Designation: D8333 – 20

Standard 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[1]

This standard is issued under the fixed designation D8333; 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 reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice provides for the sample preparation of collected water samples with high, medium, or low suspended solids to determine the presence, count, polymer type, and physical characteristics of microplastic particles and fibers. It has been designed for the preparation of samples collected from drinking water, surface waters, wastewater influent and effluent (secondary and tertiary), and marine waters using collection practice (Practice D8332). This practice is not limited to these particular water matrices; however, the applicability of this practice to other aqueous matrices must be demonstrated.

1.2 This practice consists of a wet peroxide oxidation followed by progressive enzymatic digestion to the extent necessary to remove interfering organic constituents such as cellulose, lipids and chitin that are typically found in abundance in water matrices of samples with high to medium suspended solids such as wastewater influent. For water samples with low suspended solids, such as but not limited to drinking water and tertiary treated wastewater, the oxidation and digestion steps may not be necessary.

1.3 Water samples prepared using this practice are suitable for analysis utilizing either Pyrolysis-GC/MS methods for qualitative identification and mass quantitation, or IR spectroscopy or Raman spectroscopy for identifying the quantity (number count) and composition (polymer type) of microplastic particles. If desired, microplastic particle size and shape may be ascertained with appropriate instruments such as a scanning electron microscope (SEM) and microscopy techniques.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D883 Terminology Relating to Plastics

D1193 Specification for Reagent Water

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

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D883.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *effluent, n*—any stage of treated wastewater.

3.2.2 *influent, n*—raw sewage entering a wastewater treatment facility.

3.2.3 *microplastic, n*—any solid, synthetic organic polymeric material to which chemical additives or other substances may have been added, which are particles <5 mm in their largest dimension, and fibers no longer than 15 mm in length with an aspect ratio of at least 30:1 and <500 µm in its smallest dimension.

[1] This practice 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] 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.

3.2.4 *surface waters, n*—a water body with its surface in contact with the ambient atmosphere, examples which include lakes, rivers, and streams.

3.2.5 *suspended solids, n*—refers to all matter which remain in suspension in water media and that are removed by a 0.45 µm filter.

4. Summary of Practice

4.1 This preparation practice is applicable for water samples with high to very low suspended solids. All water samples should be collected using procedure in Practice D8332, Section 7. For those water samples with high to medium suspended solids, it consists of a wet peroxide oxidation followed by progressive enzymatic digestion of sieve contents. It is appropriate for collected raw wastewater influent, secondary wastewater effluent, and surface waters samples which are high in suspended solids to remove interfering organic constituents such as cellulose, lipids, and chitin that are typically found in abundance in these water matrices. The preparation steps are completed in the following sequence:

4.1.1 Wet peroxide digestion (1 hr),

4.1.2 Centrifuge and decant excess liquid (15 min),

4.1.3 Cellulose digestion with modified Schweizer's reagent (5 min),

4.1.4 Centrifuge and decant excess liquid (5 min),

4.1.5 Schweizer's reagent quench with 30 % NH_4OH (5 min),

4.1.6 Buffer sample to pH 8 with Tris-HCl (5 min),

4.1.7 Simultaneous protease and lipase digestion (20 hrs),

4.1.8 Centrifuge and decant excess liquid (5 min),

4.1.9 Water wash (5 min), and

4.1.10 Centrifuge and decant excess liquid, add 10 mL of CH_4OH.

4.2 Samples collected using Practice D8332 are suitable for preparation using this practice.

5. Significance and Use

5.1 Large volumes of water are required to be sieved for accurate quantification of microplastics. Water with high to medium content of suspended solids can lead to an excess of inorganic and organic background material which can interfere with the ability to conduct reliable analyses. The presence of this background material can often impede the ability to accurately discern, distinguish and identify the number of microplastic particles in solution.

5.2 The digestion described in this procedure allows for significant reduction of interfering substances and contaminants, rendering a sample suitable for particle and fiber characterization and identification using either Raman and IR spectroscopic analysis or for polymeric quantification and identification by Pyrolysis-GC/MS.

5.3 For water samples with medium to low suspended solids, the oxidation and digestion steps necessary will be dependent upon the type and nature of interfering substances and contaminants and may be determined through simple trial efforts.

6. Reagents and Materials

6.1 Purity of water shall be reagent water as defined by Type IV of Specification D1193.

6.2 *Conical centrifuge tubes*, 50 mL, with lids.

6.3 *Temperature controlled orbital test tube shaker*, or similar test tube rocker.

6.4 *Laboratory centrifuge and rotor*, suitable for containing 50 mL conical centrifuge tubes.

6.5 *Analytical balance* (precision to 0.1 mg).

6.6 *Digital pH meter*.

6.7 *Protease Reagent*—Protease from *Bacilius licheniformis* – ≥ 2.4 units/g with storage temperature from 2–8°C.

6.8 *Lipase Reagent*—Lipase from *Aspergillus oryzae* – solution, ≥ 100 000 units/g with storage temperature from 2–8°C.

6.9 *Hydrogen peroxide*, 30 %.

6.10 *Tris HCl buffer* 1 M, pH 8.

6.11 *Methanol*.

6.12 *Graduated cylinder*, 25 mL, glass.

6.13 *Copper (II) hydroxide*, granular.

6.14 *Ammonium hydroxide*, 30 %

7. Procedure

7.1 *High to Medium Suspended Solids:*

7.1.1 Transfer collected sieve materials [from the 0.25 L glass collection jar (petri dish) using procedure in Practice D8332, 7.1.8 and 7.2.7] into a 50 mL centrifuge tube using 25 mL of 30% H_2O_2. Begin wet peroxide oxidation of organic matter by capping and placing centrifuge tube in the test tube rocker for 60 minutes at 20 rpm.

7.1.2 Centrifuge contents at 5000 rpm for 3 minutes to create a sediment pellet within the tube.

7.1.3 Using a disposable glass pipette, remove as much of the remaining hydrogen peroxide solution as possible from the sample without disturbing the sediment fraction.

7.1.4 Prepare a modified Schweizer's reagent consisting of 2.5 g copper (II) hydroxide added to 100 mL of 30% ammonium hydroxide. Stir contents for 10 minutes to dissolve. Contents will not dissolve completely due to an excess of copper hydroxide.

7.1.5 Initiate cellulose digestion by adding 40 mL of the modified Schweizer's reagent to the centrifuge tube containing sample. Make sure to avoid the addition of any undissolved copper (II) hydroxide crystals from the modified Schweizer's reagent.

7.1.6 Place a cap on the centrifuge tube and place in the test tube rocker for 5 minutes at 20 rpm.

7.1.7 Remove the centrifuge tube from the test tube rocker and centrifuge contents for 3 minutes at 5000 rpm.

7.1.8 Using a disposable glass pipette, remove as much of the modified Schweizer's reagent from the sample as possible without disturbing the sediment fraction.

7.1.9 Add 25 mL of 30 % ammonium hydroxide to the centrifuge tube containing sample to solvate any remaining modified Schweizer's reagent.

7.1.10 Place a cap on the centrifuge tube and place in the test tube rocker for 5 minutes at 20 rpm.

7.1.11 Remove the centrifuge tube from the test tube rocker and centrifuge contents for 3 minutes at 5000 rpm.

7.1.12 Using a new disposable glass pipette, remove as much of the 30% ammonium hydroxide solution from the sample as possible without disturbing the sediment fraction.

7.1.13 To prepare solution pH for enzymatic digestion, add 20 mL of 1M Tris HCl buffer, pH 8, to the centrifuge tube containing sample to solvate any remaining ammonium hydroxide.

7.1.14 Place a cap on the centrifuge tube and place in the test tube rocker for 5 minutes at 20 rpm.

7.1.15 Remove the centrifuge tube from the test tube rocker and centrifuge contents for 3 minutes at 5000 rpm.

7.1.16 Using a disposable glass pipette, remove as much of the Tris HCl buffer solution from the sample as possible without disturbing the sediment fraction.

7.1.17 Add 15 mL of protease solution to the centrifuge tube containing sample.

7.1.18 Add 20 mL of Tris HCl, pH 8, buffer to the centrifuge tube containing sample.

7.1.19 Add 5 mL of lipase to the centrifuge tube containing sample.

7.1.20 Place a cap on the centrifuge tube and place in the test tube rocker for 20 hours at 20 rpm and 45°C.

7.1.21 Remove the centrifuge tube from the test tube rocker and centrifuge contents for 3 minutes at 5000 rpm.

7.1.22 Using a disposable glass pipette, remove as much of the enzyme/Tris HCl buffer solution as possible from the sample without disturbing the solid fraction.

7.1.23 Wash any remaining buffer/enzyme from the sample by adding 40 mL water to the centrifuge tube containing sample.

7.1.24 Place a cap on the centrifuge tube and place in the test tube rocker for 5 minutes at 20 rpm.

7.1.25 Remove the centrifuge tube from the test tube rocker and centrifuge contents for 3 minutes at 5000 rpm.

7.1.26 Using a disposable glass pipette, remove as much of the water as possible from the sample without disturbing the sediment fraction.

7.1.27 Add 10 mL of methanol, washing any sample contents from the sides of the centrifuge tube.

7.1.28 Centrifuge contents for 3 minutes at 5000 rpm.

7.2 *Low to Very Low Suspended Solids:*

7.2.1 Transfer collected sieve materials [from the 0.25 L glass collection jar (petri dish) using procedure in Practice D8332, 7.3.7] into a 50 mL centrifuge tube using 10 mL of methanol, washing any sample contents from the sides of the centrifuge tube.

7.2.2 Centrifuge contents for 3 minutes at 5000 rpm.

7.3 Sample is now ready for transfer of sediment material onto a suitable glass microscope slide or appropriate filter for spectroscopic analysis by either micro-IR or micro-Raman analysis or for collection into an appropriate cup for Pyrolyis-GC/MS analysis.

8. Quality Assurance/Quality Control

8.1 To ensure that plastic particles are recovered and not destroyed during the preparation, obtain reference spheres of at least one resin type, preferably 150–250 µm in size, and preferably a bright color such as red, green, or yellow. Add between 5–10 particles per extraction batch after sieved materials have been placed in centrifuge tube, immediately prior to starting wet oxidation process (step 7.1 and 7.2). The reference spheres must be taken through all of the steps of the extraction.

8.2 Following completion of sample preparation in step 7.1.28 and 7.2.2, plate each sample containing the reference spheres on either a microscope slide or appropriate filter depending on the appropriate analytical method.

8.3 Use visual microscopy to verify the presence of both quantity and structural integrity of the reference spheres.

8.4 Analyst should be able to identify a minimum of 80 % of the reference spheres. Identification of >120 % of the reference sphere particles or fragments is an indication that the preparation is overly aggressive and causing fragmentation of particles. Shaker speed and centrifuge speed should be reduced until fragmentation is no longer observed.

8.5 Initially, a negative control (blank) will be determined by a petri dish, centrifuge tube, pipette and slide that have gone through the complete preparation process (handling, oxidation, digestion, centrifuging, removal, slide placement and subsequent analysis), minus sample sieve materials, for each preparation of interest.

9. Keywords

9.1 all water matrices; analytical quality assurance; calibration samples; collection procedures; count-based; drinking water; high turbidity waters; low turbidity waters; mass-based; microplastic pollution measurement; microplastics; proficiency samples; quality assurance; quality control; quantification procedures; reference samples; sample preparation; sampling procedures; suspended solids; wastewater