Designation: E 1470 – 92 (Reapproved 1998)

AMERICAN SOCIETY FOR TESTING AND MATERIALS
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Standard Test Method for Characterization of Proteins by Electrophoretic Mobility[1]

This standard is issued under the fixed designation E 1470; 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 describes a procedure for determining the electrophoretic mobility of proteins of molecular weight greater than 10 000 Daltons.

1.2 This test method uses automatic Electrophoretic Light Scattering (ELS) principles to determine the electrophoretic mobility.

1.3 The instrument[2] simultaneously measures the Doppler shifts of scattered light at four different angles to determine the electrophoretic mobility distribution of protein particles. The mobility is expressed as µm-cm/V-s (micron-centimeter/volt-second).

1.4 *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. Summary of Test Method

2.1 A carefully dispersed, dilute suspension of the protein particles is loaded into the sample cell and is positioned in the path of collimated laser light. The laser light directed onto particles moving at constant velocity under an applied electrical field. The laser light is scattered from moving particles, producing a Doppler shift proportional to the particle's velocity.

2.2 The instrument response is essentially to a sinusoidal" beat" signal produced at the detector by mixing the scattered light and a reference (unscattered) beam. The frequency of the "beat" signal is equal to the difference Doppler shift and therefore, to particle speed and direction.

3. Significance and Use

3.1 The prime purpose of this test method is to provide data expressed as either electrophoretic mobility or zeta potential distribution of protein particles.

3.2 Both sellers and purchasers of protein particles will find this test method useful to determine either mobility or zeta potential distributions for protein specifications, manufacturing control, and development and research.

4. Apparatus

4.1 The apparatus for analysis consists essentially of a laser light source, sample cell for introducing the sample, power supply source, four 256 channel spectrum analyzers, microprocessors, and computer assembly.

4.2 Sample chamber assembly, holds approximately 1 mL of sample and is composed of three basic parts. The two side pieces are made of solid silver and contain hemispherical cavities. Between the two side pieces is a fused silica glass insert, running through it is a rectangular channel (3 mm wide by 1 mm high). The channel connects the two cavities. Fluid fills both cavities and the channel. Electrophoretic Light Scattering measurements are made on particles in the channel.

4.2.1 *30 mL Plastic Accuvetts*, (disposable) for preparing the sample.

4.2.2 *Membrane Filtering Device*, 0.2 µm filters or finer.

4.2.3 *5 mL Sterile Plastic Syringe*.

4.2.4 *8 Gage Blunt Tipped Hypodermic Needle*.

4.2.5 *pH Meter*.

4.2.6 *Standard Buffer Solution*.

5. Reagents and Materials

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where specifications are available.[3] 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.

5.2 *Suspending Media*—The sample media could be any standard buffer solution (conductivity 2 µs to 200 millisiemen). The media shall be filtered through 0.2 µm or finer membrane filter. Select filter that is chemically compatible with the diluent used and with no extractables or surfactants present. The surfactants or extractables can influence the particle's surface chemistry.

5.3 *Rinse Water*—Deionized or distilled water twice filtered

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[1] This test method is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.03 on Unit Processes and Their Control.

Current edition approved March 15, 1992. Published May 1992.

[2] The Coulter® Delsa 440 instrument from Coulter Corporation has been found satisfactory. This instrument is available from Coulter Corporation, 601 W. Coulter Way, Hialeah, FL 33010.

[3] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analar Standards for Laboratory U.K. Chemicals," BDH Ltd., Poole, Dorset, and the "United States Pharmacopeia."

through the membrane device.

6. Procedure

6.1 *Sample Preparation*—Obtain the test sample in accordance with ASTM methods. Rinse polyethylene 30 mL disposable plastic accuvett with clean 0.2 µm filtered DI or distilled water. Prepare 1 % protein sample with the chosen clean suspending media. Gently mix the sample until all the protein particles are well dispersed. Measure the pH of the medium. If necessary, adjust the pH to the desired value. About 25 mL of the sample is transferred into the previously cleaned accuvett. Equilibrate this solution for 1 h by gently mixing over rollers.

6.2 *Filling the Sample Chamber Assembly*—Fill the syringe with sample suspension. Holding the syringe vertically with needle pointing up, allow any air space or bubbles to first rise to top. Gentle tapping will facilitate movement of bubbles. Expel bubbles by gently pushing syringe plunger until only bubble-free solution is observed. Insert the blunt tipped needle into the outer fill tube. Push the solution through until you see solution coming out of both of the other fill tubes. Turn the sample chamber assembly onto its side, pull the solution back into the syringe, remove the syringe and then expel the rinse solution. Repeat this procedure two or three times and then fill the sample chamber assembly with sample. Make sure that there are no air bubbles in the sample chamber. Rinse the sample chamber assembly with clean water and dry it thoroughly. Clean the glass insert with lens paper. Insert the sample chamber into its position in the instrument for electrophoretic mobility distribution analysis.

6.3 *Instrument Set-Up*—Follow the instrument manufacturer's operating instructions to set up the instrument for analysis.

7. Operating Instructions

7.1 Brief description of the operating principles of the instrument.

7.1.1 Description of various systems.

7.1.2 Description on limitations on electrophoretic mobility range, particle size range, measured conductivity range, and the temperature range.

7.1.3 Suggested maintenance procedures.

7.1.4 Performance verification procedures.

8. Verification

8.1 Verification of the performance of the instrument function may be determined by using well characterized latex particles[4] or the standard sample supply by the instrument manufacturer.

8.2 Interlaboratory comparisons shall be made using this well characterized standard.

9. Report

9.1 The report shall include the following:

9.1.1 The electrophoretic mobility or zeta potential distribution presented as mobility (µm-cm/V-s) or zeta potential (mV) versus intensity of scattered light,

9.1.2 The frequency spectrum at all four angles,

9.1.3 Conductivity (Millisiemen),

9.1.4 Temperature (OC),

9.1.5 Current (mA), and

9.1.6 Peak analysis with Mean and Mode values.

10. Resolution Limits

10.1 The base resolution of the measurement is determined by the frequency range selected (that is, 0 to 1000 Hz range implies 1000 Hz/256 channels = 3.9 Hz/channel). Only data from angles exhibiting minimally frequency shifts 2.5 times greater than the base resolution can be used.

11. Precision and Bias

11.1 *Interlaboratory, Same Operator*—Experience of several laboratories indicate that the method is capable of a precision of ± 1 % (95 % confidence level) when using the same standard material as mentioned in 8.1.

11.2 *Intralaboratory*—Experience of several laboratories indicates that the method is capable of a precision of ± 3 % (95 % confidence level) when using the same standard material as mentioned in 8.1.

11.3 *Bias*—No absolute method of electrophoretic mobility determination is recognized. Therefore, it is not possible to include a bias of results obtained by this test method.

12. Keywords

12.1 electrophoretic mobility; molecular weight; proteins

[4] Coulter Corporation, 601 W. Coulter Way, Hialeah, FL 33010.