Designation: E2865 – 12 (Reapproved 2022)

Standard Guide for Measurement of Electrophoretic Mobility and Zeta Potential of Nanosized Biological Materials[1]

This standard is issued under the fixed designation E2865; 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 guide deals with the measurement of mobility and zeta potential in systems containing biological material such as proteins, DNA, liposomes and other similar organic materials that possess particle sizes in the nanometer scale (<100 nm).

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1470 Test Method for Characterization of Proteins by Electrophoretic Mobility (Withdrawn 2014)[3]

E2456 Terminology Relating to Nanotechnology

2.2 *ISO Standards:*[4]

ISO 13099-1 Colloidal Systems — Methods for Zeta-Potential Determination — Part 1: Electroacoustic and Electrokinetic Phenomena

ISO 13099-2 Colloidal Systems — Methods for Zeta-Potential Determination — Part 2: Optical Methods

ISO 13321 Particle Size Analysis — Photon Correlation Spectroscopy

3. Terminology

3.1 *Definitions*—Definitions of nanotechnology terms can be found in Terminology E2456.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *Brownian motion, n*—is the random movement of particles suspended in a fluid caused by external bombardment by dispersant atoms or molecules.

3.2.2 *dielectric constant, n*—the relative permittivity of a material for a frequency of zero is known as its dielectric constant (or static relative permittivity).

3.2.2.1 *Discussion*—Technically, it is the ratio of the amount of electrical energy stored in a material by an applied voltage, relative to that stored in a vacuum.

3.2.3 *electrophoretic mobility, n*—the motion of dispersed particles relative to a fluid under the influence of an electrical field (usually considered to be uniform).

3.2.4 *isoelectric point, n*—point of zero electrophoretic mobility.

3.2.5 *mobility*—see electrophoretic mobility.

3.2.6 *redox reaction, n*—a chemical reaction in which atoms have their oxidation number (oxidation state) changed.

3.2.7 *stability, n*—the tendency for a dispersion to remain in the same form for an appropriate timescale (for example, the experiment duration; on storage at 358K).

3.2.7.1 *Discussion*—In certain circumstances (for example water colloid flocculation) instability may be the desired property.

3.2.8 *van der Waals forces, n*—in broad terms the forces between particles or molecules.

3.2.8.1 *Discussion*—These forces tend to be attractive in nature (because such attractions lead to reduced energy in the system) unless specific steps are undertaken to prevent this attraction.

3.2.9 *zeta potential, n*—the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle.

[1] This guide is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.02 on Physical and Chemical Characterization.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

[4] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

3.2.10 *zwitterionic, n*—a molecule with a positive and a negative electrical charge.

3.2.10.1 *Discussion*—Amino acids are the best known examples of zwitterions.

4. Summary of Practice

4.1 *Introduction*—It is not the intention of this guide to spend any significant time on the theory of zeta potential and the routes by which a particle acquires charge within a system. Indeed it may be more appropriate to deal only with the movement or mobility of particles under an electrical field where conversion to zeta potential is not even attempted. The relevant text books (for example, see Hunter **(1)**[5]) should be consulted along with the more academic ISO references (ISO 13099-1 and ISO 13099-2). The IUAPC report **(2)** is also very useful, albeit fairly theoretical, but it does contain a section (4.1.2) entitled 'How and under which conditions the electrophoretic mobility can be converted into ζ-potential'. The Corbett and Jack paper **(3)** contains excellent practical advice for measurement of protein mobility and is recommended.

4.2 Test Method E1470 is based around a sole vendor's equipment, but this does not deal with the basis of the measurement or provide guidance in the practice of the measurement. It is one intention of this guide to address those deficits.

4.3 The following aspects need emphasis:

4.3.1 Zeta potential is a function of the particulate system as a whole – so the environment that the particle resides in (pH, concentration, ionic strength, polyvalent ions) will directly influence the magnitude and, in certain circumstances, the sign of the acquired charge. In particular, small quantities (parts per million) of polyvalent ions (for example calcium ions (Ca^{2+}), iron (III) ions (Fe^{3+})) or other impurities can significantly affect the magnitude of the zeta potential. It is obvious, but often ignored, that there is no such concept of the zeta potential of a powder.

4.3.2 The calculation of zeta potential from mobility measurement typically refers to the unrestricted mobility of a particle in suspension. In crowded environments (that is high concentration) particle-particle interactions occur and the movement may be hindered. In this circumstance, although a movement can be detected and measured, it may provide interpretation issues when a conversion to zeta potential is attempted.

4.3.3 Zeta potential tends only to be important in the sub-5 μm (and thus relevant to the sub-100 nm region considered in this text) region where van der Waals attractive forces are of a similar order of magnitude as inertial forces. Thus if sedimentation (function of size and density of the particle with respect to the medium it resides) is occurring or has occurred, the system is clearly not ideal for a zeta potential or mobility measurement. With significant settling the measurement of mobility is obviously compromised. The lower limit for measurement of electrophoretic mobility is in effect determined by the signal to noise which is a complex function of size, concentration and relative refractive index of the particulate system. An unambiguous statement of the lower size is therefore not possible.

4.3.4 Zeta potential and its (assumed) relation to system stability are reasonably well understood in aqueous systems. The classic examples are indicated in Thomas Riddick's text **(4)**. The obvious or stated link with formulation or product stability is not obvious for organic media where the counterions will be strongly bound to the particle surface and the position of the diffuse layer will be difficult to identify in an (effectively) insulating external medium. Again, what is often forgotten, is that conductivity is required in the 'background' solution (typically 0.001 $molL^{-1}$ sodium chloride (NaCl) is utilized) so that an electrical field can be correctly applied without effects such as electrode polarization (causing voltage irregularities) occurring. Mobility or zeta potential measurements should not be made in de-ionized water. In non-polar dispersant liquids, conversion of observed mobility to zeta potential may need some understanding of the position and thickness (single atom or molecule?) of the double layer, but this is not relevant to measurements in (aqueous) biological media.

4.3.5 It is mobility (movement) that is usually measured and the conversion to zeta potential relies on application of the Henry equation. (See also Fig. 1).

$$U_E = \frac{\varepsilon\zeta\, f(\kappa\alpha)}{6\pi\eta} \quad (1)$$

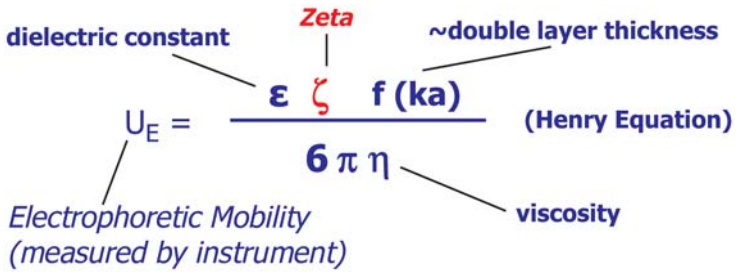

FIG. 1 Equation (1)

[5] The boldface numbers in parentheses refer to a list of references at the end of this standard.