Designation: E 1772 – 95 (Reapproved 2001)

Standard Test Method for Particle Size Distribution of Chromatography Media by Electric Sensing Zone Technique[1]

This standard is issued under the fixed designation E 1772; 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 test method is valuable for the measurement of particle size and covers determination of the particle size distribution of chromatography media in the overall size range of approximately 1 to 450 µm using the electric sensing zone (ESZ) apparatus. This instrument uses an electric current path of small dimensions that is modulated by individual particle passage through an aperture and produces individual pulses of amplitude proportional to the particle volume **(1)**.[2]

1.2 The values stated in SI units are to be regarded as the standard.

1.3 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[3]

E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods[4]

E 456 Terminology Relating to Quality and Statistics[4]

2.2 *Other Document:*

Manufacturer's Operating Manual for Particle Size Distribution Analyzer[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *equivalent volume diameter*—the diameter of a sphere with a volume equal to that of the actual particle.

3.1.2 *volume weighted (mass) median diameter*—a number distribution, $n\,(d)$, is measured, and $N = \sum_{\mathrm{i}=1}^{C} n\,(d_i)$ is the total number of particles in the C classes and $n\,(d_i)$ is the number of particles in class i (corresponding to diameter d_i).

3.1.2.1 *Discussion*—From n (d), the corresponding volume distribution can be calculated:

$$f(d_i) = n(d_i)\frac{\pi}{6}d_i^3/V = n(d_i)d_i^3/\sum_{i=1}^{C} n(d_i)d_i^3 \tag{1}$$

where:

$$V = \frac{\pi}{6}\sum_{i=1}^{C} n(d_i)d_i^3 \tag{2}$$

is the total particle volume.

A cumulative volume distribution, $F(d_i)$, is defined by

$$F(d_i) = \sum_{x_i \leq d_i} f(x_i) \tag{3}$$

The *volume weighted (mass) median diameter* is the diameter, d_{50}, given by

$$F(d_i) = 0.5 \tag{4}$$

that is, the diameter that divides the particle volume into two equally sized halves.

4. Summary of Test Method

4.1 A carefully dispersed, dilute suspension of the particles in a beaker filled with electrolyte is placed in the counting position on the instrument sample stand. The suspension is forced through a restricting aperture. Each particle passing is recorded on an electronic counter according to selected particle size levels.

4.2 The instrument determines the particle volume (liquid displacement); therefore, the equivalent spherical diameter is commonly used to express the particle size.

5. Significance and Use

5.1 It is important to recognize that the results obtained by this test method or any other test method for particle size determination using different physical principles may disagree. The results are strongly influenced by physical principles used by each method of particle size analysis. The results of any particle sizing method should be used only in a relative sense and should not be regarded as absolute when comparing results obtained by other methods.

5.2 Both sellers and purchasers of chromatography media will find the test method useful if mutually acceptable to all parties concerned to determine the particle size distributions for materials specifications, manufacturing control, and development and research.

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[1] This test method is under the jurisdiction of ASTM Committee E48 on Biotechnology and is the direct responsibility of Subcommittee E48.03 on Unit Processes and Their Control.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this test method.

[3] *Annual Book of ASTM Standards*, Vol 11.01.

[4] *Annual Book of ASTM Standards*, Vol 14.02.

[5] Available from Coulter Corp., Scientific Instruments, P.O. Box 169015, MC 195-10, Miami, FL 33116-9015.

6. Apparatus

6.1 *Electronic Particle Counter*, using the ESZ technique, for example, Multisizer II from Coulter Electronics Ltd., Elzone 280 from Particle Data Inc., or their equivalent.

6.1.1 The apertures are selectable from 50, 70, 100, 140, 280, 400, and 560-µm diameter. The measuring range for each sensor is approximately 2 to 80 % of the aperture diameter **(2)**.

6.2 *Microscope*—Zeiss Axioplan from Carl Zeiss, Germany, or similar microscope.

6.3 *Filtering Device*—Peristalic pump with 0.22-µm membrane filters (Sterivex-GS Millipore Corp.).

6.4 *Ultrasonic Bath*, 50 to 100 W and 60 to 80 kHz.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type I of Specification D 1193.

7.3 *Dispersing Media*—Non-ionic dispersants such as polyethylene sorbitan monolaurate (2 % solution) have been found applicable for chromatography media.

7.4 *Electrolyte*—For materials with hydrophilic surfaces, 9 g/L sodium chloride in water has been found suitable. For materials with charged groups (for example, ion exchangers), 117 g/L sodium chloride in water is normally sufficient. Consult the instrument manufacturer's manual for other electrolyte solutions and dispersing agents.

7.5 *Distilled Water*.

8. Procedure

8.1 *Sample Preparation*—If the sample is in the form of a powder, the container should not be filled to more than a quarter of the container height. Mix the powder using the twist and turn method (turn the container upside down and at the same time twist a quarter of a revolution). Allow enough time for the powder to settle, and repeat the procedure 50 times. Take approximately 100 mg of powder, and place in a round-bottomed sample beaker. Add a suitable dispersing agent and electrolyte solution and sonicate. Check the particle concentration. It should be at 5 % coincidence (Table 1).

8.1.1 If the concentration is too high, dilute the sample with electrolyte solution. If the concentration is too low, prepare a new sample starting with dry powder.

8.1.2 If the sample is in the form of suspension, there should be enough space in the container to allow homogenization of the suspension. Shake the container vigorously until the entire suspension is homogenous. Immediately withdraw approximately 5 mL of suspension using a Pasteur pipette. Place the suspension in a round-bottomed sample beaker, and dilute slowly with electrolyte solution. Sonicate for 1 min. Check the particle concentration. Adjust if necessary.

TABLE 1 Coincidence Chart

Aperture Diameter, µm	Manometer Volume, mL	Maximum Count for 5 % Coincidence
50	0.05	16000
70	0.05	5830
100	0.5	20000
140	0.5	7290
280	2.0	3640
400	2.0	1250
560	2.0	455

8.2 *Calibration for Particles in Suspension*—Wet sieve a portion of the material under test in water with sieves that are as close as possible together in size and with a size range that covers the peak of the particle size distribution. Transfer the sieved material to a suitable container, and substitute the water for that electrolyte solution that shall be used when measuring the material using the ESZ instrument. Transfer a suitable portion to a microscope slide, and cover with a cover slip. Measure the diameters of at least 400 particles using the microscope. Calculate the volume weighted (mass) median diameter.

8.2.1 Analyze the calibration fraction with the ESZ instrument. Calibrate the instrument using the known volume weighted (mass) median diameter **(3)**. At the same time, calibrate the ESZ instrument with standard uniform particles, such as latex (see the instrument manufacturer's manual). The latex shall then be given a reference size relative to the material under test and used for subsequent calibration checks.

8.2.2 Follow the same procedure for dry powders, except dry sieve a portion of the powder using suitable sieves and size the particles in a dry state using a microscope to obtain the volume weighted (mass) median diameter.

8.3 *Measurement*—Follow the instrument manufacturer's operating instructions to set up the instrument for analysis. Place the sample beaker on the sample platform of the sampling stand. Accumulate particle pulses in the instrument at a constant flow through the aperture for 30 s.

9. Report

9.1 The instrument automatically presents the acquired population data into several kinds of size distributions, one of which is the volume weighted (mass) versus equivalent volume diameter.

9.2 The histograms can be presented in either differential or cumulative form.

9.3 The statistics such as mean, median, mode, standard deviation, and coefficient of variation can be determined using the standard statistical formulae, as noted in the operator's manual, or using the manufacturer's computer software.

10. Precision and Bias

10.1 *Precision*:

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[6] *Reagent Chemicals, American Chemical Society Specifications*, 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. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

10.1.1 *Repeatability*—Duplicate determinations by the same operator should be considered suspect if they differ by more than 2 % of the average value of the determinations (see Practice E 177 and Terminology E 456).

10.1.2 *Reproducibility*—Duplicate determinations by each of two laboratories should be considered suspect if they differ by more than 4 % of the average value determinations (see Refs **4-6**).

10.2 *Bias*—Bias cannot be determined because there is no method for reference (see 5.1).

11. Keywords

11.1 chromatography media; electric sensing zone; particle size

REFERENCES

(**1**) Scarlett, B., "Theoretical Derivation of the Response of a Coulter Counter," *2nd European Symposium on Particle Characterization*, Nuremburg, 1979, pp. 681–693.

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(**3**) Goransson, B., and Dagerus, E., "Image Analysis As a Tool for Calibration of Electrical Sensing Zone Instruments in the Size Measurement of Porous Spherical Particles," *Particle Size Analysis*, Lloyd, P. J., ed., John Wiley and Sons Ltd., 1988, pp. 159–166.

(**4**) Alliet, D. F., and Behringer, A. J., "A Performance Reliability Study on the Model C. Coulter Counter in the Characterization of Polymer Materials: Particle Size Analysis," *Proceedings of the Conference of the Society of Analytical Chemists*, London, 1970, pp. 353–365.

(**5**) Kinsman, S., and Coulter, J. R., "Particle Size Measurements Using the Resistance Change Principle," *American Ceramic Society 63rd Annual Meeting*, Toronto, Canada, April 26, 1961.

(**6**) Wood, W. M., and Lines, R. W., "Particle Size Analysis Using Coulter Counters," *Journal of the Society of Cosmetic Chemists*, Vol 17, 1966, pp. 197–211.