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Designation:D8402-23

Standard Practice for Development of Microplastic Reference Samples for Calibration and Proficiency Evaluation in All Types of Water Matrices with High to Low Levels of Suspended Solids

This standard is issued under thefixed designation D8402;the number immediately following the designation indicates the year of original adoptionor,in the case of revision,the year of last revision.A number in parentheses indicates the year of last reapproval.A superscript epsilon(e)indicates an editorial change since the last revision orreapproval.

1.Scope

1.1 This practice describes manufacturing methods to create microplastic particles from pellets of common polymers and the preparation of microplastic reference samples for calibration and proficiency evaluation of microplastic collection practices,preparation practices,and identification methods.

1.2 This practice does not describe methods for controlling or characterizing the shapes of particles.The procedures have been observed to yield irregularly shaped particles,the use of which in many cases will serve to remove the analytical bias inherent with using distinctive manufactured spherical beads. Other procedures should be used if spheres or elongated fibers are desired.

1.3 This practice does not describe handling procedures for waste generated when executing the procedures described herein.It is the responsibility of the user of this practice to follow applicable laws and regulations when manufacturing and disposing of microplastic particles,and to establish appropriate procedures to minimize the amount of waste generated.

1.4 The values stated in SI units are to be regarded as standard.No other units ofmeasurement 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 limitationsprior 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

D1921 Test Methods for Particle Size(Sieve Analysis)of Plastic Materials

D5905 Practice for the Preparation of Substitute Wastewater

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

2.2 ISO Standards:3

ISO 8573-1:2010 Compressed air —Part 1:Contaminants and purity classes

3.Terminology

3.1 For definitions of terms used in this practice,refer to Terminology D883.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 glass transition temperature,n—a temperature above which an amorphous material transitions from a relatively brittle material to a viscous,supercooled liquid

3.2.2 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

3.2.3 suspended solids,n—includes all matter that is removed by a 0.45 μm pore size filter.

4.Summary of Practice

4.1 Polymer pellets are processed in a laboratory grinder that utilizes a spinning blade to fragment the pellets into

[1]This practice is under the jurisdiction of ASTM Committe 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.ForAnmualBook of ASTM Standards volume information,refer to the standard's Document Summary page on the ASTM website.

3Available from American National Standards Institute(ANSD),25W.43rd St., 4th Floor,New York,NY 10036,http://www.ansi.org.

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powders containing particles of various sizes.This practice does not describe methods of separation of the mixed-size powder into fractions based on size ranges.Test Methods D1921 describes methods for dry sieve separation of polymers that could be used to separate the powders described in this practice.If particle fractions under approximately 20 µm in diameter are desired,suspension of the particles in liquid followed by filtration or sedimentation methods may be more effective than dry sieve separation.

4.2 Polymers with a glass transition temperature (Tg)below room temperature require a different grinding procedure than polymers with Tg above room temperature.Polymers with high Tg tend to undergo brittle fracture during grinding,while polymers with low T,tend to be stretched into very elongated structures,and special steps must be taken to grind them effectively.

4.3 Two grinding procedures are described:

4.3.1 Procedure A—This method uses a blunt-edged blade to fragment brittle polymers with a Tgabove room temperature, such as polystyrene (PS),polyvinyl chloride (PVC),and polyethylene terephthalate (PET).

4.3.2 Procedure B—This method uses liquid nitrogen to embrittle polymers with Tg below room temperature,such as polyethylene (LDPE,HDPE)and polypropylene (PP).Cryo-milling or cryo-crushing is the process of cooling or chilling a material and then reducing it into a smaller particle size.While the use of a specialized cryo-mill may be considered to ensure production of a wide size distribution of particles in this procedure,the cooled polymers are coarsely fragmented with a blunt-edged blade and then fragmented into smaller particles with a sharp cutting blade.

4.4 Microplastic reference sample preparation includes two approaches,(1)count-based reference samples for %recovery and (2)mass-based proficiency samples,which possess some inherent variability between samples.

4.4.1 Count-based Microplastics Reference—40 mL vials of DI H_2O containing 20 to 40 microplastic particles are shipped at room temperature to recipient laboratories to spike their own water matrix samples.Because a lab's water samples may already contain microplastics,the reference microplastics for each vial should be documented with saved microscopy images,so their morphologies can be checked against the particles identified by the recipient lab.A 100%transfer efficiency of particles to vials should be demonstrated.This method is best suited to low numbers of reference samples due to the effort of documenting and transferring individual par- ticles.As such,this approach is most applicable to generating reference samples for one's own laboratory or partner labora- tories.In addition,due to the relatively low numbers of microplastics per sample,it will yield the most environmen- tally realistic concentrations when spiking already collected samples such as filters or subsamples for analysis,rather than the original water volumes,which can be orders of magnitude larger.

4.4.2 Mass-based (Gravimetric)Microplastic Standards— Gravimetric reference samples are prepared in large batches of simulated water matrices.Bulk quantities of reference micro-plastics are measured in terms of mass on a balance and added to a simulated water matrix substitute,for example,Practice D5905.Measurable amounts of microplastic(>1 mg)are diluted into several liters of simulated water matrices,then split into several 1 L reference samples.This approach is typically used to prepare reference or proficiency samples for other common environmental analytes such as organic solvents or asbestos.Even with the homogenization step described in **12.6,** uniform microplastic counts may be more difficult to achieve in split samples compared to other analytes,so assessments of the standard deviations of both the microplastic mass/sample within the batch,as well as the equivalent number of particles per sample,are critical for quality control.Depending on the standard deviation,this approach may only be suitable for order of magnitude assessments of accuracy.In addition,the completed reference samples should be analyzed by one or more reference laboratories to determine the truemean value of the reference samples.Alternatively,the mean count may be determined from all participant labs.Despite the inherent variability,this approach is capable of generating many more reference microplastics per batch.As such,it may be a desirable when creating samples for large numbers of laboratories,or if environmentally realistic microplastic con-centrations are required in very large water volumes.If shipping 1 L vessels of non-DI water,the procedure must include packing safely with ice and providing any needed shipping documentation.This approach will also yield a more direct comparison to the results ofmass-based methods such as Pyr-GC/MS.

5.Significance and Use

5.1 These procedures can be used to generate microplastic particles as a simulation of microplastic particles found in the natural environment.Suitable uses may include evaluation of microplastic detection and imaging methods.Use of reference samples will support estimation of ambient and flux concen-trations in drinking water,wastewater and natural environments,investigations of microplastic particle degradation,and ingestion of microplastics by animals in the contexts of food safety and human health risk assessment.

6.Apparatus

6.1 Glass Sample Jars—Clean glass jars to store polymer powder.If polymer lids are used,lid composition should be recorded in case potential contamination is discovered;lids with polymer foam liners should be avoided.

6.2 Timer—A timer with a resolution of 1 s or less to measure time intervals during grinding

6.3 Laboratory Grinder—A spinning blade grinder with interchangeable blades and a maximum speed of 20000 rpm to 30000 rpm and output power of approximately 100 w.4 The blade height within the grinding chamber should be adjustable during grinding.Coffee grinders or other consumer-grade

4Cooling times described in this standard were established by tests performed with aspecific make and modelof grinder-thus,cooling times as stated may vary with other devices.

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grinders or mills should be avoided.The following grinder accessories are required:

6.3.1 Impact Blade—A blunt-edged blade to break apart particles by impact.

6.3.2 Cutting Blade—A blade with sharpened edges.

6.3.3 Liquid Nitrogen-tolerant Grinding Chamber-A chamber that is specified by the manufacturer to be safe for use with liquid nitrogen.

*6.3.4 Stainless Steel Spatulas—For dispensing polymer ma-*terials into the grinder,and for transporting powder from the grinder bowl to glass sample jars

*6.4 Vacuum Cleaner—A vacuum cleaner with a high-*efficiency particulate air(HEPA)filter to remove particle dust from the grinder and workbench.

6.5 Compressed Air—A source of Class O compressed air as defined by ISO 8573-1:2010.

6.6 Sonicator—A bath-type ultrasonic cleaner that applies frequency of 40 kHz±10 kHz.The sonicator is used to clean polymer dust from grinder parts and spatulas.

6.7 Type I Reagent Grade Water—Water conforming to Specification D1193 for use as sonicator bath water.

6.8 Infrared Thermometer—Non-contact thermometer to monitor the temperature of polymers during manufacturing

6.9 Bin with Tight-fitting Lid—A container to store jars of manufactured microplastics and prevent inadvertent release of particles

6.10 Certified clean 40 mL glass vials with sealed caps.If caps contain polymer seals,the polymer type should be recorded.

6.11 Aluminum foil

*6.12 Stereo-zoom microscope,with camera and image stor-*age capability.

6.13 Fine-tipped,stainless steel tweezers,probe,pin,or equivalent micro-tool.

6.14 Ultrapure H_2O

6.15 Clean glass petri dish bottom.

6.16 Analytical balance,with precision and calibration weights appropriate for the microplastic masses to be weighed (not required for count-based approach).

7.Materials

7.1 Polymer Pellets—Polymer pellets with documented chemical and material properties may be obtained from chemical suppliers.Pellets with diameters ranging from approximately two to five millimeters are recommended.To minimize bias,all the plastic particles should be free of dyes,pigments, plasticizers,or other additives.The following is a list of common polymers in pellet form available for purchase and use in reference sample preparation:

7.1.1 Polyvinyl chloride (PVC),
7.1.2 Polyethylene (PE),
7.1.3 Polypropylene (PP),
7.1.4 Polystyrene (PS),and
7.1.5 Polyethylene terephthalate(PET).

7.2 Liquid Nitrogen—For causing low Tg polymers to become brittle,which facilitates efficient grinding.

8.Interferences

8.1 The cooling times specified in Procedures A and B depend on the particular grinder used and should be increased if particles become heated above 40℃,as measured with the infrared thermometer.Clumped or charred particles indicate that longer cooling times are needed.

9.Grinding Procedure A

9.1 Install the impact blade on the grinder.

9.2 Fill the grind chamber with approximately 40 mL of pellets of a single polymer type.

9.3 Grind for 10s,then pause for 10 s to allow cooling. Repeat this cycle five times.While grinding,traverse the blade up and down through the vertical extent of the grind chamber, applying 8 to 10 traversals per 10 s grind period.

9.4 Before opening the grinder,retract the blade as far as possible from the bottom of the mixing chamber.Briefly turn on the grinder once or twice in this position to dislodge particles from blade.

9.5 Pause for 1 min to allow particles suspended in the air to settle,then open the grinding chamber.

9.6 If an undesirable quantity of large pellets remain,wait approximately 10 min for the grinder bowl to cool to within 1℃ of room temperature as measured with the infrared thermometer,then repeat 9.3 and 9.4.

9.7 Use a metal spatula to slowly transfer the ground plastic to a glass sample jar

9.8 Store the glass sample jar in a sealed containment bin with a tight-fitting lid.

9.9 Remove the grinder blade and grinding bowl from the grinder body.

9.10 Use the vacuum to thoroughly remove visible polymer powder,and optionally use a jet of compressed air to remove remaining particles that were not removed by vacuuming.

9.11 Ultrasonically clean the blade,bowl,and other detachable grinder components by submerging them in the sonicator for at least 10 min.The sonicator should be filled with Type I reagent grade water.Alternatively,the sonicator bath may be filed with tap water,and the parts to be cleaned may beplaced in a glass jar that contains enough Type I reagent grade water to submerge the parts,with the jar suspended in the tap water above the bottom of the tank such that the top of the jar is exposed above the bath waterline

10.Grinding Procedure B

10.1 Follow **9.1 and 9.2** of Procedure A.

10.2 Fill the grind chamber with liquid nitrogen such that it completely submerges the pellets,then wait for all of the liquid nitrogen to evaporate.

10.3 Follow 9.3-9 5. of Procedure A.

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10.4 Wait approximately 10 min for the grinder bowl to cool to room temperature,then remove the impact blade and replace it with the cutting blade.Do not use liquid nitrogen with the cutting blade

10.5 Repeat 9.3-9.6 of Procedure A with the cutting blade installed in the grinder

10.6 Repeat steps 9.7-9.11 of Procedure A.

11.Count-Based Reference Sample Preparation Procedure

11.1 Plan a range of different target concentration levels for the set of reference samples plus one verification QA sample.

11.1.1 For example,to create a set of six vials at three different concentrations:

11.1.1.1 Two Reference Samples—Two microplastics of each of the five types(10 particles/vial).

11.1.1.2 Two Reference Samples—Four microplastics of each of the five types (20 particles/vial).

11.1.1.3 Two Reference Samples—Six microplastics of each of the five types (30 particles/vial).

11.1.1.4 One Verification Sample—Four microplastics of each of the five types (20 particles/vial).

11.1.2 If mass-based analyses (for example,Pyr-GC/MS) are to be assessed,an estimate should be made of the target mass levels.The following equation assumes spherical particles and is thus intended only to determine approximate target levels;it is not suitable for assessing proficiency:

$$MMp,rer=NMp,ret\times m \quad (1)$$

$$mp=3.14159\times PMp\times d^3/6 \quad (2)$$

where:

MMp ret =estimate of target mass levels,g,
NMP,ref =number of reference particles of the given microplastic type,
mp =estimated mass of a single reference microplastic particle,g,
PMP =density of the microplastic polymer type,g/μm³ (g/μm³=[g/cm³]/[1×10¹²(μm³/cm³)]),and
dp =average reference particle diameter for the microplastic type,μm

11.2 To prepare each sample:

11.2.1 Cover the work surface on stereo-zoom stage with a fresh sheet of aluminum foil.

11.2.2 Use tweezers or needle,or both,and stereo-zoom microscope to transfer approximately four individual microplastics of each of the five types(20 particles total)to a glass petri dish bottom,and document them with stereo-zoom camera image(s).

11.2.2.1 Segregate particles of each microplastic type together in clusters in dish so that each cluster type can be documented clearly in images

11.2.2.2 Acquire stereo-zoom microscope camera image(s) of only those particles that will be transferred into the vial.

11.2.2.3 Set aside any excess microplastic particles in the petri dish,clearly clustered by plastic type,for additional preparations

11.2.2.4 This method is not suitable for particles smaller than 50 μm.

11.2.3 Fill the vial with approximately 35 mL of ultrapure H,O.

11.2.4 Carefully transfer each documented particle to water in a glass vial with clean tweezers or an equivalent micro-tool.

11.2.5 Rinse tweezers with approximately 5mL of ultrapure H_2O into the vial and ensure no particles remain under the microscope.

11.2.6 Cap the vial.

11.2.7 Use a marker to label the cap with a temporary identifier.

11.2.8 Record the number of microplastics present in this vial for each microplastic type,nMP,rer(#).

11.3 Verify transfer efficiency with verification sample prior to shipping to laboratories:

11.3.1 Transfer the verification sample vial's contents into a 1L flask of DI water.(This sample is not intended to be representative of a real water sample;it is intended to assess transfer efficiency only.)

11.3.2 Rinse the empty vial three times with ultrapure or DI water and pour rinses into the 1 L flask.

11.3.3 Filter the contents of the 1 L vial using Practice D8333 filtration procedures described in 7.2

11.3.4 Analyze the contents of the 1 L vial using IR or Raman spectroscopy

11.3.5 Verify 100 %transfer efficiency of all previously documented particles or investigate the method if<100%.

11.4 Create blind sample IDs for distribution to the recipient labs:

11.4.1 Randomize order of vials,then label each vial with sample ID generated by LIMS.

11.4.2 Record correlation between temporary codes and LIMS sample IDs.

11.4.3 Remove temporary marker codes with isopropyl alcohol.

11.5 Pack the reference sample vials for each recipient (for example,laboratory)with padding in a small box;no ice or refrigeration is necessary

11.6 Within each box of vials,include the list of blind LIMS sample IDs.

11.7 Send the following instructions to each recipient:

11.7.1 Enclosed are a set of microplastic vials for spiking all water matrix media samples to test analytical recovery.These vials contain a range of microplastic concentration levels.For each,the plastic types and morphologies of all particles in the vial have been recorded.To spike a water media sample:

11.7.1.1 Select one vial and record its ID.

11.7.1.2 Pour the entire contents of the vial into the sample

11.7.1.3 Triple rinse the empty vial with DI water into the sample.

11.7.1.4 Mix the spiked sample thoroughly.

11.7.1.5 Analyze the spiked sample using your lab's microplastic SOP.The results should include,for each identified microplastic:

(1)Plastic type,and

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(2)Image with scale bar to document the particle morphology and size.

12.Mass-Based (Gravimetric)Proficiency Sample Procedure

12.1 For gravimetric analyses (for example,Pyr-GC/MS), determine the target mass per reference sample Mref

12.2 If count-based analyses (for example,IR or RAMAN) are to be assessed in addition to gravimetric analysis,an estimate should be made of the target number of particles per mass microplastic.The following equation assumes spherical particles and is thus intended only to determine approximate target levels;it is not suitable for assessing proficiency:

$$N(\#)=Met/m \quad (3)$$

$$mp=3.14159\times PMp\times d3/6 \quad (4)$$

where:

Mer =measured mass of one type of microplastic in the reference sample,g,

mp =estimated mass of a single reference microplastic particle,g

PMP =density of the microplastic polymer,g/um³(g/μm³=[g/cm³]/[1×10¹²(μm³/cm³)j],and

dp =average diameter of the microplastic particles,μm.

12.3 Weigh out the mass of each type of microplastic to be added to the batch,Mbtch,MP

12.3.1 Mbatch,MP=Mrer×T,where T is equal to the number of reference sample splits to be generated from the batch,and Mrer is the mass determined in 12.1 or 12.2.

12.3.2 The laboratory's analytical balance shall be maintained as per laboratory policy to insure accuracy and stability of mass measurements.

12.4 Prepare a simulated water sample batch according to the requirements of the analytical laboratories.

12.5 Add the measured microplastic masses of each type to the simulated water sample batch.

12.6 Homogenize the microplastics within the water sample batch as per laboratory policy(for example,shaking or swirling)before each split,and then split it immediately into the number of reference samples assumed in 12.3 . Alternatively,depending on balance capabilities and desired target concentrations,each sample may be prepared individually without splitting.

12.7 Deliver at least five of the reference sample splits to one or more reference laboratories and assess the mean and standard deviation microplastic concentrations of the reference sample splits.

12.7.1 The mean and standard deviation should be calculated in terms of mass or counts of each microplastic type,or both,depending on whether count-based or mass-based analysis methods are to be assessed.

12.7.2 Use the mean and standard deviations to calculate acceptance ranges for laboratories analyzing these samples.

12.8 Pack the reference samples for each recipient (for example,laboratory)with any needed shipping documentation as appropriate for shipper regulations.If shipping vessels of non-DI water,the procedure must include cold shipment to control microbial growth.

12.9 Within each shipment,include the list of LIMS sample IDs and the following instructions:Enclosed are a set of microplastic reference samples in simulated water matrices to test analytical recovery.For each,the plastic types and concentration has been recorded.Analyze these samples using your lab's microplastic SOP to calculate the concentration of each type of microplastic detected.

13.Reference Sample Calculations

13.1 The following equations are used to assess the accuracy of count-based analysis techniques that report the number of microplastics of each type detected (for example,microspectroscopy)

13.1.1 To calculate the equivalent water sample concentrations,NMp(#/gallons):

$$NMp=nMp/V_s \quad (5)$$

where:

nMp =the count for each microplastic type,and

Vs =the water volume of the spiked sample in gallons.

13.1.2 The analytical sensitivity,S,is determined by the water volume of the spiked sample and the minimum detection limit (MDL)of 1 particle:

$$S=MDL/V \quad (6)$$

13.1.2.1 For example,for a 0.1 gal subsample,S(#/gallons) =1/0.1 =10 microplastic particles/gallon is the minimum concentration detectable.

13.1.3 The percent recovery for each microplastic type is:

$$RMp(\%)=[nMp.ab(\#)nMpe(\#)]\times 100\% \quad (7)$$

13.1.3.1 For count-based reference samples,disregard any lab-detected microplastics with images that do not match the documented particles in the original reference sample spikes. These particles were likely present in the laboratories'sample matrix or the result of contamination.

13.1.3.2 For mass-based reference samples,nMP,rer is equal to the count-based mean determined by the reference laboratories.Use the standard deviation determined by the reference laboratories to help interpret recoveries significantly different than 100%.

13.2 The following equations are used to assess the accuracy of mass-based analysis techniques that report the mass of each polymer detected (for example,Pyr-GC/MS).

13.2.1 To calculate the equivalent water sample concentrations,MMp:

$$MMp=mMp/V \quad (8)$$

where:

MMP =water sample concentrations,g/gal

mMPp=mass of each microplastic type,g,and

vg=water volume of the spiked sample,gal.

13.2.2 The analytical sensitivity,S,is determined by the water volume of the spiked sample and the minimum detection limit(MDL),a mass determined by the instrument conditions:

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$$S=MDL/V \quad (9)$$

where:

S =analytical sensitivity,#/gal,
MDL =minimum detection limit,and
V_8 =water volume of the spiked sample,gal.

13.2.2.1 For example,for a 0.1 gal subsample and a 1 pg instrumental detection limit,S(#/gal)=1/0.1 =10 pg microplastic/gallon is the minimum concentration detectable.

13.2.3 The percent recovery for each microplastic type is:

$$RMp(\%)=[mMp,Iab/mMp,re]\times 100\% \quad (10)$$

13.2.3.1 For mass-based reference samples,mMP,reris equal to the mass-based mean determined by the reference laboratories.Use the standard deviation determined by the reference laboratories to help interpret recoveries significantly different than 100 %.

13.2.3.2 For count-based reference samples,mMP,rer is equal to the estimated mass of each microplastic type determined in 11.1.2

14.Keywords

14.1 all water matrices;analytical quality assurance;calibration samples;collection procedures;count-based;effluent; grinders;HDPE;high turbidity waters;influent;LDPE;low turbidity waters;mass-based;microplastic;microplastic contamination;microplastic fibers;microplastic particles;microplastic pollution;microplastics;microplastics in drinking water;microplastics in wastewater;PE;PET;polymers;PP; proficiency samples;PS;PVC;quality assurance;quality control;quantification procedures;raw sewage;reference samples;sample preparation;sampling procedures;simulation samples;stereo-zoom microscope with photo imager;suspended solids

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