method is in control, analyze an LCS of a 10 µm or 20 µm particle size standard diluted to contain at least 1000 particles per extraction batch. The LCS shall be taken through all of the steps of the analytical method, including sample preservation and pretreatment. The value obtained for the LCS should match the expected particle size within 20 % and the count should be within 70 % to 130 % of the expected concentration. If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch shall be reanalyzed, or the results qualified with an indication that they do not fall within the performance criteria of this test method.

12.5 *Matrix Spike (MS)*—To check for interferences in the specific matrix being tested, perform a MS at least once on a representative sample from each sample location or type being tested by spiking with a known number of particles of a known particle size and taking it through the complete sample preparation and analytical method.

12.5.1 The spike concentration plus the background concentration of the analyte should not exceed 130 %. The spike shall produce a concentration in the spiked sample 3 to 10 times the background concentration or 10 to 50 times the detection limit of the test method, whichever is greater.

12.5.2 Calculate the percent recovery of the spike (*P*) using:

$$P = (A - B)/A \times 100 \quad (2)$$

where:

A = analyte concentration (number of particles) in spiked sample, and

B = analyte concentration (number of similar resin particles) in unspiked sample.

12.5.3 The percent recovery of the spike shall fall within limits to be specified in advance by the user or within 50 % to 130 % if no values are specified. If the percent recovery is not