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New ISO Standards for Zeta Potential Determination - A Review

Two new ISO standards have recently been published that focus on zeta potential analysis; ISO 13099 Colloidal systems – Methods for zeta potential determination, Part 1: Electroacoustic and electrokinetic phenomena and Part 2: Optical methods. This white paper reviews these two new standards and explains how these documents relate to the HORIBA SZ-100 particle size and zeta potential analyzer.

ISO Standards

The International Organization for Standardization (ISO) is a network of the national standards institutes of over 160 countries that creates standards documents on a broad range of practices (1). ISO Technical Committee 24 (which the author is a member of) has created over 30 standards for particle characterization to date with over 10 more currently in process. A total of three standards will be created on the topic of zeta potential, Part 1 is an overview and introduction, Part 2 is specific to optical methods, and part 3 will be specific to acoustic methods. This document reviews Part 1 and Part 2 as these are of interest to our SZ-100 customers since this is an optical based technique.

Part 1: Electroacoustic and electrokinetic phenomena

Forward, Introduction, Scope, Terms and definitions, Symbols, Section 4, Theory: general comments

The *Forward, Introduction*, and *Scope* explain the organization of the three standards, define the basic principles of zeta potential, and describe how the results are applicable to colloidal systems. A useful reference is given for the IUPAC Technical Report (2) on the same subject, which provides additional background, theory and definitions. In many regards the IUPAC Technical Report provides more detailed information than these ISO standards since it was created without any limits on size (53 pages) or format. Twenty eight technical terms are defined in the next section, followed by forty nine symbols. Section 4, *Theory, general comments*, begins by explaining that there is no single theory that is valid for all real world systems, but rather a collection of elementary and advanced theories for various conditions. This white paper will only address elementary theories applicable to optical

instruments designed to measure zeta potential – excluding streaming current measurements on flat surfaces and all references to electroacoustics.

Zeta Potential Principles

In order to summarize some of the differences between the elementary and advanced theories it is first required to understand the basic principles and terms used in the field of electrokinetics. Figure 1 presents a particle with a negatively charged surface surrounded by an aqueous solution containing ions.
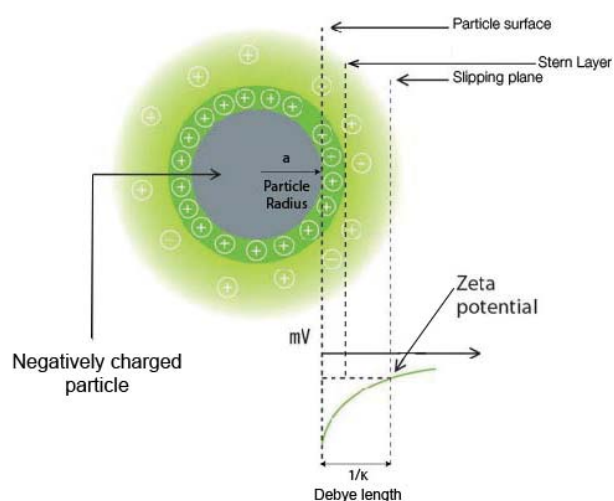

Figure 1: The EDL model

The charge on the surface of a particle influences the ionic environment in the region close to the particle surface. This ionic environment is typically described as an electric double layer (EDL) – the Stern layer of ions firmly attached adjacent to the particle surface, and the diffuse layer further away from the particle surface, but still attracted to the particle such that these ions will move with the particle. Beyond the double layer the ions are in equilibrium with the solution. The boundary between the electric double layer and the ions in equilibrium in the solution is called the slipping plane. Zeta potential is defined as the potential measured in mV at the slipping plane distance from the particle surface.

The particle has radius a, or diameter 2a. The distance from the particle surface to the slipping plane is known as the Debye length and is typically defined using 1/this distance: $1/\kappa$ or κ^{-1}. The relationship between the size of the particle (a) and the Debye length ($1/\kappa$) is an important concept. As seen in Figure 2, if the particle radius and Debye length are equal, then the product $\kappa a = 1$, known as the Hückel approximation. The most common situation is when the particle radius is larger than the EDL thickness, so $\kappa a >> 1$, known as the Smoluchowski model. This is the default model in most zeta potential calculations and is generally considered valid for samples with particle size > 100 nm in an aqueous dispersion with ionic strength > 10^{-3} mol/l. The opposite extreme is when the EDL is much larger than the particle diameter, $\kappa a << 1$, often valid for nano-disperions at low ionic strength and samples dispersed in organic solvents. In the images shown in Figure 2 there is only a single particle and single EDL. In the real world the EDL's may over lap, further complicating the theoretical models required to describe the dispersion accurately – leading to some of the advanced theories mentioned in ISO 13099-1.

The κa relationship is easily set in the SZ-100 software where the default setting is Smoluchowski, with options for either Hückel, or the ability to enter any value that better describes the particular sample being analyzed.
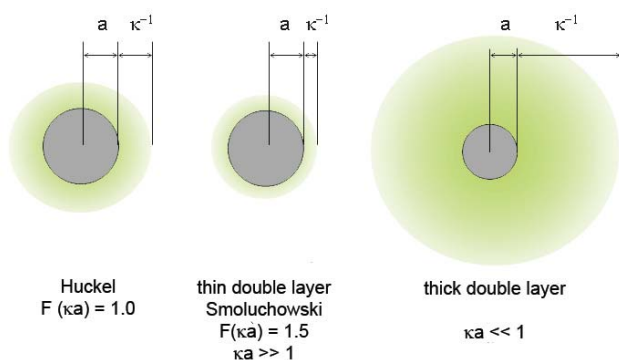

Figure 2: Particle radius (a) and Debye length ($1/\kappa$) relationship

Section 5, *Elementary theories, Smoluchowski's limit for electrokinetics.*

This section of the standard defines the applicability of Smoluchowski theory as having three restrictions:

- The EDL is thin compared to the particle size, $\kappa a >> 1$.
- The surface conductivity is negligible, $Du << 1$.

- The interface does not conduct normal electric current between phases.

Next, equations are shown to calculate electrophoresis, electroosmosis, and streaming current or potential.

Section 6, *Elementary theories, Smoluchowski's limit for electroacoustics,* and section 7, *Advanced theories,* are not reviewed in this document since we are focusing on optical techniques such as the SZ-100.

Section 8, *Equilibrium dilution and other sample modifications*, explains why dilution is important because zeta potential is not a property of the particles alone, but on the surface chemistry of the complete dispersion. Therefore, care must be taken when diluting samples to minimize any alteration to the surface chemistry. The optimum approach to dilution is the so-called equilibrium dilution procedure, using the same liquid as the original system (minus any particles) as the diluent. Two methods are described for collecting the ideal diluent:

- Sedimentation or centrifugation to obtain the supernatant, suitable for larger particles with sufficient density contrast
- Dialysis membranes, suitable for nano and bio colloids

Annex A, *Electric double layer models*

Detailed technical descriptions in ISO standards typically appear in the Annex sections. Annex A describes the more common theories used to model the EDL, with attention given to the most commonly used Gouy–Chapman–Stern model. Sections are devoted to flat surfaces, spherical isolated EDL's (the model used in the SZ-100), and overlapping double layers. Modelling the EDL as isolated (not overlapping) is considered valid up to 40 wt %, as long as κa is very large.

Annex B, *Surface conductivity*, describes an approach to model and measure surface conductance, not a parameter utilized in optical systems.

Annex C, *Debye length*, describes several approaches to determine the parameter $1/\kappa$. One equation that can be used to determined k is provided:

$$\kappa^2 = F^2 \sum_i \frac{c_i z_i}{\varepsilon_m \varepsilon_0 RT} \quad (1)$$

Where:

F is the Faraday constant
R is the gas constant
T is the absolute temperature
ε_m is the relative permittivity of the liquid
ε_0 is the dielectric permittivity of vacuum
c_i is the molar concentration of the ith ion species
z_i is the absolute valency of the ith ion species
For a symmetrical electrolyte $z^+ = -z^- = z$

Annex D, *Advanced electrophoretic theories*, gives very basic descriptions of other theories and provides references for further reading. Theories covered include O'Brien and White numerical theory (3), Henry–Ohshima theory (4,5), Hückel–Onsager theory for thick EDL (6), Dukhin–Semenikhin and O'Brien theories for thin EDL (7-9), and Overbeek electrophoretic theory for overlapping EDL (10).

Annex E, *Advanced electroacoustic theories*, is not reviewed here since we are only considering optical techniques.

Part 2 -Optical Methods

Forward, Introduction, Scope, Terms and definitions, Symbols

The *Forward, Introduction*, and *Scope* again explain the organization of the three standards and the intent of Part 2; "This part of ISO 13099 specifies two methods of measurement of electrophoretic mobility of particles suspended in a liquid: video microscopy and electrophoretic light-scattering." This white paper will only review the content applicable to electrophoteric light scattering (ELS) since only this material is important to SZ-100 users.

The *Terms and definitions* and *Symbols* sections are fairly similar to Part 1, but include additional values specific to optical methods.

Section 4, *Principles* introduces the basic concepts of how these measurements and calculations are performed. A sample is placed in a cell with two electrodes some distance, d, apart as shown in Figure 3.
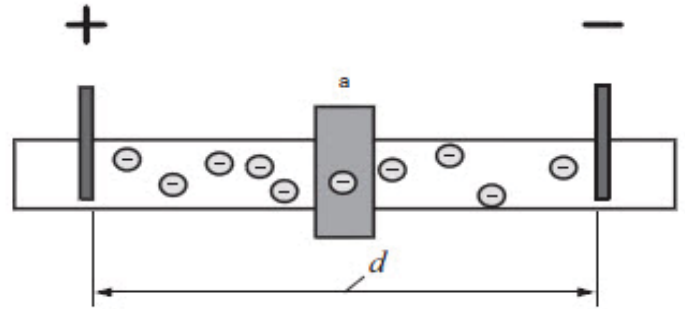

Figure 3: A zeta potential cell,
from ISO 13099 – Part 2
a = measurement zone
d = distance

An electric field is applied between the electrodes, causing two movements: the particles move towards the electrode of opposite charge, called electrophoresis, and the liquid streams along the capillary walls (if they are charged), called electroosmosis. The resulting particle velocity is the superposition of the electrophoretic and electroosmotic flow.

Section 5, *Microscopic methods* is not reviewed in this white paper.

Section 6, *Electrophoretic light scattering (ELS) method* explains that the particle motion created by the applied electric field can be optically detected due to Doppler shifts in the scattered light. From the frequency shift distribution it is possible to determine the electrophoretic mobility rapidly, accurately, and automatically "without the need to use standard particles for calibration".

Cell designs are discussed in section 6.2, along with the potential challenges of electroosmotic flow, Joule-Thompson heating, and electrolysis. Because of this "choosing an appropriate type of cell and electrode material, sufficiently prompt temperature control, and properly applied field duration and field strength are all important factors to ensure correct and reproducible results." See the comments on the SZ-100 design below for information about the cells used in the SZ-100.

Other cell design characteristics are mentioned such as reversing the applied electric field to minimize electrode polarization and making the measurement at the stationary layer where there is no electroosmotic flow to interfere with particle motion.

Comments on the SZ-100 design:

The SZ-100 measures zeta potential from the Doppler shift created by particle motion in an applied electric field, as described in this section of the standard. The disposable zeta potential cells used in the SZ-100 provide the latest design and technology to minimize the concerns expressed in this section of ISO 13099. The close positioning of patented porous carbon electrodes creates measurement conditions that greatly reduce the required applied voltage strength, minimizing the heating that causes difficulty with organic samples such as proteins. The carbon electrodes are gentler on samples and have a much longer lifetime than other designs. Electroosmotic effects are minimized by the geometric design of the cells, see Figure 4.
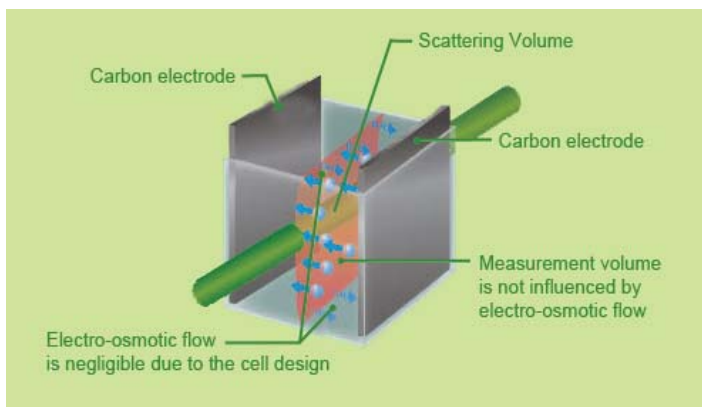

Figure 4: The SZ-100 cell design

Section 6.3 *Reference beam optical arrangement* describes the technique used in the SZ-100 design, shown in Figure 5.
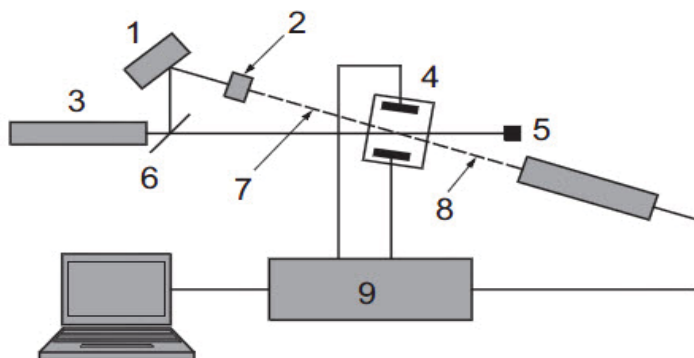

Figure 5: Optical schematic for the reference beam technique, from ISO 13099 – Part 2.

1: optical modulator
2: attenuator
3: laser
4: cell with electrodes
5: beam stop
6: beam splitter
7: reference beam
8: scattered or reference light
9: Processor

The laser is split in two, the incident and reference beam. The incident beam enters directly into the cell measurement zone to illuminate the sample particles. The reference beam merges with the scattered light at the detector, a PMT in the case of the SZ-100 at an angle of 17°, utilizing heterodyne detection.

Section 6.4 describes the cross beam optical approach not utilized on the SZ-100, so this is not reviewed in this document.

Section 6.5 *Signal processing* describes several approaches including spectrum analysis, autocorrelation function (utilized in the SZ-100), phase analysis light scattering (PALS), and modulated Brownian motion power spectrum analysis. All approaches are described and are, therefore, in compliance with the standard.

Section 6.6 *Determination of electrophoretic mobility* presents formula for calculating the mobility from the Doppler frequency shift for the cross beam and reference beam optical configurations. Equation (2) below is shown for the reference beam optics used in the SZ-100.

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi n E \sin(\theta/2) \sin[(\theta/2) + \xi]} \quad (2)$$

Where:

μ	is the electrophoretic mobility
$\Delta\omega$	is the Doppler frequency shift
λ_0	is the laser wavelength
n	is the medium refractive index
E	is the electric field strength
θ	is the angle between the incident and scattered light
ξ	is the angle between the scattered light and the orientation of the electric field

Section 7, *Calculation of zeta potential* references 13099-1 for detailed descriptions for calculating zeta potential from the electrophoretic mobility and then shows equation (3) as a widely used approach:

$$\mu = \frac{2\zeta\varepsilon}{3\eta_0} f(\kappa a) \quad (3)$$

Where:

ζ is the zeta potential
ε is the dielectric permitivity
η_0 is the medium viscosity
$f(\kappa a)$ describes the ratio of the particle radius to the EDL

The assumptions required for the validity of equation 3 are described followed by a short description of the $f(\kappa a)$ function, previously described in this white paper.

In section 8, *Operational procedures*, the content perhaps most useful to the user new to zeta potential begins. A summary and personal interpretation of helpful comments is provided below:

Instrument location	Place the instrument in a clean environment on a surface without vibration.
Dispersion liquids	Ensure chemical compatibility between the medium and the cell.
Measurement cell	Clean cells that have been previously used, control the temperature.
Sample inspection	Look for sedimentation, if visible, then the measurement is questionable.
Preparation	Avoid changing the surface chemistry during preparation.
Diluton	Don't just use DI water, follow the equilibrium dilution procedure described above. Author's comments; if this is not possible a dilute (1 -10 mM) KCl solution is far preferred over DI water.

Section 8.2, *Verification* describes a procedure any customer can use to prove the system is operating properly. Any system that is tested according to these procedures should generate acceptable zeta potential results.

The Reference materials section explains one of the basic challenges with this measurement technique, namely the lack of a universally accepted certified reference material (CRM). Although NIST supplies the sample standard reference material (SRM) 1980, few instrument suppliers or users make frequent use of this material. Most instrument suppliers ship a reference material (RM) of their own design with the instrument for verification purposes. The ISO document notes that RM sample should have the following characteristics:

- Be sufficiently homogeneous and stable for the stated time and temperature range.
- The accepted electrophoretic mobility value was obtained by several operators and rigorously proven.
- The material should be well documented in terms of sampling procedure, dilution, if required, and measurement protocol.

Any sample that meets these requirements can be used as a reference material (RM). Note that ISO 13099 only talks about electrophoretic mobility values in this section, not calculated zeta potential results. This is because the actual measured value is the electrophoretic mobility. But in practice, most/all instrument manufacturers provide a zeta potential value for the RM and this is what customers use to verify the system.

The repeatability of the system is tested by measuring the RM three times and passes the expected performance if the coefficient of variation (COV) is <10% (provided the reference mobility value is > 2 x 10^{-8} m^2/V s).

Not all customers test for intermediate precision, but if different test portions are analyzed then the system passes the expected performance if the COV is < 15%.

The term "trueness" is used rather than accuracy, but the system passes the expectations if the mean value of the three measurements is within 10% of the published value for the RM. Figure 6 shows results from the SZ-100 for the NIST 1980 SRM sample, reference value 2.53 µm•cm/V•s ± 0.12 µm•cm/V•s, conforming to ISO 13099 expectations.

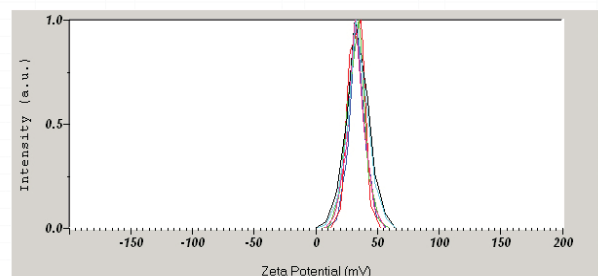| No.	Sample Name	Zeta Potential (Mean)(mV)	Electrophoretic Mobility mean(cm2/Vs)
17	NIST SRM1980_06.nzt	33.4	0.000257
18	NIST SRM1980_01.nzt	32.7	0.000252
19	NIST SRM1980_02.nzt	32.5	0.00025
20	NIST SRM1980_03.nzt	33.9	0.000261
21	NIST SRM1980_04.nzt	32.7	0.000252
22	NIST SRM1980_05.nzt	34.2	0.000263
Mean		33.2	0.000256
S. D.		0.7	0.000005
COV		2,11	1,95

Figure 6: SZ-100 result for NIST SRM 1980

Sources of error described in section 8.3 include the following:

- Contamination of the current sample by the previous sample
- Inappropriate sample preparation procedure
- Inappropriate sample
- Inappropriate liquid medium
- Poor temperature stabilization
- Condensation on the illuminated surfaces
- Particles, fingerprints or scratches on the optical surfaces
- Too large a potential applied
- Incorrect entry of parameters by the operator
- Air bubbles
- Cell coating damage
- Inappropriate theory

Section 8.3.13 on *Sample stability consideration* states that it is always advisable to conduct a series of measurements to demonstrate that the sample is stable.

Section 8.4 *Test report* lists the other sample information that should be included in a result, along with the average values of zeta potential and electrophoretic mobility.

Annex A, *Electroosmosis within capillary cells* describes the effect of liquid flow relative to the charged surface of a cell. This description may be interesting for customers new to the field, but in practice all zeta potential cells (including those used in the SZ-100) take this into account and users never act or enter values into the controlling software due to this phenomenon.

Conclusions

The two new ISO standards, 13099 Part 1 and 2, provide an excellent introduction to the theory and practice of zeta potential measurements. The most useful practical information for the new user may be the sections describing dilution and verification procedures. As with any analytical technique, a zeta potential analyzer should be subjected to verification tests on a regular basis. The frequency of verification testing is not addressed in the ISO standard, in accordance with common practice for these documents. Instruments used in a research environment are sometimes verified on an annual basis. Systems used for regular QA testing (uncommon for zeta potential analyzers at the moment) are typically verified on a more regular basis.

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