National Institute of Standards & Technology

Report of Investigation

Reference Material 8634

Ethylene Tetrafluoroethylene for Particle Size Distribution and Morphology

This Reference Material (RM) is intended for use in validating the counting, sizing, and morphological analysis of liquid-borne particles over an approximate size range of 1 µm to 30 µm. RM 8634 has been developed to closely mimic the optical properties of aggregated proteinaceous particles but may also be applicable in the validation of non-optical instruments. A unit of RM 8634 consists of one vial containing approximately 20 mL of a suspension of ethylene tetrafluoroethylene (ETFE) particles in a solution of 0.02 % sodium azide and 0.02 % surfactant [4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol]. The ETFE particles were prepared by wet abrasion of the bulk polymer against a diamond abrasive pad, followed by filtration and dilution. The particle size distribution is highly polydisperse, and the particles have an irregular morphology.

Reference Values: A NIST reference value is a non-certified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with associated uncertainty that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1,2]. Particle size distribution and morphological reference values are provided in Tables 1 and 2. Values are metrologically traceable to the measurement methods and calibration procedures used.

Information Values: The density of the ETFE source material, prior to the abrasion process, was determined gravimetrically by use of ASTM D792-13 [3]. The density value obtained was 1 707 kg/m^3, which is consistent with the specific gravity range 1.70 to 1.77 reported by ETFE manufacturers. Information values cannot be used to establish metrological traceability.

Expiration of Value Assignment: RM 8634 is valid, within the measurement uncertainty specified, until **15 December 2024**, provided the RM is handled and stored in accordance with instructions given in this report (see "Instructions for Storage, Handling, and Use"). The value assignment is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM: NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Value assignment measurements were performed by S. Telikepalli, K. Steffens, M. Carrier, C. Montgomery and D. Ripple of the NIST Biomolecular Measurement Division, and N. Ritchie of the NIST Materials Measurement Science Division.

Management support was provided by M. Tarlov and D. Ripple of the NIST Biomolecular Measurement Division.

Statistical analysis was provided by J. Lu of the NIST Statistical Engineering Division.

Support aspects involved with the issuance of this RM were coordinated through the NIST Office of Reference Materials.

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Particle Size Distribution Reference Values: The particle size and number concentration were measured using three optical-microscopy methods. Repeatability and all known particle-size and number-concentration biases of the three methods have been accounted for in the stated uncertainty; however, some biases have not been fully evaluated by comparison with independent methods. The results can be expressed as the complementary cumulative distribution $N(d)$, which gives the number of particles per milliliter of solution of diameter greater than or equal to d. Here, d is the equivalent circular diameter equal to the diameter of the circle with the same area as the projected geometric cross section of a particle, with any holes filled. The particles were measured either aligned in the shear flow of a liquid passing through a flow cell of rectangular cross section or after undergoing gravitational settling.
The reference values for number concentration as a function of equivalent diameter are given by:

$$N = b_0 \exp\left[-\left(b_1 d\right)^{b_2}\right]$$

with

b_0 = 96 727.7 mL^{-1}
b_1 = 0.983 21 µm^{-1}
b_2 = 0.523 46

The inverse of the $N(d)$ function is:

$$d = \left(\frac{1}{b_1}\right)\left[\ln\left(\frac{b_0}{N}\right)\right]^{1/b_2}$$

Table 1 gives the values of $\ln[N(d)]$ and $N(d)$ at discrete values of d, along with the expanded uncertainties of these values. The expanded uncertainty of N is calculated using the approximation $U(N) \approx N \cdot U(\ln N)$.

Table 1. Reference Values for the Complementary Cumulative Distribution $N(d)$ in RM 8634

Diameter, d (µm)	$\ln(N \cdot \text{mL})$[a]	N[a][b] (mL^{-1})
1	10.49 ± 0.31	35 900 ± 11 223
2	10.05 ± 0.27	23 270 ± 6 322
3	9.72 ± 0.25	16 615 ± 4 209
5	9.18 ± 0.25	9 682 ± 2 381
7	8.73 ± 0.22	6 215 ± 1 380
10	8.17 ± 0.23	3 538 ± 823
15	7.39 ± 0.23	1 618 ± 373
20	6.72 ± 0.22	832 ± 181
25	6.14 ± 0.22	462 ± 102
30	5.60 ± 0.24	270 ± 65

[a] Values are expressed as $x \pm U(x)$, where x is the reference value and $U(x)$ is the expanded uncertainty of the reference value with a coverage factor of 2. To propagate this uncertainty, treat the reference value as a normally distributed random variable with mean x and standard deviation $U(x)/2$.
[b] The propagation of uncertainty is based on the linear approximation $U(N) \approx N \cdot U(\ln N)$.

Morphological Parameter Reference Values: The morphology of the ETFE particles was characterized using two optical microscopes, corrected for diffraction biases, and a scanning electron microscope. The morphological parameters reported for this RM, as measured from binary images processed from the original images, can be expressed in terms of basic length measurements of the binary image as follows. The ellipse ratio equals the ratio of minor and major axes (L_{min} and L_{maj}) of the ellipse with the same area and ratio of second moments as the measured particle:

$$f_{ell} = \frac{L_{min}}{L_{maj}}$$

The aspect ratio equals the ratio of minimum and maximum Feret diameters (F_{min} and F_{max}):

$$f_{asp} = \frac{F_{min}}{F_{max}}$$

The compactness equals the ratio of equivalent circular diameter, d, to the maximum Feret diameter:

$$f_{comp} = \frac{d}{F_{max}}$$

Figure 1 displays these definitions graphically:

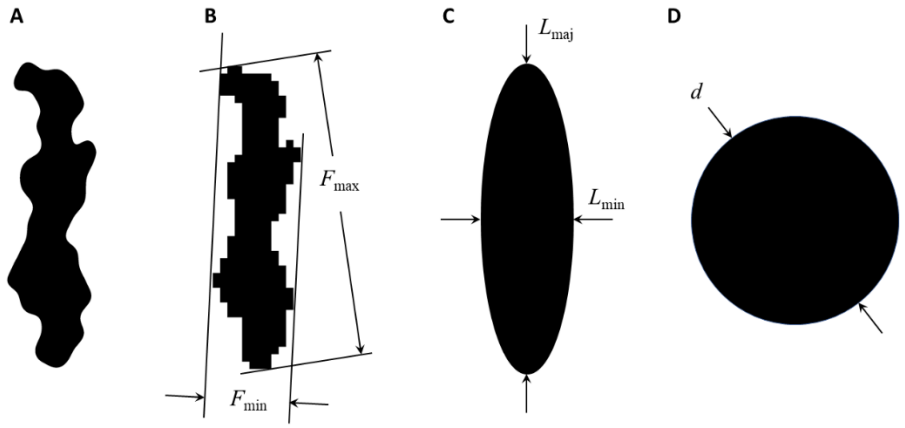

Figure 1. Characteristic dimensions of a binary particle image. From the left, A) the theoretical binary particle image after applying a binary threshold, B) the binary image as obtained with finite pixel size, C) the Legendre ellipse fit to the particle image, and D) a circle of equivalent circular diameter.

Table 2 gives the mean values of the morphological parameters and the expanded uncertainty of these values with a coverage factor of two. The values of each parameter are uniform throughout the equivalent diameter range of 1.2 µm to 25 µm, to within the stated uncertainties. The ETFE particles are highly variable in morphology, and the standard deviation of the measured values characterizes the variability of the ETFE particle population. Table 2 also gives the mean standard deviation *SD* of the particle population and the expanded uncertainty of the mean standard deviation.

Table 2. Reference Values for Morphological Parameters in RM 8634

Parameter	Mean Value[(a)]	*SD*[(a)]
f_{ell}	0.531 ± 0.060	0.180 ± 0.010
f_{asp}	0.568 ± 0.069	0.143 ± 0.017
f_{comp}	0.605 ± 0.055	0.103 ± 0.021

[(a)] Values are expressed as $x \pm U(x)$, where x is the reference value and $U(x)$ is the expanded uncertainty of the reference value with a coverage factor of 2. To propagate this uncertainty, treat the reference value as a normally distributed random variable with mean x and standard deviation $U(x)/2$.

INSTRUCTIONS FOR STORAGE, HANDLING, AND USE

Storage and Handling: RM 8634 should be kept refrigerated at 2 °C to 8 °C for long term storage. The vial should be allowed to warm to room temperature overnight prior to use. RM 8634 may be left in the dark at room temperature for up to one month if measurements are being conducted for several days. Do not subject RM 8634 to vortexing, motorized tumbling, or centrifuging. Such treatment is ineffective at resuspending the particles and can lead to changes in the particle size distribution. Do not subject RM 8634 to freezing temperatures.

Use: ETFE particles will settle to the bottom of the vial. At the beginning of each day of analysis, the ETFE particles must be resuspended. An effective way of resuspending particles is to first ensure that the vial is firmly sealed, then hold the vial horizontally in your hand, with your forearm vertical, and shake back and forth **vigorously** for 20 s (at about 2 back-and-forth shakes per second). Because of the surfactant in the formulation, this procedure will generate a significant amount of foam. Allow the vial to sit for 60 min to 90 min for the foam to dissipate. Slowly tipping and rotating the vials 10 times every 30 min can increase the rate of foam dissipation. Do **not** sonicate to degas the solution.

After short-term storage and between measurements, particles will still be in suspension, but there will be some sedimentation. First ensure that the vial is firmly sealed, then hold the vial horizontally in front of you. Tip the vial gently from side to side 10 times, while slowly rotating the vial with each tip. With each tip, the air bubble in the vial should go from one end of the vial to the other. This short-term resuspension method should be conducted before transferring material or taking a measurement whenever the vial has sat for more than 10 s. After performing the long-term-storage resuspension (vigorous shaking, as described above), we have confirmed that the short-term-storage method suffices to keep particles in suspension over the course of 5 h at a minimum.

The vial and cap are fabricated from perfluoroalkoxy (PFA), a fully fluorinated polymer. Care must be taken to tighten the caps firmly to eliminate leakage. At the same time, excessive torque can lead to production of additional large particles. Friction on the threads of the PFA vials occasionally generates particles. These particles are not an appreciable fraction of the ETFE particles, except at large (>50 µm diameter) sizes. For this reason, the counts at large particle sizes may have poor repeatability.

In cases when multiple draws will be taken from the RM 8634 vial within a month, thread debris can be minimized by first resuspending the ETFE particles and then transferring the particle solution to precleaned Polyethylene Terephthalate Glycol (PETG) vials. The PETG vials are highly resistant to sonication and have minimal thread debris. Long-term storage in PETG is not recommended because extraneous small particles (<2 µm typically) may appear after one month.

The RM 8634 particle solution may be transferred by pipette, provided the suspension is mixed just prior to transfer. Pipette tips should either be confirmed to contribute low particle loads (e.g., pipette tips with built-in barriers) or be thoroughly rinsed before use.

The RM 8634 particle solution may be diluted with water/glycerol mixtures to reduce the optical contrast of the ETFE particles, as described in reference 4. The RM 8634 solution should not be diluted by more than a factor of one part of RM 8634 to three parts of diluent (by volume), in order to ensure that there is sufficient surfactant in the final solution to prevent ETFE agglomeration.

The RM 8634 particles themselves are highly inert and unlikely to interact with any solution that they are added to.

Reference 4 gives procedures on using RM 8634 to determine the bias of particle counting instruments and to determine the particle size distribution of other lots of ETFE particle suspension.

ETFE particles are denser than either commonly used polystyrene latex microspheres or proteinaceous particles. Consequently, sedimentation can alter measured particle size distribution values. Guidance on protocols to minimize the effects of sedimentation are found in reference 4.

Safety: Please refer to Safety Data Sheet.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2020 Edition; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Aug 2021).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/publications/guides (accessed Aug 2021); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Aug 2021).

[3] ASTM D792-13 Standard Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement (2013) ASTM, West Conshohocken PA.

[4] Ripple, D.; Telikepalli, S.; Steffens, K.; Carrier, M.; Montgomery, C.; Ritchie, N.; Lu, Z.Q.J.; *Reference Material 8634: Ethylene Tetrafluoroethylene for Particle Size Distribution and Morphology*; NIST Special Publication 260-193; U.S. Government Printing Office: Washington, DC (2019); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-193.pdf (accessed Aug 2021).

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