Designation: E 1470 – 92 (Reapproved 1998)

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

[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."