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New Standard 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

ABSTRACT

Microplastic particles and fibers are pervasive in the environment, including wastewater effluent, the ocean water column, sediments, animal tissue, and drinking water. This pervasiveness has led to product bans for small plastics (e.g., microbeads used in cosmetic products) and large plastic items that can degrade into microplastics (e.g., bags and straws). In addition, there are new and planned requirements to monitor microplastics in the environment, wastewater effluent, and drinking water. Implementing monitoring programs requires reliable standardized methods and best practice guidelines. Although the quantification and characterization of microplastics in samples are common, the results are not necessarily reliable or comparable because standard field and laboratory methods for collection and identification do not yet exist and the reference materials necessary for quality assurance are not readily available.

A new test method, being developed by ASTM Committee D19 on Water, provides for the determination of microplastic particles. The method describes the procedures for characterizing fiber concentration, size, and shape using image analysis of sample extracts containing particles between 10 and 100 µm. Light is transmitted through a cell containing particles in liquid medium. The particles create shadows as they pass through the field of vision of a camera thus producing a multitude of images. The images are then used to measure size, shape, and concentration.

The method is to be used as a complementary technique for other microplastic particle and fiber polymer identification methods including, but not limited to, WK67565 Spectroscopic Identification and Quantification of Microplastic Particles and Fibers in all High and Low Turbidity Water Matrices including Municipal Wastewater Using IR and Raman Spectroscopy and WK67788 - New Test Method for Identification of Polymer Type and Quantity (Mass) Measurement of Microplastic Particles and Fibers in Waters with High-to-Low Suspended Solids Using Pyrolysis-Gas Chromatography/Mass Spectrometry: Py-GC/MS currently under development by ASTM Committee D19.

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Introduction

In June 2017 in Phoenix AZ, members of the US EPA presented at the summer meeting of ASTM Committee D19 on Water, Subcommittee D19.06 on Organics, describing the problem of microplastic particles in the environment and the need for standardized methods. The presentation led to the establishment of a task group and subsequent work on numerous standards to encompass sampling, sample preparation, preparation of reference materials, and various techniques used to qualitatively identify and quantitate microplastic particles. The task group chose to first work on sampling and sample preparation resulting in ASTM D8332-20 *Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers*[i] and 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*[ii].

These two practices provide the foundation for determinative methods of analysis. These determinative methods include analysis of microplastic particles by Py-GC/MS, currently WK67788, and analysis by IR and Raman spectroscopy, currently WK67565.

The Py-GC/MS method quantitates microplastic particles by determining the concentration and providing a qualitative identification of individual plastics; however, this technique does not provide qualitative information such as size, shape, color, or particle size distribution. The IR method, on the other hand, provides qualitative identification using IR spectral analysis and enables the counting and sorting of individual particles. Concentrations can only be estimated by approximating the volume of the particles and calculating the mass based on the plastic's specific gravity. The IR method also allows analysts to manually view individual particles through the microscope to count and sort (some manufacturers have automated this process). Thus, the IR and Raman method is limited by the ability of the analyst to see the individual particles (Figure 1); the smaller the particles, the harder they are to see.

This limitation of the IR method led us to propose Work Item WK72349 *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*, referred to as the DIA method.

The DIA method complements the IR method by automatically assisting in the estimation of particle size, size distribution, concentration, and particle shape. This method focuses on smaller size microplastics between 10 and 100 µm.

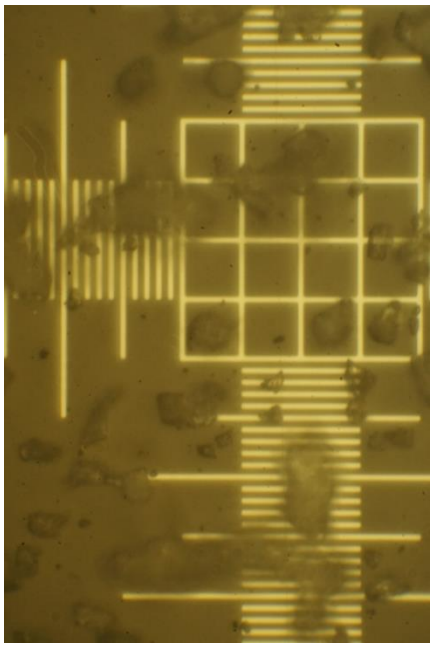

Figure 1: IR microscope slide image showing a mixture of particles with calibration lines 10 µm apart (boxes are 50 µm2)

Materials and Methods

Description of the Proposed Method

The proposed DIA test method provides for the characterization of microplastic particle and fiber size and shape with the capacity to perform image analysis and concentration for samples collected using Practice D8332 and prepared using Practice D8333. The proposed method is restricted exclusively to particles between 10 and 100 µm. Operationally, light is transmitted through a flow cell containing particles in a 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.

The DIA method is applicable to microplastic particles in municipal wastewaters, including sewage, treated effluent, the rivers in which effluent is discharged, ambient waters, finished drinking water, and bottled water. The required digestion procedure included in D8333 removes most non-plastic material, such as natural fibers and cellulose, leaving microplastics such as polyethylene (HDPE, LDPE, PP, PVC, PUR, PET, and PS) to be measured. Other sample preparation techniques, such as flotation with high specific gravity solutions as proposed by ISO TC147, may be used after digestion using D8333, providing further removal of interferences. The result of the D8333 procedure is a methanolic solution containing suspended plastic particles. The quantity and size distribution of microplastic particles present in the prepared sample are measured using the DIA method, and theoretical estimates can be inferred for the larger body of water.

Apparatus

A Shimadzu iSpect DIA-10 (Figure 2) imaging analyzer capable of determination of size distribution, shape, and particle counting in sizes of 10 to 100 µm was used for this study.

Figure 2: Shimadzu iSpect DIA-10 Dynamic Image Analyzer

The system utilizes a microcell and advanced optics to detect particles accurately and efficiently. The iSpect DIA-10 uses a telecentric lens that maintains a constant image magnification. This allows the accurate determination of particle size regardless of the location of the particle in the field of view. An autofocus function increases the imaging efficiency which makes it possible to accurately detect foreign objects and obtain repeatable concentration. Samples are added to the system using a disposable pipettor tip (Figure 3).

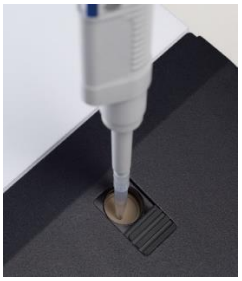

Figure 3: Adding Sample to the iSpect DIA-10

Pump operation and particle imaging are performed automatically according to the analytical conditions selected. In addition, particle images are monitored and measured in real time while the sample flows through the unit. Software-generated results, including scatterplots, histograms, and particle images, are displayed by the instrument. In the proposed ASTM method, an aliquot of sample is diluted in a high viscosity glycerin solvent to slow the flow of particles and improve precision.

Sampling and Sample Digestion Procedure

In accordance with Practice D8332, water samples with high, medium, or low suspended solids are passed through sieves of sufficient mesh size to capture the smallest desired particle size. For waters with high or medium suspended solids content, using a series of sieves with increasingly smaller mesh size prevents clogging and allows for the collection of desired particle size fractions. Water flowing through the sieves is metered to record the total volume, which is used to calculate the particles per unit volume. Contents from each sieve fraction are rinsed and transferred in a minimal amount of reagent water to 250 mL collection jars sorted by sieve size. These sample fractions are then sequentially digested using procedures in Practice D8333 to remove interferences leaving only microplastics.

Practice D8333 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 wastewater. The oxidation and digestion steps used depend on the type and nature of interfering substances and contaminants and substantially reduce the interfering substances and contaminants, rendering a sample suitable for particle and fiber characterization. Once the digestion is complete, the microplastic particles are suspended in 10 mL of methanol, and only the 10 to 100 µm sieve fractions are analyzed.

Analysis Procedure

After sample preparation by D8333, 250 µL of well-mixed sample in methanol is added to 750 µL of a glycerin dilution solvent contained in a 15 mL tube. The sample is mixed using a 10 mL pipet by pulling and dispensing 4 mL at least 20 times. Then, a volume of the well mixed sample is introduced into the instrument previously programmed to collect size distribution and particle counts. Images, or shadows, are saved for later particle shape determination.

Results and Discussion

For calibration verification, the particle counts and particle size of purchased reference materials (RMs) were determined and compared to theoretical values. For this validation, we obtained microbead RMs of 10, 20, 50, and 100 µm. These RMs are certified in particle size with an approximate concentration. To verify linearity, each RM was diluted to four different concentrations ranging between about 15 and 2000 particles per milliliter (Table 1).

Table 1: Reference materials (RMs) with particle size and concentration

Reference Material	Size (µm)	Particles per mL
Microparticle, polystyrene	10	1.82E+08
Microparticle, polystyrene	20	2.27E+07
Microparticle, polymethacrylate	50	1.25E+06
Microparticle, monodispersed polystyrene	100	3.64E+04

Scatterplots of the theoretical particles per mL versus the measured particles per mL for each of the four RMs were created in Microsoft Excel with a trendline; the equation of the line and R-squared value is displayed on the chart. The initial scatterplot for the 100 µm bead data is shown in Figure 4 with a closer look at the lower concentrations shown in Figure 5. The data were similar for all other bead sizes.

The cause of the inaccuracy in counts at the lower concentrations can be seen by examination of a scattergram that plots area-based diameter versus the aspect ratio (Figure 6). In this plot, about 85% of the particles are 100 µm and approximately 15% of the particles are less than 80 µm with most being 20 µm or less. These smaller contaminant particles are sufficient in number to affect measurement at the lower concentrations.

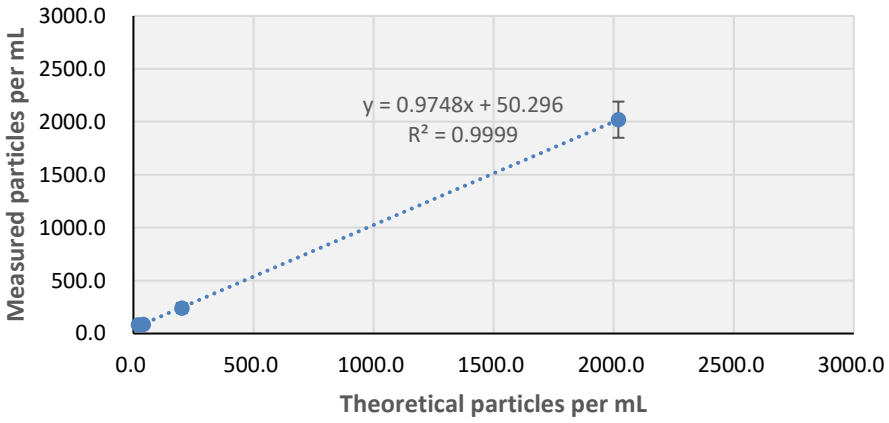

Figure 4: Initial scatterplot of 100 µm bead RM data

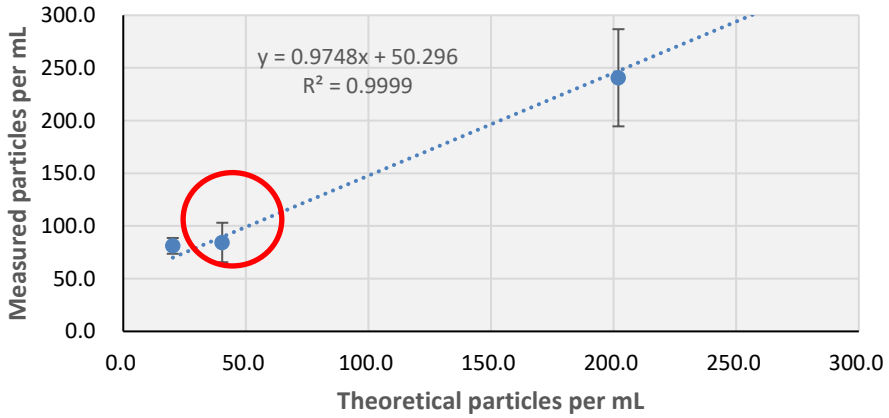

Figure 5: Scatterplot of the 100 µm bead RM at lower concentrations only

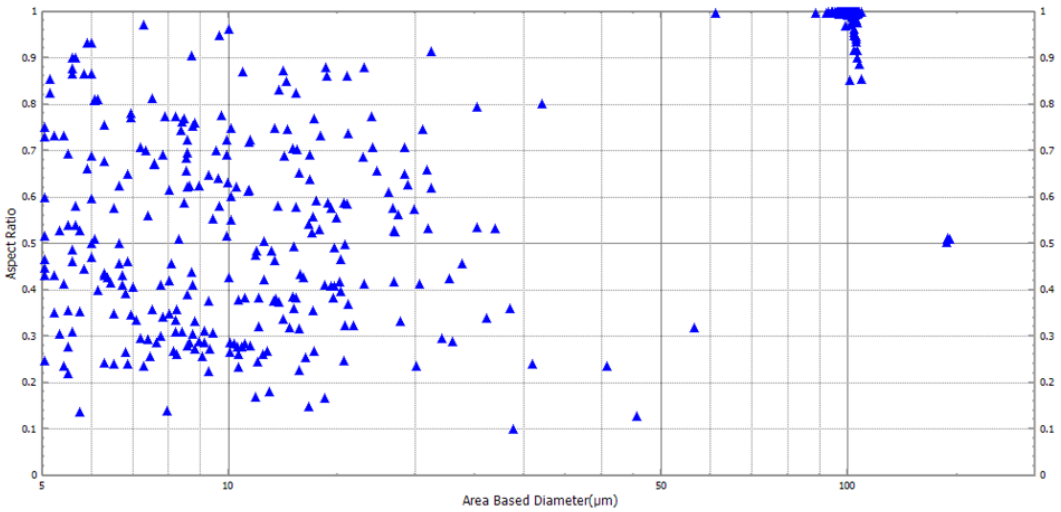

Figure 6: Area-based diameter versus aspect ratio scatterplot of the 100 µm bead RM

Blank Evaluation

To investigate the source of the smaller particles found in the RMs, we examined the dilution solvent and pipetting techniques more closely. To determine the existence of microplastic particle contaminants in the blank, we added dilution solvent to three separate 15 mL plastic tubes prewashed with methanol. The results were 1 particle per mL, 10 particles per mL, and 4 particles per mL. Next, we prewashed the 15 mL tubes as before, and then pipetted 8 mL of solvent, rinsing the pipet tip by pulling in and pushing out the solvent 20 times. This test was performed twice and revealed 11 particles per mL in the first test and 22 particles per mL in the second test. (Figure 7). The particles flushed from the pipet tip ranged in size from 5 to 50 µm with most 20 µm or less.

The data from method blanks show that some small particles are introduced by the plastic tips used when mixing and transferring sample. We analyzed seven separate blanks according to the method and showed an average of 5.8 particles per mL with a standard deviation of 4.6 (Figure 8).

We estimate the detection limit to be approximately 10 to 15 particles per mL. The concentration of particles introduced into the blank is enough to limit quantitation to no lower than approximately 30 particles per mL.

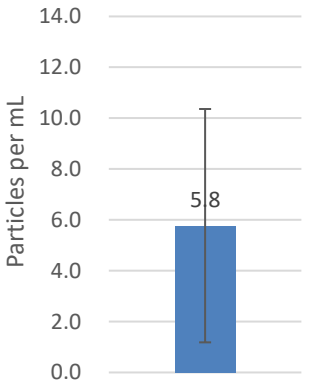

Figure 8: Distribution of particles per mL in a blank

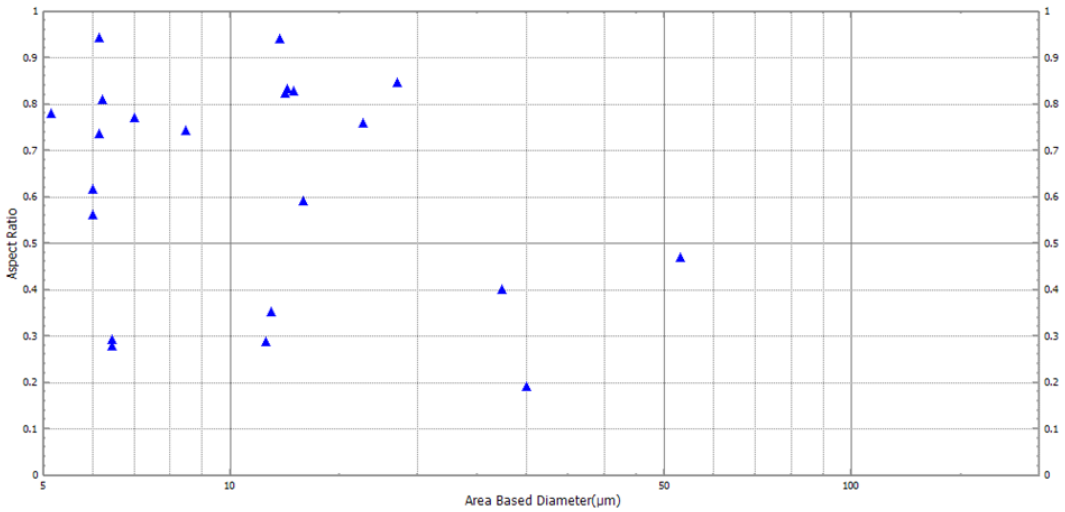

Figure 7: Area-based diameter versus aspect ratio scatterplot of dilution solvent from 15 mL tube prewashed and mixed 20 times

Correcting Results to Account for Smaller Particles

The concentration of particles introduced by the blank, however, does not account for the large percentage of small particles in the RMs, such as shown in Figure 6. In addition, the shape of these smaller particles is not spherical (Figure 9).

Figure 9: Images showing the area equivalent diameter (in µm) of the contaminating particles

We conclude, therefore, that the RMs are contaminated with smaller sized particles, either from the manufacturing process or abrasion after manufacture and during storage or transport.

Data were reprocessed, this time excluding all particles smaller than 80 µm for the 100 µm RM, smaller than 40 µm for the 50 µm RM, and outside the range of ±10% for the 20 µm and 10 µm RMs. We prepared regression plots using Microsoft Excel, comparing the theoretical concentration versus the measured concentrations. Figure 10 shows the corrected 100 µm bead RM, and Figure 11 shows the lower concentrations of the corrected 100 µm bead RM.

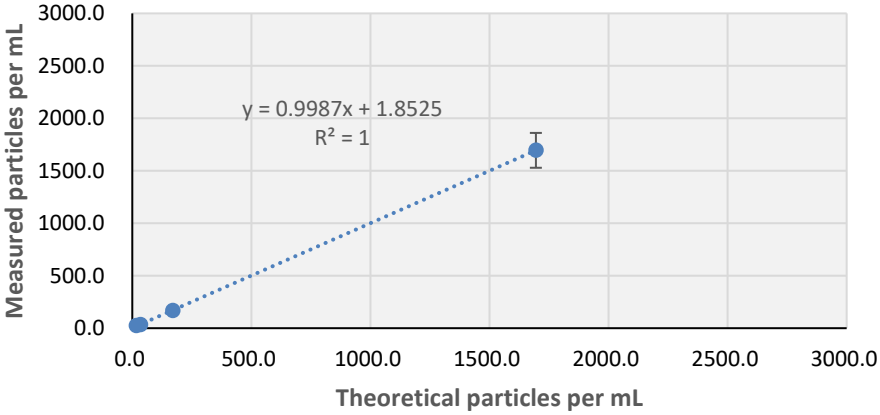

Figure 10: Corrected scatterplot of 100 µm bead RM data showing each concentration analyzed in triplicate, a trend line, the regression equation and the r-squared value

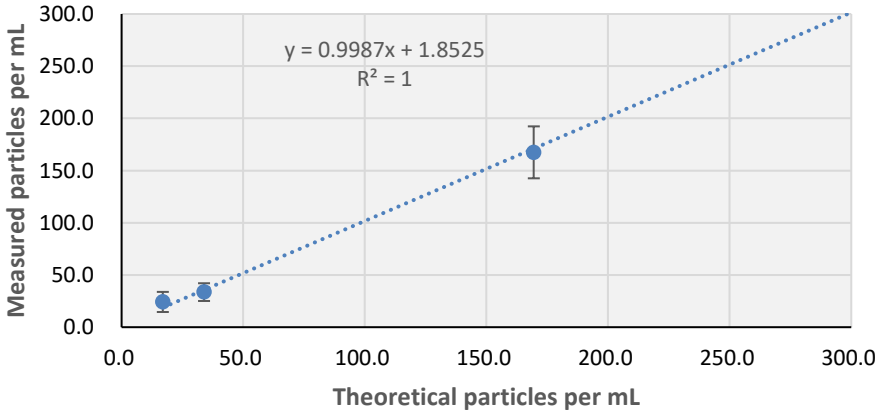

Figure 11: Corrected scatterplot of the 100 µm bead RM, showing lower concentrations analyzed in triplicate, a trend line, the regression equation and the r-squared value

Figures 12, 13, and 14 show the low-level concentration scatterplots of the corrected data for the 10 µm, 20 µm, and 50 µm RMs, respectively.

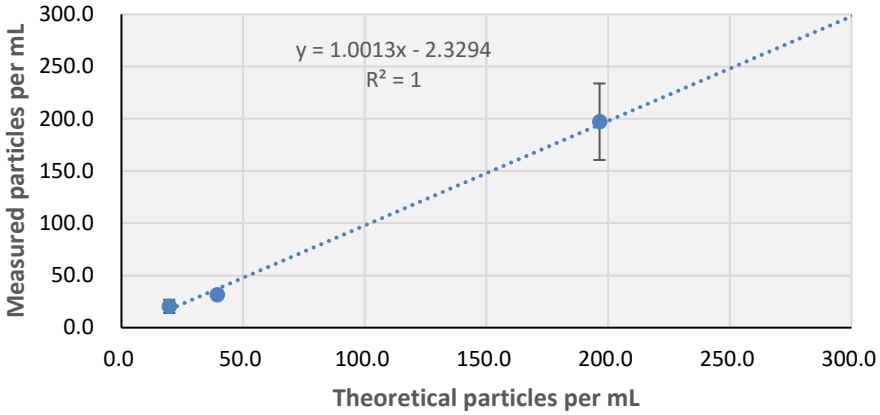

Figure 12: Corrected scatterplot of the 10 µm bead RM, showing lower concentrations analyzed in triplicate, a trend line, the regression equation, and the r-squared value

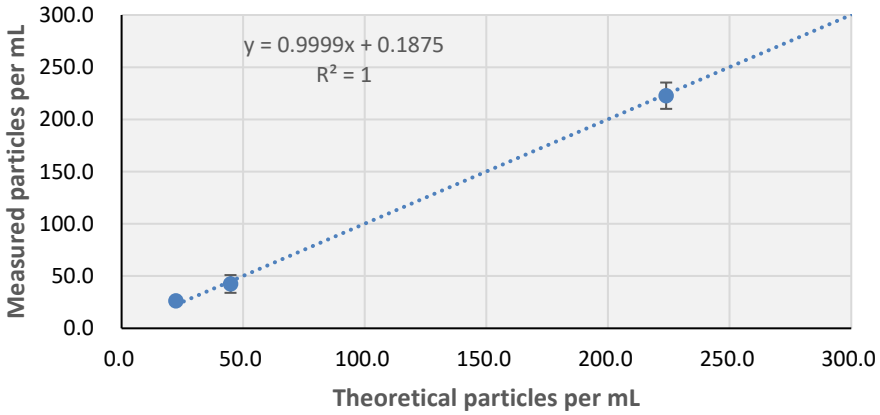

Figure 13: Corrected scatterplot of the 20 µm bead RM, showing lower concentrations analyzed in triplicate, a trend line, the regression equation, and the r-squared value

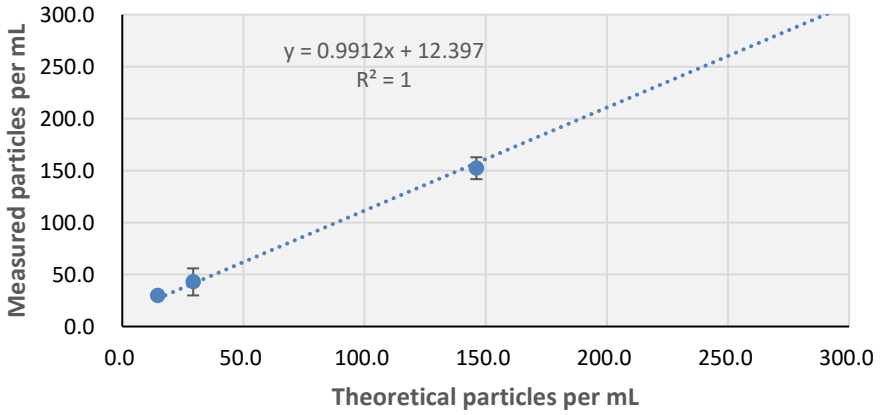

Figure 14: Corrected scatterplot of the 50 µm RM, showing lower concentrations analyzed in triplicate, a trend line, the regression equation and the r-squared value

Final Data Analysis

Each RM was analyzed in quadruplicate at three different concentrations for precision, and one concentration near the estimated 30 particle quantitation limit was analyzed in seven replicates. A summary of these data is shown in Table 2.

Table 2: Precision for various particle sizes and concentrations

Particle Size, µm	Theoretical Concentration, particles per mL	n	Measured Concentration, particles per mL	Standard Deviation	RSD, %
100	1700	4	1700	166	9.8
	170	4	168	24.9	14.8
	34	7	34	8.5	25.3
	17	4	24	9.6	39.8
50	1460	4	1460	78.1	5.3
	146	4	152	10.5	6.9
	29	7	43	13	30.3
	15	4	30	2.5	8.4
20	2240	4	2240	104.7	4.7
	224	4	223	12.6	5.7
	45	7	42	8.5	20
	22	4	26	1.6	6
10	1960	4	1960	28.5	1.4
	196	4	197	36.6	18.6
	39	7	31	2.4	7.6
	20	4	20	6.3	31.1

Conclusions and Next Steps

We examined the preliminary validation of a new ASTM work item for the analysis of microplastics between 10 and 100 µm by direct imaging analysis. The data demonstrate acceptable precision and bias within the scope of the method. Thus far, we have analyzed reference materials of plastic beads of known diameter and concentration. Next steps will include analysis of actual sample matrices after sampling and preparation by Practices D8332 and D8333.

List of Abbreviations

GCMS	gas chromatography mass spectrometry	PP	polypropylene
HDPE	high-density polyethylene	PS	polystyrene
IR	infrared	PUR	polyurethane
LDPE	low-density polyethylene	PVC	polyvinyl chloride
PET	polyethylene terephthalate		

References

i. Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers, ASTM D8332-20 (West Conshohocken, PA: ASTM International)
ii. 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, ASTM D8333-20 (West Conshohocken, PA: ASTM International)

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