Designation: E2865 – 12

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

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E1470 Test Method for Characterization of Proteins by Electrophoretic Mobility (Withdrawn 2014)[3]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*—is the random movement of particles suspended in a fluid caused by external bombardment by dispersant atoms or molecules.

3.2.2 *dielectric constant*—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*—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*—point of zero electrophoretic mobility.

3.2.5 *mobility*—see electrophoretic mobility.

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

3.2.7 *stability* —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*—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*—the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle.

3.2.10 *zwitterionic*—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

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

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).

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

where:

U_E = the electrophoretic mobility (measured by instrument),
ε = the dielectric constant of the dispersion medium,
ζ = the (calculated) zeta potential,
$f(\kappa a)$ = Henry's function (see below), and
η = the viscosity of the medium (measured or assumed).

4.3.5.1 It is important to specify the units of measurement as failure to get these correct will lead incompatibility of units on the right and left hand side of the above equation. The normal SI units (metre, kilogram, second) are not often utilized in this area as they are too large for practical purposes (diffusion distances of one metre are not routinely encountered!) — see additional unit information in Ref. **(5)**. We need to remember that the mobility and diffusion coefficient are a flux (and thus

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

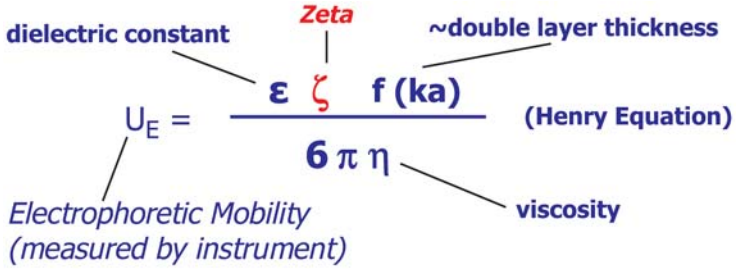

FIG. 1 Equation (1)

area) per unit time. The mobility will be scaled by the field (volts/distance). Ref. (**5**) recommended units for electrophoretic mobility are $m^2\ s^{-1}\ V^{-1}$. This can be expressed as $(ms^{-1})/(Vm^{-1})$ or a velocity per unit field. In practice, the electrophoretic mobility, U_E, has more convenient units of $\mu m^2/Vs$ Often mobilities are expressed in confused units (for example, the oft-utilized $\mu mcm^{-1}/Vs$ because this gives rise to mobility values in the convenient ±10 region). Mobilities expressed with a negative sign imply a negative zeta potential.

4.3.5.2 ε is the dielectric constant of the dispersion medium dimensionless/no units as it is a ratio of the relative permittivity of the material to vacuum whose relative permittivity is defined as 1.

4.3.5.3 f(κα) is usually referred to as "Henry's function" where α is the radius of the particle. κ is referred to as the Debye parameter and can be calculated from the electronic charge, Boltzmann's and Avogadro's constants, the absolute temperature and the ionic strength. The charged region around a particle falls to about 2 % of the surface charge at a distance approximately 3/κ from the particle. For ionic strength around 0.01 $molL^{-1}$ then 3/κ is around 10 nm and for ionic strength around 10^{-5} $molL^{-1}$ then 3/κ is around 280 nm (see Koutsoukos et al. (**6**)). 1/κ can be envisioned as the "thickness" of the electrical double layer (the Debye length) and thus the units of κ are reciprocal length. Thus f(κα) is dimensionless and usually assigned the value 1.00 or 1.50. For particles in polar media the maximum value of f(κα) is taken to be 1.5 (Smoluchowski approximation) and for particles in non-polar media the minimum value of f(κα) is 1 (Hückel approximation). It is the former that we are considering in this text. The literature does indicate intermediate values for f(κα) but in most biologically relevant media the value of 1.5 is the most appropriate.

4.3.5.4 In terms of viscosity, η, the SI physical unit of dynamic viscosity is the pascal-second (Pa·s), (equivalent to $N{\cdot}s/m^2$, or kg/(m·s)). Water at 293 K has a viscosity of 0.001002 Pa·s. The cgs physical unit for dynamic viscosity is the poise (P). It is more commonly expressed, particularly in ASTM standards, as centipoise (cP). Water at 293 K has a viscosity of 1.0020 cP.

NOTE 1—At room temperature (assumed 298 K) in water, all of the expressions are constants except for the (measured) mobility and the equation defers to:

$$\text{Zeta potential} = K^{*}\text{electrophoretic mobility},\ U_E \sim 12.85^{*}U_E \quad (2)$$

where the value of K (collective proportionality constant) is ~12.85 if the zeta potential is to be stated in mV and this falls out naturally from the Henry equation if the deprecated $\mu mcm^{-1}/Vs$ unit is used for electrophoretic mobility.

4.3.5.5 As well as movement under the constraint of an electric field, some degree of Brownian motion will also occur and may need to be considered. In biological media of relatively high ionic strength the Hückel model (f(κα) = 1) for zeta potential calculation is inappropriate and the value of f(κα) should be calculated from the measured size and the known ionic strength (or measured conductivity) (see Fig. 2).

4.3.6 Systems of positive charge tend to provide more measurement difficulties from a practical perspective than those of inherent negative charge. This is because most organic media including plastic sample cells are inherently negatively charged at neutral pH and may attract particles of opposite charge removing them from suspension and altering the wall potential. It is useful to have some form of automation for pH adjustment – for example a titrator. This eases the adjustment of pH and additive concentration.

4.3.7 It is of no value to state a zeta potential value without description of the manner in which it was measured together with vital measurement parameters. Zeta potential without a stated pH, ionic compostion, and electrolyte concentration value is close to meaningless.

4.4 *Biological Molecules and Entities*—Again, a few obvious points will need mentioning:

4.4.1 Many materials such as proteins contain charges and may be zwitterionic (contain both positive and negative charges). These molecules can be quite labile and may absorb

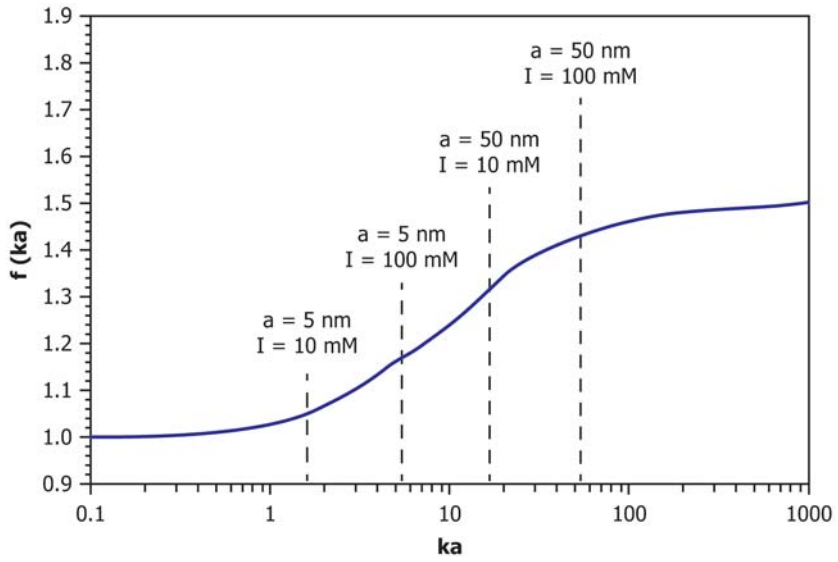

FIG. 2 Graphical Representation of the Henry Function and the κa Values for Four Example Particle Size and Ionic Strength Combinations

and decompose readily under an electrical field at the electrode with the deposition of carbon (shown as electrode darkening) and gas evolution. This is a conventional redox reaction and is virtually impossible to eliminate if organic materials interact with or contact metal electrodes—the electrical field over the length of an adsorbed molecule is enormous in relation to that between the electrodes themselves. Protocols need to be aware of this possibility and seek to minimize it after appropriate investigation of the magnitude of the phenomenon. It may be virtually impossible to eliminate such decomposition for some molecules unless specific routes are taken—for example, isolation of the electrodes from the biological molecules with a porous membrane that allows ions but not larger molecules to pass through. Measurements taken quickly and at lower voltages in combination with a reduced electrode spacing (thus reducing the field) may also help in this regard but resolution will almost certainly be lost. Many hours are required in order for proteins to diffuse a few tens of millimeters; a distance between detection point and electrodes somewhat typical of many capillary based laser Doppler electrophoresis systems. It is the slow timescales associated with the diffusion coupled with measurement times of the order of minutes to tens of minutes associated with laser Doppler electrophoresis that is the enabling factor for the implementation of any diffusion barrier technique. Detection of aggregates by measurement in the forward scattering direction combined with visual inspection of (polished metal) electrodes for blackening will be good indicators of sample degradation. Obviously on a blackened oxide surface such 'deposits' will not be evident. The consequence in the measurement is typically a drift to more negative values and instability in the measurements themselves. Rapid measurements and those avoiding Joule heating may alleviate the problem somewhat but the only real solution is to prevent the protein interacting or adsorbing onto the electrode itself.

4.4.2 Biological materials may be in low concentration, may be small and are invariably of low relative refractive index (RRI) in comparison to inorganic particles and colloids. The practical aspects of this are that the scattered signal may be weak with the consequence that the mobility detected by a Doppler shift may be difficult to isolate from any background noise.

4.4.3 The availability of material may be small and thus representative sampling may need to be considered—is one drop really indicative of a bigger system? It is only possible to measure a few μL of sample with specific experimental set-ups as the electrodes need to be of a finite size and distance apart. In many instances a few millilitres of solution or suspension will make life easy, especially if flushing of a cell is needed, but this is not always available. If the material can be held as a 'plug' it may be possible to work with considerably less quantity.

4.4.4 Biological material is often contained in buffered solutions of relatively high ionic concentration. For example, phosphate buffered saline (PBS) is constituted of 0.0032 $molL^{-1}$ disodium hydrogen phosphate (Na_2HPO_4), 0.005 $molL^{-1}$ monopotassium phosphate (KH_2PO_4, 0.0013 $molL^{-1}$ potassium chloride (KCl), 0.135 $molL^{-1}$ NaCl, and is adjusted to pH 7.4. This has implications of Joule heating when voltage is applied across such a solution and the propensity of decomposition is increased in such scenarios—60 seconds between measurements is often recommended to allow appropriate cooling. Chloride ions often present as NaCl (say 0.9 $molL^{-1}$) can be aggressive to some electrode systems (especially the platinum group metals) and the electrode material may need investigation. The current passing through the measurement zone can be reduced by appropriate reduction of voltage or by reducing the distance between the electrodes but this is not a universal panacea.

5. Significance and Use

5.1 The magnitude of zeta potential of a system in aqueous media is often an indicator of formulation stability or a means to understanding protein charge of the system and this is the usual reason for measurement. Oft-quoted values of stability when a threshold of +30 mV or –30 mV is reached are common. This arises from Riddick's text **(4)** and it is worth reproducing his table in full:

Stability Characteristics	Average ZP, mV
Maximum agglomeration and precipitation	0 to +3
Range of strong agglomeration and precipitation	+5 to –5
Threshold of agglomeration	–10 to –15
Threshold of delicate dispersion	–16 to –30
Moderate stability	–31 to –40
Fairly good stability	–41 to –60
Very good stability	–61 to –80
Extremely good stability	–81 to –100

5.2 It is noted that –30 mV represents only 'moderate stability'—nowhere in Riddick's text **(4)** are these qualitative terms further defined: for example what is 'delicate dispersion?'. It is also noted that positive values greater than +5 mV are not noted in the table, the assumption being that it is the modulus rather than the sign of the charge that is responsible for the qualitative stability terms listed above. For smaller systems typically <1 μm, a higher magnitude of charge may be needed to confer stability in the system and nanometre-sized material may require in excess of 100 mV for adequate stability.

6. Reagents

6.1 *General:*

6.1.1 As each system is different, it is difficult to be specific about reagents. In many instances, though, an automated titrator is useful in order to add specific amounts of additive. It would be usual to have some route of pH adjustment via the addition of acid or base. All reagents should be made up in de-ionized water (18.2 MΩcm; 293K) typically extracted from a high specification laboratory deionizer and preferably filtered to 0.22 μm or better and free from dissolved gas. It should be noted that water characterized by conductivity measurement alone may contain quantities of organics (for example amines washed out from an ion exchange column) that may significantly alter the measured zeta potential value. In certain circumstances, if the quality of the water supply is suspected, measurement in a source of distilled water may provide insight as to a potential problem and the organic content of the DI water can be assessed with a technique such as total organic carbon (TOC).

6.1.2 Other reagents, such as surfactants (wetting agents) and stabilizers (often ions such as PO_4^{2-}) are often utilized but

these may pose problems as they can linger around after rinsing and tend not to react well with protein samples. Ethanol works well for cleaning systems and is easily rinsed away.

6.2 *pH Adjustment:*

6.2.1 It is important to choose an appropriate pH modifier that does not cause sample degradation. Typically 0.001 $molL^{-1}$ sodium hydroxide (NaOH) (for base) and 0.001 $molL^{-1}$ hydrogen chloride (HCl) (for acid) are used for pH adjustment. A typical range would be from pH 2 to 12. Below pH 2, stainless steel will be attacked by HCl with evolution of hydrogen – although, of course, the material itself may not be in contact with stainless steel at any time. Nitric acid (which passivates stainless steel) is a possible alternative in such circumstances and the early literature abounds in measurements in potassium nitrate (KNO_3) at different concentrations and pH's. Above pH 12, glass is leached and slowly dissolved. The chemistry of the situation needs to be understood. Bringing the pH below around 3 (by adding acid) of an iron oxide sol will cause dissolution. Excessive pH change or heating of proteins can cause degradation commonly called denaturation. Salts, organic solvents, and heat can cause similar effects.

6.3 *Other Additives:*

6.3.1 As well as a function of pH, it is common to be studying zeta potential with controlled additions of some additive. In the study of colloidal coagulation in the water industry, additions of aluminum ions (Al^{3+}) or iron (III) (Fe^{3+}) are common and the determination of the isoelectric point gives some indication as to the best conditions for sedimentation. In a number of industries, for example ceramics and coatings, the effect of addition of phosphate (PO_4^{2-}) as a stabilizer is often studied.

6.4 *Sample Dilution:*

6.4.1 Zeta potential is related to charge on a particle within a system. Zeta potential is calculated from the mobility with the assumption that the particle is free to move in an unhindered manner. In a more crowded or concentrated scenario the particle will have hindered mobility due to particle-particle interactions and thus the conversion or interpretation of mobility from a zeta potential perspective becomes difficult.

6.4.2 In light scattering measurement of electrophoretic mobility, light needs to enter and leave the system. Thus, many samples as received may be too concentrated for direct measurement. In this situation, if dilution is deemed to be necessary, it is ideally carried out in the mother liquor – the same ionic environment within which the particles are contained. Large extraneous matter can be obtained by centrifuging but even with ultracentrifugation it will not be possible to obtain the analyte-free supernatant in most biological systems with dissolved protein molecules. Alternatively, in certain circumstances dictated by the size and density of the particles, if the particles are allowed to settle, a supernatant can be withdrawn and this used for mobility measurement – again only applicable to dust and large contaminant removal. Generally speaking the zeta potential is relatively independent of particle size and a suitable and stable result can be obtained in such circumstances. It is usual, and emphasized in ISO 13321 that a concentration ladder should occur and the effect of dilution on the system interpreted.

7. Procedure

7.1 *General Practice:*

7.1.1 Verify the performance of the instrument to be used in line with the manufacturer's recommendations and with an appropriate standard. A single NIST[6] standard exists for electrophoretic mobility (SRM 1980 (**7**)) and it is recommended that, in the lifetime of the instrument, an appropriate verification test is carried out with this material. SRM 1980 (**7**) is a positive mobility standard containing 500 mg dm^{-3} microcrystalline goethite (α-FeOOH) and 100 mol g^{-1} phosphate in 0.05 mol dm^{-3} sodium perchlorate electrolyte solution at pH 2.5. The suspension is diluted prior to use. The certified mobility value is 2.53 ± 0.12 μm cm V^{-1} s^{-1}. A more convenient secondary standard, for example, an appropriate polystyrene carboxylated latex or colloidal silica, can be used for routine validation. Coffee creamer (as it contains casein; isoelectric point at pH ~4.6) is a useful and inexpensive pseudo-standard material especially if a zeta potential – pH titration experiment is contemplated. As it contains no disulfide bridges and has relatively little tertiary structure, it cannot denature. Caesin is also relatively hydrophobic, making it poorly soluble in water.

7.1.2 Measure the pH and temperature of the solution. The concentration of species to be measured as well as the concentration of ions (phosphate, chloride) is useful. The viscosity and dielectric constant of the system need to be known – often this is incorporated and calculated within software assuming aqueous environments.

7.1.3 The temperature of the system needs to be maintained at a known and controlled point. Typically a precision of ±0.1 K is required on this parameter.

7.2 *Cleanliness of System and Wetting of Electrodes:*

7.2.1 Small amounts of impurity (especially polyvalent ions or some organic materials) can markedly change the value of zeta potential and it is common practice to flush systems and all glassware with de-ionized water and with absolute alcohol to ensure cleanliness.

7.2.2 Metal electrodes (and polished metals in general) do not wet well in water and this will cause polarization effects at the electrodes if not corrected. A common practice is to wet the electrodes in a lower (than water) viscosity medium such as absolute ethanol before addition of de-ionized water. Dilute solutions of surfactant can often accomplish the same task but are not recommend for reasons stated earlier – they tend not to react well with protein samples and are difficult to thoroughly remove. Oxide coated electrodes (for example palladized or platinized) do not usually suffer from this (lack of wetting) issue. Contact of many organic materials with electrodes under voltage conditions (whether alternating currents (AC) or direct currents (DC)) is liable to lead to "surface-induced denaturation" (see, for example, Ostatná et al. (**8**) and Ref. (**9**)) and blackening of the electrodes. According to the above reference, in the vicinity of the electrode surface a non-homogenous electric field exists (up to about $10^9 Vm^{-1}$) in the inner double layer (its width corresponding to a few atomic diameters)

[6] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, http://www.nist.gov/srm.

adhering to metal phase. Coating of the electrodes with various absorbed species has been utilized in order to attempt to prevent this mechanism but these all have moderate or little effect as does limiting the amount and duration of applied potential difference. The only real route to prevent protein denaturation is to avoid direct contact of the organic with the electrode. The can be accomplished in a number of ways, the main route being to shield the electrodes by means of some semi-permeable membrane that permits the passage of ions but not the organic molecules of interest. This membrane should be easily wetted – materials such as polytetrafluoroethylene (PTFE) are prone to bubble formation that is virtually impossible to eradicate. The more extreme the pH, ionic strength and temperature, the greater the propensity to denaturation. On metal electrodes, denaturation is easily observed and any period of stability is often followed by drift (usually to more negative values) of zeta potential and blackening of the electrodes. Obviously with palladized or platinized electrodes such decomposition is not visible or obvious. Reducing the voltage at the electrodes and taking limited and quick measurements is one route to obtain reasonable zeta potential results. However, these will possibly compromise resolution and stability and this is normally a prerequisite for quality control. The use of disposable measurement cells is obviously useful as recovery of blackened electrode surfaces is tedious at best and almost impossible in the worst cases. Dip cells and quartz block cells, as well as the more routine capillary cells (glass or plastic; cleanable or disposable) are routinely used and one cell system may prove to be more robust and usable for a given application. The influence of the applied voltage is complicated and very much depends upon the geometry of the system. For example, capillary electrophoresis systems routinely operate at high voltages, with minimum destruction of the sample. With regard to light scattering, the applied voltage, along with the cell geometry and electrode separation distance, defines the "field" that is experienced by the sample in the measurement zone. In order to get a usable signal, it is necessary to have electrophoretic motion, which is related to the field strength. So it is routine to increase the voltage to increase field to increase the mobility to increase the signal to a level such that it can be distinguished from signal arising from simple diffusional motion. The catch is, that the applied voltage also defines the potential across the metallic electrodes, which if large enough can induce protein denaturation and degradation via disulfide bond cleavage, deamidation of side chain ammonium groups, and oxidation of histidine residues to aspartic acid, all of which would make the total charge balance more negative, while simultaneously disrupting the structure, composition, rigidity, stability, etc. of the protein. Ideally, the measurements should be conducted under conditions such that the sample never comes into contact with the electrode.

7.2.3 Finally, if the system is to be unused for a long period of time, it is recommended to keep non-disposable systems wetted (clean with non-ionic surfactant solution and store in DI water) during non-use, especially if prolonged, is recommended.

7.3 *Sample Introduction:*

7.3.1 Follow the manufacturer's instructions for set-up of the system including appropriate parameters. Dilute and filter the sample, if appropriate. Record and document appropriate information relating to the sample. After cleaning the cell some of the sample may need to be flushed through the system. In certain circumstances the material can be passed into the cell via an appropriate filter to remove large material that may interfere with the measurement by sedimentation. Note that the presence of an electrical field will help keep material in suspension for a longer period of time than in the absence of such a field. This means that the upper size limit for a zeta potential measurement is likely to be larger than that specified for a Brownian motion dynamic light scattering (DLS) experiment. Allow sufficient time for temperature equilibration (two minutes is typical) before voltage is applied to the system.

7.4 *Measurement:*

7.4.1 Measurements are best taken as short groups or sets in order to assess reproducibility. Measurements should be spaced in time to avoid effects of Joule heating in high ionic environments. Inspect the electrodes in the system for bubble formation and blackening. Select dilutions, timescales and voltages that lead to acceptable and pre-defined measurement stability normally assessed by calculation of an pre-defined acceptable coefficient of variation (100 × standard deviation/mean) for a consecutive group of measurements.

7.4.2 Dispose of all samples and cells in an appropriate and safe manner.

7.5 *Interpretation:*

7.5.1 Many companies provide software that contains some form of expert advice or evaluation of the generated result including quality factors (often available throughout the experiment). These diagnostics provide excellent advice in the event of any issues. Often raw acquired data can be inspected directly too.

8. Precision and Bias

8.1 *General:*

8.1.1 As this is a guide only, no precision and bias testing is mandatory but some general comments can be made. A number of round robin exercises have been conducted on zeta potential methods and the technique is well-accepted in the literature. The method is sensitive to small amounts of impurity, to minor changes in experimental technique and to many other hard-to-define parameters. Accordingly precisions of a maximum of 10 % relative standard deviation (minimum five measurements) in the reported zeta potential values may be considered acceptable in many circumstances.

8.2 *Measurement of NIST traceable standard (SRM 1980 (7)):*

8.2.1 A round robin exercise on a prepared goethite sample found a mobility of 2.53 ± 0.12 m cm V^{-1} s^{-1} (95 % two-sided confidence limits; 116 measurements total) giving a zeta potential of ~ +32.5 mV at 250°C (Smoluchowski approximation; f(κa) = 1.5). This variation (~0.5 % RSD) certainly represents the best case conditions for measurement where the ultimate care was taken. This has been well documented (**10**) and the certificate is available from the SRM portion of the NIST website.

9. Report

9.1 The following should be seen as a minimum:

9.1.1 Time and date of measurement,

9.1.2 Name of operator,

9.1.3 Description of instrument – make and model,

9.1.4 Date of last measurement of a verification or other standard and the result (pass/fail and target values with actual result),

9.1.5 Temperature of measurement (Kelvin),

9.1.6 Composition of dispersion or dilution medium,

9.1.7 pH,

9.1.8 Viscosity of medium (Pa.s),

9.1.9 Dielectric constant (assumed, calculated or measured),

9.1.10 Voltage utilized and measured current (Volts and mA are typical units here),

9.1.11 Measured conductivity (SI units: Siemens/m. Often seen in the non-SI form as mS/cm),

9.1.12 Minimum of 5 consecutive measurements specifying the mean (typically in mV) and standard deviations (typically as Relative Standard Deviation or Coefficient of variation) of these measurements, and

9.1.13 Report the mobility and the assumption (for example, Hückel or Smoluchowski approximation of the Henry equation) used in calculating a zeta potential, if the latter is reported.

10. Keywords

10.1 electrophoretic mobility; isoelectric point; zeta potential; zwitterion

REFERENCES

(1) Hunter, R.J., *Zeta Potential in Colloid Science Principles and Applications*, Academic Press, 1981.

(2) Delgado, A.V., González-Caballero, F., Hunter, R.J., Koopal, L.K, and Lyklema, J., "Measurement and interpretation of electrokinetic phenomena (IUPAC Technical Report)," *Pure and Applied Chemistry*, Vol 77, No. 10, 2005, pp. 1753–1805.

(3) Corbett, J.C.W., and Jack, R.O., "Measuring protein mobility using modern microelectrophoresis," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, Vol 376, 2011, pp. 31–41.

(4) Riddick, T.M., *Control of Colloid Stability through Zeta Potential: With a Closing Chapter on its Relationship to Cardiovascular Disease*, Volume 1, Livingston Publishing Company, 1968.

(5) "Quantities and units for electrophoresis in the clinical laboratory (IUPAC Recommendations 1994)," prepared for publication by G. Férard, *Pure and Applied Chemistry*, Vol 66, No. 4, 1994, pp. 891–896.

(6) Koutsoukos, P.K., Klepetsanis, P.G., and Spanos, N., "Calculation of Zeta-Potentials from ElectroKinetic Data" *Encyclopedia of Surface and Colloid Science*, Second Edition, Volume 2, edited by P. Somasundaran, CRC Press Taylor and Francis Group, 2006, pp. 1097–1114, p. 1100.

(7) SRM 1980—Positive Electrophoretic Mobility standard from NIST. See certificate at: https://www-s.nist.gov/srmors/view_cert.cfm?srm=1980

(8) Ostatná, V., Černock, H., and Paleek, E., "Protein Structure-Sensitive Electrocatalysis at Dithiothreitol-Modified Electrodes," *Journal of the American Chemical Society*, Vol 132, No. 27, 2010, pp. 9408–9413.

(9) Armstrong, F.A., *Encyclopedia of Electrochemistry*, Vol 9, 2002.

(10) Hackley, V.A., et al., "A standard reference material for the measurement of particle mobility by electrophoretic light scattering," *Colloids and Surfaces A: Physiochemical and Engineering Aspects*, Vol 98, 1995, pp. 209–224.