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Colloidal systems — Methods for zeta-potential determination —

Part 2: Optical methods

Systèmes colloïdaux — Méthodes de détermination du potentiel zêta —

Partie 2: Méthodes optiques

Reference number
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Foreword

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ISO 13099 was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

ISO 13099 consists of the following parts, under the general title *Colloidal systems — Methods for zeta-potential determination*:

— *Part 1: Electroacoustic and electrokinetic phenomena*

— *Part 2: Optical methods*

The following part is under preparation

— *Part 3: Acoustic methods*

Introduction

Zeta-potential is a parameter that can be used to predict the long term stability of suspensions and emulsions and to study surface morphology and adsorption on particles and other surfaces in contact with a liquid. Zeta-potential is not a directly measurable parameter. It can be determined using appropriate theoretical models from experimentally determined parameters, such as electrophoretic mobility. Optical methods, especially electrophoretic light scattering, have been widely used to determine electrophoretic mobility of particles or macromolecules in suspension or in solution. The purpose of this part of ISO 13099 is to provide methods for measuring electrophoretic mobility using optical means and for calculating zeta-potential.

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Colloidal systems — Methods for zeta-potential determination —

Part 2: Optical methods

IMPORTANT This part of ISO 13099 shall be read in conjunction with ISO 13099-1, which gives a comprehensive overview of the theory.

1 Scope

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. Estimation of surface charge and determination of zeta-potential can be achieved from measured electrophoretic mobility using proper theoretical models, which are described in detail in ISO 13099-1.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13099-1, *Colloidal systems — Methods for zeta-potential determination — Part 1: Electroacoustic and electrokinetic phenomena*

ISO Guide 30: *Terms and definitions used in connection with reference materials*

3 Terms, definitions and symbols

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1.1
Brownian motion
random movement of particles suspended in a liquid cause by thermal movement of medium molecules

3.1.2
Doppler shift
change in frequency and wavelength of a wave for an observer moving relative to the source of the wave

3.1.3
electric surface potential
difference in electric potential between the surface and the bulk liquid

NOTE Electric surface potential is expressed in volts.

3.1.4
electrokinetic potential
zeta-potential
ζ-potential
ζ
difference in electric potential between that at the slipping plane and that of the bulk liquid

NOTE Electrokinetic potential is expressed in volts.

3.1.5
electroosmosis
motion of liquid through or past a charged surface, e.g. an immobilized set of particles, a porous plug, a capillary or a membrane, in response to an applied electric field, which is the result of the force exerted by the applied field on the countercharge ions in the liquid

3.1.6
electroosmotic velocity
$\upsilon_{\mathbf{eo}}$
uniform velocity of the liquid far from the charged interface

NOTE Electroosmotic velocity is expressed in metres per second.

3.1.7
electrophoretic mobility
μ
electrophoretic velocity per electric field strength

NOTE 1 Electrophoretic mobility is positive if the particles move toward lower potential (negative electrode) and negative in the opposite case.

NOTE 2 Electrophoretic mobility is expressed in metres squared per volt second.

3.1.8
electrophoretic velocity
$\upsilon_{\mathbf{e}}$
particle velocity during electrophoresis

NOTE Electrophoretic velocity is expressed in metres per second.

3.1.9
slipping plane
shear plane
abstract plane in the vicinity of the liquid/solid interface where liquid starts to slide relative to the surface under influence of a shear stress

3.2 Symbols

a particle radius

D diffusion coefficient

E electric field strength

k_B Boltzmann constant

I light intensity

N_A Avogadro's number

n medium refractive index

R_{cap} capillary radius

$S(\omega)$ frequency power spectrum of scattering

Γ characteristic Lorentzian half peak width

ε medium permittivity

ζ electrokinetic potential (zeta-potential)

η_0 medium viscosity

θ angle between incident light and scattered light

θ' angle between two cross-beams

κ reciprocal Debye length

λ wavelength

μ electrophoretic mobility

μ_{eo} electroosmotic mobility of liquid

ν frequency

ξ angle between scattered light and electric field direction

τ delay time in autocorrelation function

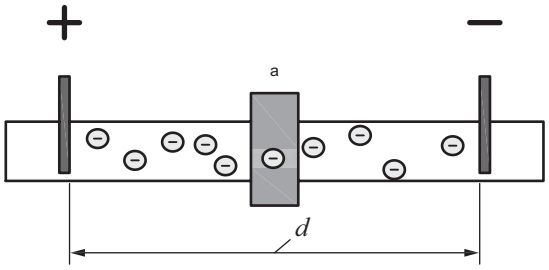
φ volume fraction

ω rotational frequency ($= 2\pi\nu$)

4 Principles

A suspension of particles having a given electrokinetic charge is placed in a cell which has a pair of electrodes placed some distance apart (Figure 1). This cell can be in the form of either a cylindrical or rectangular capillary with electrodes at either end, or a pair of electrodes at a known fixed distance apart that are dipped into a cuvette or other vessel. A potential is applied between the electrodes. Due to the process of electrophoresis, particles carrying a net negative charge are drawn towards the electrode of opposite sign and vice versa. In addition, if the capillary walls are charged, then an effect called electroosmosis causes the liquid to stream along the capillary walls. The direction and velocity of this flow depends on the sign and magnitude of the wall charge. The resulting velocity of the particle in the frame of references associated with the cell is superposition of the electrophoretic velocity and the velocity of electroosmotic flow. Here it should be noted that the time taken for the particle to reach the terminal electrophoretic velocity after the application of the electric field is much shorter than the period of time needed to fully establish the electroosmosis flow throughout the whole cell. This difference is exploited in some implementations. The velocity of the particles measured at a specific position can be determined using either video microscope or electrophoretic light scattering through a laser Doppler arrangement. Both the velocity and the direction of the moving particles in the frame of references associated with the cell are determined. Provided that the distance between the electrodes is known together with the applied electric potential, then the electrophoretic mobility can be established, from which a zeta-potential can be calculated using established theories. Alternatively, calibration with particles having a known zeta-potential can be used to eliminate the need to determine the unknown cell constant of a particular cell.

There are two distinctively different approaches to monitor particle motion in the electric field. Historically, the first deals with particle images observed through a microscope. It is referred to as the “microscopic method”, or alternatively as “microelectrophoresis”. The second relies on measuring light scattered by particles and extracting information on electrophoretic mobility from the Doppler frequency shift of the scattered light. This method is called the “electrophoretic light-scattering method”. For optical techniques, a cell constant for many types of cells has to be determined, through either calculation or measurement of a solution of known conductivity.



Key

d distance

a Measurement zone.

Figure 1 — Schematic diagram of electrophoresis measurement

5 Microscopic methods

The main principles of these methods can be traced back over two centuries (Reference [1]) following the development of microelectrophoresis. A light source illuminates particles migrating under the influence of a d.c. or a.c. electric field. The illuminated particles can be observed due to scattering. This illumination can be arranged either as a bright field or as a dark field or both (Reference [2]). The contrast afforded by the bright field illumination is inadequate to illuminate particles with sizes smaller than about 0,2 μm. Dark field illumination is suitable for capturing images of moving nano-particles with sizes down to nanometre scale.

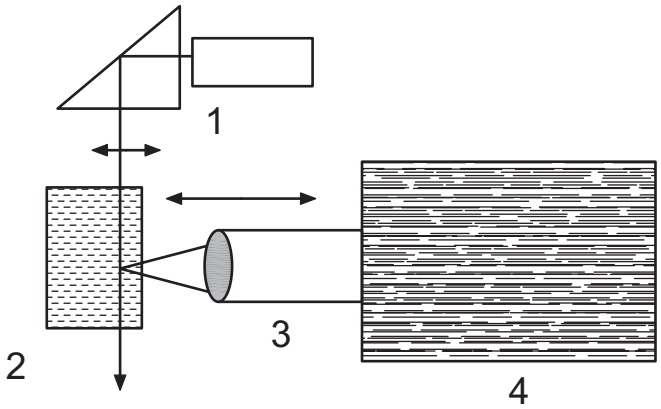
There are several approaches to the treatment of microscopic images of the moving particles. Depending on the degree of operator involvement, it can be classified as manual, semi-automatic and automatic. Manual methods track the movement of one or several individual particles by eye and a stopwatch and therefore are typically time consuming, tedious to employ and inaccurate.

In the semi-automatic methods, particles are tracked through a microscope manually while the apparatus either scans the illuminating light or moves a prism reflecting the illuminated image of particles. When the light-scanning velocity or prism-moving velocity is semi-automatically adjusted so that the particle image as viewed in the microscope is static, such a velocity is the electrophoretic velocity of particles (References [3][4]). These methods are only applicable to samples having a homogeneous electrophoretic mobility.

There are designs combining the manual microscopic observation with automatic electrophoretic light-scattering signal analysis to measure samples of polydisperse electrophoretic mobility (References [5][6]).

The appearance of modern charge-coupled devices (CCD) and computers has made it possible to capture images, transfer the images sequentially to a computer, and then using sophisticated image analysis to reconstruct trajectories of particles moving under the influence of an electric field from the time-stamped video frames. Only particles confined to video visibility can be measured. In order to record accurate moving distances, from the time duration between frames and the distance each particle moved, the velocity of each particle is calculated and combined with the applied field strength, and its electrophoretic mobility is obtained. Dark field illumination extends this method to nano-particles. This method allows application of electric field for very short periods of time, which resolves the problems of thermal convection and electrochemical contamination. Concentration of particles shall be very low in order to track individual particles.

A 90° laser scattering device is a typical optical arrangement of modern instruments. The laser serves as the illumination of the microscope focal plane. Both laser beam and microscope axis are perpendicular to the electric field. In Figure 2, the field direction is perpendicular to the plane of the drawing. Laser illumination and microscope require alignment with the stationary layer to avoid electroosmosis, which is explained in Annex A. It is necessary precisely to locate this position in order to accurately measure the electrophoretic motion of particles (Reference [7]).



Key

1	laser	3	microscopic objective
2	cell channel cross-section	4	video camera

Figure 2 — A typical electrophoresis video microscope

6 Electrophoretic light-scattering (ELS) method

6.1 General

Electrophoretic light scattering (ELS) is an indirect ensemble method for measuring electrophoretic mobility via the Doppler shifts in scattered light. In an ELS experiment, coherent incident light illuminates dispersed particles in a liquid that are subjected to an applied electric field. Charged particles move towards either the anode or the cathode, depending on the sign of their net charge. Because of the motion, the frequency of scattered light from particles is shifted due to the Doppler effect. From the frequency shift distribution, the particle electrophoretic mobility distribution can be determined. ELS provides rapid, accurate, automatic, and highly reproducible electrophoretograms of complex particulate samples suspended in either aqueous or non-aqueous media without the need to use standard particles for calibration (Reference [8]).

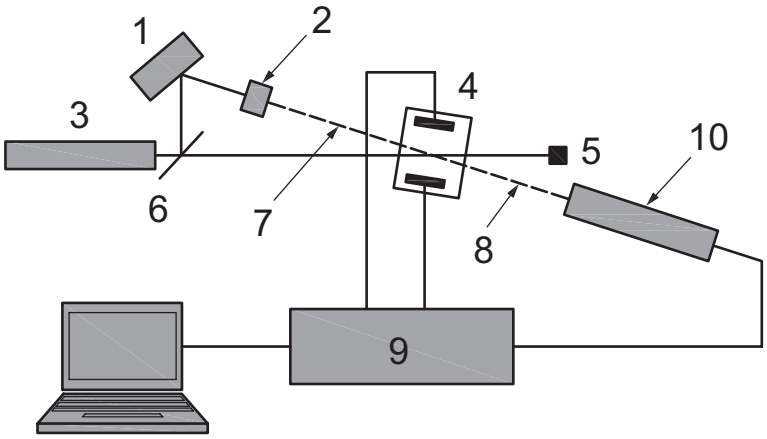
6.2 Cell design

Many designs of measurement cell have been employed. All cells have at least three functions: holding the sample containing the particles to be measured; supplying an electric field to the sample; and providing an entrance and an exit for the incident light and scattered light, respectively. Some cells are designed with liquid flow capability so that automatic titration can be performed with an additional device. In some implementations, special cell designs, e.g. utilizing a transparent electrode and multiple refraction for both incident and scattered light (Reference [9]), have been implemented to facilitate measurements of electrophoretic mobility at moderate concentrations. The electric field at the place of measurement shall be stable, homogenous, and parallel. To achieve that, either the two electrodes have to be placed very close to each other, in the case of cuvette cells, or the field path has to be confined, in the case of capillary cells. The voltage applied to the electrodes induces a current in the liquid if ions are present. This current can well be sufficiently high to cause Joule–Thompson heating of the liquid and lead to electrolysis at the electrodes. Therefore, 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.

To reduce polarization on the electrodes and maintain homogenous distribution of particles in the sample, the applied field direction is regularly reversed with an intervening off-time to minimize heating effects. In capillary cells, because of electroosmosis of liquid caused by charges on the walls, particles do not move in a static liquid. The liquid moves in a parabolic form across the closed capillary. Measurements are therefore taken at the so-called stationary layer where there is no liquid movement or multiple measurements are taken across the capillary to separate the liquid movement from the electrophoretic motion of the particles. Some implementations offer disposable cells.

6.3 Reference beam optical arrangement

A typical example for a small angle light-scattering arrangement is shown in Figure 3.



Key

1	optical modulator	6	beam splitter
2	attenuator	7	reference beam
3	laser	8	scattered or reference light
4	sample cell with electrodes	9	processor
5	beam stop	10	photoelectric detector

Figure 3 — One configuration of the reference beam optics

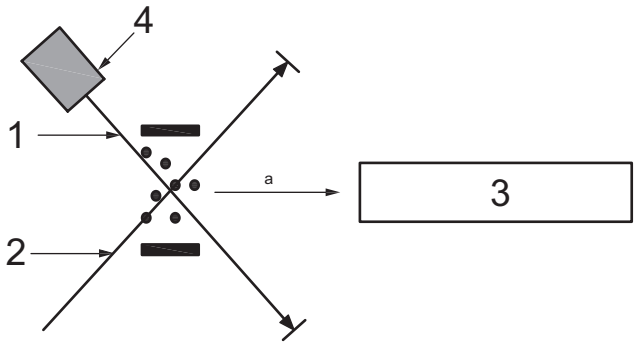
A small angle scattering optical arrangement incorporating heterodyne detection is often employed. The scattering angle, typically between 15° and 30°, exploits the advantage that the spectral broadening due to Brownian motion is reduced. With non-spherical particles, rotational diffusion may increase the spectral broadening. Means are provided in the measurement cell for the introduction of a pre-dispersed sample. The cell may be temperature controlled; if it is not, the temperature shall be accurately known since viscosity, permittivity, and refractive index of the liquid are all temperature dependent. A voltage is applied between the electrodes of the cell, whose spacing is defined, to set up a potential gradient. In some implementations, extra monitoring electrodes are employed at a defined spacing to provide a direct measure of the potential gradient. Light from a coherent laser source of known wavelength is split into two beams, one called the incident beam and the other the reference beam. The incident beam enters the cell directly or is refracted through a cell window to illuminate particles in the sample. The reference beam, which may or may not go through the cell, merges with the scattered light through conventional or fibre optics at the surface of the photoelectric detector, which is either a photomultiplier tube or an avalanche photodiode. One or both of the laser beams pass through a form of optical modulator to shift its frequency by a few hundred hertz from the original laser frequency so that the two beams acquire a desired frequency difference. This moves the origin of the Doppler shift caused by the velocity of the particles away from zero and enables the particle moving direction to be recognized and low frequency environment interference to be minimized. The detector aperture may be variable so as to control coherent detection and the scattering volume. The detected signal is passed to a signal processing unit that can be a digital correlator, a spectrum analyser or an amplitude-weighted phase structure function system. The voltage applied to the measuring cell can be reversed or pulsed and reversed as determined by the processor which also synchronizes the data collection. The final control is often by a desktop computer which calculates the zeta-potential.

6.4 Cross-beam optical arrangement

Another now less common optical arrangement is the cross-beam method. See Figure 4. In the cross-beam method, the main beam is split into two beams of equal intensity, the frequency of one or both beams being modulated. The two beams are made to cross symmetrically to enter one side of the cell. A detector is located at the other side of the cell in between the two beams. The scattering from each particle is a product of the illumination of both beams yet at different scattering angles. The Doppler shift resulting from scattered light is

independent of either scattering angle and depends only on the cross-beam angle. Another simplified way to view the arrangement is to understand that since the two beams are coherent, they form an interference pattern in the cell. The spacing between these fringes depends upon the wavelength and the angle between the two beams. The detection of electrophoretic motion is through particle movement in the strip-like fringe pattern.

An optical modulator is used to impose a movement of the fringes across the particle at some known frequency and in a defined direction. A particle in motion through the moving fringes resulting in a measured frequency greater than the modulator frequency indicates that its movement is against the fringe moving direction. A measured frequency lower than the imposed fringe frequency indicates that the particle is moving in the same direction as the fringe motion. The fringe movement is always made greater than the anticipated maximum particle velocity. By this means, both the velocity and the direction (charge sign) are determined.



Key

1	beam 1	3	photoelectric detector
2	beam 2	4	optical modulator

[a] Scattered light.

Figure 4 — Configuration of cross-beam optics

6.5 Signal processing

6.5.1 Spectrum analysis

Consider a particle sample that is polydisperse both with respect to size and electrophoretic mobility. The particles are undergoing Brownian motion together with electrophoretic motion under the influence of a d.c. field of defined strength.

The spectrum, also called an electrophoretogram, for the reference beam optical arrangement can be written as follows (Reference [8]):

$$S(\omega) = 2\pi I_{\mathrm{L}}^2 \delta(\omega) + 2I_{\mathrm{L}} \sum_{i=d_{\min}}^{d_{\max}} \sum_{j=\Delta\nu_{\mathrm{s,min}}}^{\Delta\nu_{\mathrm{s,max}}} \frac{I_{\mathrm{s},ij}\Gamma_i}{\left[\omega \mp \left(\omega_{\mathrm{M}} + 2\pi\Delta\nu_{\mathrm{s},ij}\right)\right]^2 + \Gamma_i^2} \tag{1}$$

where

I_{L} is the reference beam intensity;

$I_{\mathrm{s},ij}$ is the scattered intensity from particles of ith size and jth mobility;

Γ is the characteristic Lorentzian half peak width at half height from particles of the ith size, which for spherical particles is related to particle diameter;

ω is the rotational frequency;

ω_{M} is the modulator frequency;

$\Delta\nu_{\mathrm{s},ij}$ is the frequency shift caused by the electrophoretic motion of particles with ith size and jth mobility.

The symbol $\mp$ in the denominator denotes that there are two peaks in the spectrum. One is located in the negative frequency region that cannot be observed and the other in the visible positive frequency region. If by choosing a large modulator frequency, ω_M, so that the sum ($\omega_M + 2\pi\Delta\nu_{s,ij}$) is always positive, a negative sign can be used instead.

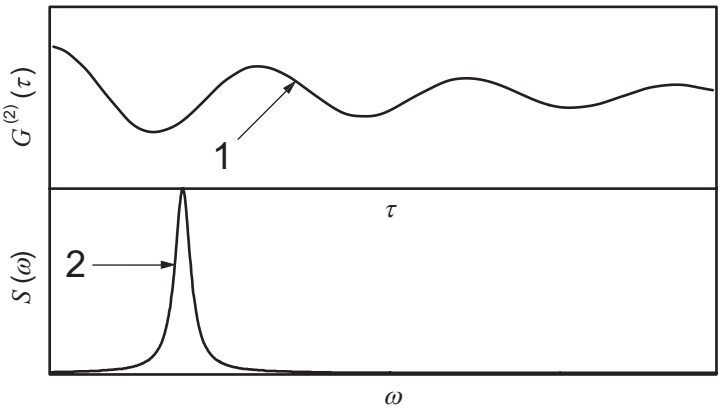
According to Formula (1), the electrophoretic spectrum of any particulate sample has an intrinsic broadening caused by Brownian motion of the particles in addition to any spectrum of electrophoretic mobility velocities. The broadening due to Brownian motion becomes more pronounced as the particle size reduces or when the scattering angle increases. One strategy of measuring the degree of broadening of the frequency spectrum as a result of the Brownian motion is to conduct a measurement with the applied field switched off. By subtraction of the Brownian motion only spectrum from the total spectrum a distribution of electrophoretic mobility can in certain circumstances be established (Reference [10]).

6.5.2 Autocorrelation function

The autocorrelation function is a Fourier transform of the frequency power spectrum. Formula (2) shows the intensity–intensity autocorrelation function in the reference beam optics as a function of delay time, τ:

$$G^{(2)}(\tau) = I_L^2 + 2I_L \sum_{i=d_{min}}^{d_{max}} \sum_{j=\Delta\nu_{s,min}}^{\Delta\nu_{s,max}} I_{s,ij} \exp\left(-\Gamma_i |\tau|\right) \cos\left[\left(\omega_M + 2\pi\Delta\nu_{s,ij}\right)\tau\right] \quad (2)$$

Figure 5 shows a typical autocorrelation function together with an electrophoretic velocity spectrum. In the autocorrelation function, the cosine wave is due to oriented electrophoretic motion and the decay is due to random Brownian motion. In the spectrum, the peak location is related to optical modulator and electrophoretic motion of the particles, and the peak shape is caused by Brownian motion of the particles, the mobility velocity spectrum and any finite laser beam width restrictions.



Key

1	autocorrelation trace	$G^2(\tau)$	autocorrelation function	τ	delay time
2	spectrum	$S(\omega)$	frequency power	ω	rotational frequency

Figure 5 — A typical autocorrelation function and spectrum from electrophoretic light scattering

6.5.3 Phase analysis light scattering (PALS)

The electrophoretic mobility of some particles in a non-polar solvent is very small, resulting in very small differences between the modulator frequency and the Doppler frequency shifts from electrophoretic motion. Such frequency differences can be less than 1 Hz. For particles suspended in a solution of high ion concentration, only a very small field can be applied between the electrodes before the Joule–Thompson heating affects measurement. Therefore, again, very small Doppler frequency shifts require detection.

In these instances, because of small frequency shift, intensity or spectrum analysis of scattered light no longer has enough resolution and phase analysis of scattered light becomes a better choice (Reference [11]). PALS is a method of exposing the very small shift in frequency caused by the additional small frequency component

induced by the electrophoretic mobility of the particles. In PALS processing (also called amplitude-weighted structure function processing), the modulator frequency is synthesized within the processor, to provide sine or cosine waves. The signal detected is multiplied by the sine or cosine waves to create two derived functions: the in-phase component and the quadrature component. The resolved arctangent vector from these two components has an arbitrary amplitude but its rate of change is the phase difference per time.

In principle, this technique is able to resolve frequency differences as low as 0,001 Hz. However, due to noise and other limitations, this is not generally possible. Frequency shifts as low as 0,002 Hz have been observed, nonetheless. PALS can only provide a mean electrophoretic mobility value.

In some implementations, a combination of PALS and spectrum analysis coupled with both fast and slow applied voltage reversals is employed to both prevent cell electrode polarization and separate the effects of electrophoresis and electroosmosis. By these means, both the mean value and the spectrum of electrophoresis values are obtained (Reference [12]).

6.5.4 Modulated Brownian motion power spectrum method

This method utilizes a frequency spectrum analysis of the scattered light from particles in suspension under an alternating electric field to measure the average amplitude of particle electrophoretic mobility, while the sign of mobility is determined by a separate d.c. measurement. A single point calibration with a traceable mobility standard is needed.

6.6 Determination of electrophoretic mobility

The relation between the Doppler frequency shift of scattered light and particle electrophoretic mobility, μ, depends on the optical arrangement of the instrumentation. Formulae (3) and (4) apply for the reference beam optics and cross-beam optics, respectively (Reference [8]).

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi nE\sin(\theta/2)\sin\left[(\theta/2)+\xi\right]} \tag{3}$$

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi nE\sin(\theta'/2)} \tag{4}$$

where

$\Delta\omega$ is the Doppler frequency shift;

λ_0 is the laser wavelength in vacuum;

n is the refractive index of the medium;

E is the electric field strength;

θ is the angle between the incident light and the scattered light;

ξ is the angle between the scattered light and the orientation of the electric field;

θ' is the angle between the two beams.

7 Calculation of zeta-potential

For a detailed description of various theories applied for calculating zeta-potential, refer to ISO 13099-1.

For non-conducting spheres, Formula (5), an extension of the Henry equation between zeta-potential, ζ, and electrophoretic mobility, μ, is widely used (Reference [13]):

$$\mu = \frac{2\zeta\varepsilon}{3\eta_0} f(\kappa a) \tag{5}$$

where

η_0 is the viscosity of the medium;

κ is the reciprocal of Debye double layer thickness;

ε is the dielectric permittivity of the medium;

a is the sphere radius;

$f(\kappa a)$is a monotonic function varying from $f(\kappa a)_{\kappa a \to 0}$ = 1 to $f(\kappa a)_{\kappa a \to \infty}$ = 3/2.

Formula (5) assumes that:

a) the total electric field a particle experiences is a superposition of the applied field and the field due to the charge on the particle;

b) the distortion of the field induced by particle movement (i.e. the relaxation effect) can be ignored;

c) the inertial terms in the hydrodynamic equation are negligible;

d) the surface potential is much smaller than $k_B T/e$.

There are many refined or more rigorous models between ζ and μ without having to impose restrictions a) to d). However, the calculation using these procedures requires tedious computation and prior knowledge of some other parameters related to the sample, whose values are often unknown or difficult to obtain.

Relationships between electrophoretic mobility and zeta-potential for other types of particles in dilute suspensions can be found in the literature and their applications in practice are fairly limited. Since most samples are polydisperse in size and thus have different κa values, it is impractical to apply complex and different conversions for each component in the distribution in order to obtain the complete zeta-potential distribution.

When κa >> 1, typical for large particles in aqueous suspension, $f(\kappa a)$ takes the value of 3/2 in Formula 6, leading to the Smoluchowski equation.

When κa << 1, typical for small particles in organic liquids, $f(\kappa a)$ takes the value of 1 in Formula (5). The equation is then called the Hückel equation.

For a comprehensive overview of these theories, refer to ISO 13099-1.

8 Operational procedures

8.1 Requirements

8.1.1 Instrument location

The instrument should be located in a clean environment that is free from excessive electrical noise, mechanical vibration and temperature fluctuations, and sheltered from direct sunlight and airflows. The operating area should conform to local health and safety requirements. The instrument should contain a rigid internal optical bench with a vibration damp setup and be installed on a rigid table or bench to avoid the necessity of realignment of the optical system at frequent intervals.

WARNING The radiation within instruments equipped with a laser can cause permanent eye damage. Never look into the direct path of the laser beam or its reflections. Avoid blocking the laser beam with reflecting surfaces. Observe relevant local laser radiation safety regulations.

8.1.2 Dispersion liquids

Ensure that the sample cell is compatible with the medium to be used. The electrophoretic mobility of the particles is wholly dependent upon the chemical characteristics of the suspending liquid. Both the pH and specific ion concentration of the dispersing liquid are vital characteristics to be controlled if concentrated suspensions are to be diluted for measurement. The conditions the particles are currently experiencing within a concentrated suspension shall be entirely matched by the diluents.

8.1.3 Measurement cell

Depending upon the apparatus available, there may be a choice of cell designs. Some cells are disposable. To avoid the possibility of contamination, carefully rinse any cell previously used for tests. A measurement conducted on a solution at low ion concentration may be distorted if the previous measurement was conducted at high ion concentration. It is quite a challenge to adequately flush out all of the residual ions from the earlier test. The condition of the electrode surface may well have been compromised by previous measurements, leading to errors in the assessment of the potential gradient. If the cell walls remain contaminated, then it is possible that the electroosmotic flow in a capillary cell is not as expected.

Careful temperature control of the cell is important, as the liquid viscosity is a function of temperature, e.g. in the case of aqueous samples, the viscosity changes by about 2 %/°C. The terminal velocity the particles obtain depends upon the liquid viscosity.

The potential applied between the electrodes may be a d.c. voltage applied for a finite time before being reversed in polarity. When highly conducting fluids are required, the d.c. voltage may only be applied for a short period of the time employed before reversal. This method is employed to suppress Joule–Thompson heating effects. This method of operation may result in pulses that are so short that side-peak artefacts appear in the spectrum. PALS processing is sometimes required under these conditions (Reference [11]).

8.1.4 Sample inspection, preparation, dilution, and concentration

8.1.4.1 Sample inspection

Inspect the material to be analysed, visually or with the aid of a microscope, to check whether the particles have been dispersed adequately and whether any sedimentation occurs upon standing. If particles sediment faster than the measurement duration, measurement is not possible, since there are no particles left in the laser beam. For polydisperse samples, large particles can settle, leaving only smaller ones to be measured.

The electrophoretic mobility measured in a sample is only valid for a batch of material if the test sample is representative for that batch and has been sampled adequately.

8.1.4.2 Preparation

Considerable care should be exercised to avoid changing the electrophoretic mobility of the sample to be tested during sample preparation for measurement. Any handling device such as a glass beaker or syringe can have surfaces that attract specific ions from the sample or that add contaminants left behind from earlier cleaning processes. Additionally, some materials from the handling device may “leak” into the sample. Disposable plastic preparation beakers and pipettes are preferred as long as they are chemically compatible with the sample.

8.1.4.3 Dilution

In some implementations, when using special cell designs, even at small scattering angles, measurements of electrophoretic mobility at moderate concentrations is possible (Reference [9]). In most cases, apparatus that employs a small angle scattering optical arrangement requires that the sample be diluted so as to permit the scattered light to pass through the measurement cell.

Zeta-potential not only is a property of particles, but also depends on the chemical equilibrium between particle surface and the liquid. Any variation in the liquid chemical and ionic composition affects this equilibrium and, consequently, zeta-potential.

This presents a problem for methods that require extreme dilution of the sample. Dilution can change the chemical composition of the liquid, if special measures are not taken. The sample preparation shall follow a procedure so that zeta-potential is not changed from the original system to the diluted sample.

This procedure requires, upon dilution, that not only particles and their surfaces remain identical between the original and diluted systems, but also liquids remain identical. This condition is not easy to satisfy if both dilution and surfactant stabilization of the sample are involved. Sample preparation procedures can affect liquid composition tremendously.

The sample preparation shall use the so-called equilibrium dilution procedure, which uses the same liquid as in the original system as a diluent. After dilution, the only parameter that has changed is the particle concentration. Only sample preparation based on equilibrium dilution provides zeta-potential values that are identical between the original system and the diluted sample.

There are two approaches to collecting the liquid used for dilution. The first is extraction of a supernatant using sedimentation or centrifugation. This supernatant can be used for diluting the initial sample to the degree that is optimal for the particular measuring technique. This method is suitable for large particles with sufficient density contrast. It is not very convenient for nano-particles and soft biological systems.

The other approach, more suitable for nano- and bio-colloids, is to employ dialysis. Dialysis membranes are required that are penetrable for ions and molecules, but not for colloidal particles.

A detailed report of precisely how the sample was handled and how the diluent was prepared shall accompany the result. Several complete dilutions and measurements of the sample should be made to demonstrate that the method adopted was at least stable and consistent.

8.2 Verification

8.2.1 Reference materials

Consult ISO Guide 30 for guidance on using reference materials.

A reference material (RM) for electrophoretic mobility should be sufficiently homogeneous and stable with respect to the electrophoretic mobility value that has been tested for changes with time and temperature. The accepted electrophoretic mobility value shall have been obtained by several operators and rigorously proven. The material should be well documented in terms of sampling procedure, dilution, if required, and measurement protocol.

A certified reference material (CRM) for electrophoretic mobility is used for the purpose of calibrating equipment, validation of measurement methods, and the assignment of values to materials. It should be accompanied by a certificate with the electrophoretic mobility being certified by a procedure which establishes traceability to an accurate realization of the electrophoretic mobility unit and accompanied by an uncertainty at a stated level of confidence. At the time of publication, no CRMs are available and many materials available do not provide an independent traceability to the electrophoretic mobility unit.

8.2.2 Repeatability

To achieve the desired repeatability of electrophoretic mobility measurement, follow these steps:

a) set up the instrument adequately, select the proper settings for operating conditions and allow all parts sufficient warm-up time;

b) follow the measurement protocol given for the material;

c) perform three consecutive measurements on the same test portion or diluted sample at an appropriate concentration.

An instrument is considered as meeting the requirement of this part of ISO 13099 if the coefficient of variation (CV) for the mean electrophoretic mobility values from each measurement for a reference material is less than 10 %, provided that the absolute value of the reference material mobility is higher than 2×10^{-8} $m^2/V•s$.

8.2.3 Intermediate precision

The test of intermediate precision shall follow the procedure specified in 8.2.2 except that different test portions or diluted samples at an appropriate concentration shall be used.

An instrument is considered as meeting the requirement of this part of ISO 13099 if the CV for the mean electrophoretic mobility values from each measurement for a reference material is less than 15 % provided that the absolute value of the reference material mobility is higher than 2×10^{-8} $m^2/V•s$.

8.2.4 Trueness

The trueness of operation of instruments shall be determined using CRMs supported by national standards bodies or other centres of excellence certified as meeting recognized national or International Standards of measurement competence, or a reference material, as defined by ISO Guide 30, of hard spheres from a reliable source, homogenous and stable having a defined electrophoretic mobility determined by optical means at specified conditions. The test of intermediate precision shall follow the procedure specified in 8.2.3.

An instrument is considered to meet the requirements of this part of ISO 13099 if the mean electrophoretic mobility value for three measurements is within 10 % of the published value provided that the absolute value of the reference material mobility is higher than 2×10^{-8} $m^2/V•s$. If a larger error is obtained, advice should be sought and the operation of the instrument examined.

8.3 Sources of measurement error

8.3.1 Contamination of the current sample by the previous sample

A residue of the previous sample may remain in the cell due to inadequate flushing. If the previous suspension had a high ion concentration while the current sample has a low ion concentration, flushing may not be adequate. A separate cell kept exclusively for high ion concentration sample measurements is preferred.

8.3.2 Inappropriate sample preparation procedure

The zeta-potential of a particle system is heavily influenced by the suspending fluid. If the test sample is to be obtained from a concentrated suspension, then the ionic condition of the liquid phase of the concentrated suspension shall be maintained if a true zeta-potential of that concentrated suspension is to be established. A supernatant can be produced either by filtration or sedimentation. Alternatively, a buffered suspension mimicking the concentrated state may be created. Any glassware or other vessels used to create a supernatant or equivalent suspending media shall be clean and free from ionic contamination.

8.3.3 Inappropriate sample

Particles have to be suspended in the sample cell and maintained in the light beam during the entire measurement in order for the mobility to be measured. Therefore, particle density determines the largest particle that can be measured for a certain time period using a particular method and instrument setup. Capillary cells have very small distances for particles travelling before they settle on the floor of the capillary. Accessible measurement time using a capillary cell is usually much shorter than that using a parallel-plate cell for sedimenting particles. Particles also have to produce enough scattering in order to be detected which then determines the smallest particles at a given concentration that can be measured.

The PALS method permits the lowest mobility determination for very small particles. The reason for this is that the influence of Brownian motion is considerably reduced because the PALS processing is averaged over a very large number of cycles to report the first moment of mobility, largely removing the diffusive term from the result.

8.3.4 Inappropriate liquid medium

The medium should be clear and non-absorbing at the wavelength of the laser used. It should not be too high a viscosity preferably lower than 10 mPa•s. It should not be volatile at the measurement temperature.

8.3.5 Poor temperature stabilization

The viscosity of water varies by about 2 %/°C around room temperature. The terminal velocity obtained by the particles experiencing the electric field gradient is limited by viscous drag. Therefore, accurate comparisons of zeta-potential determinations shall be established at known, reported values of temperature. Test samples may be initially created in the laboratory at a different temperature to that existing within the measurement machine. Time shall be allowed for the injected sample to reach thermal equilibrium.

8.3.6 Condensation on the illuminated surfaces

For measurements performed below room temperature, take precautions to avoid possible condensation on the interfaces through which light passes. Purging with dry air or nitrogen is recommended.

8.3.7 Particles, fingerprints or scratches on the optical surfaces

The adverse effect of additional flare from a scratch or contamination of the cell optical surface has a lesser effect when using the reference beam optical arrangement. It is a potential problem when using the cross-beam method and can prevent measurement altogether if serious.

8.3.8 Too large a potential applied

When using a suspension of high ionic strength, such as red blood cells in physiological saline, the induced Joule–Thompson heating effect can cause thermal convection and be problematic. The temperature of the suspension in the measuring volume could be quite different from the set temperature. This results in an incorrect viscosity value. The heated electrodes may burn cells on to their surface in extreme cases and air bubbles produced by electrolysis may disturb particle movement. To minimize this effect, the voltage applied should be reduced and for a short time within each cycle of reversal with synchronized data collection.

8.3.9 Incorrect entry of parameters by the operator

Errors are introduced when parameters such as permittivity, viscosity, refractive index, temperature, conductivity, and electric field are incorrectly entered.

8.3.10 Air bubbles

Air bubbles can arise from the filling process, from dissolved air, or due to electrochemical reactions, e.g. electrolysis occurring at the electrode surfaces. Bubbles adhering to the walls of a capillary cell can distort the electric field leading to uncertainty of the position of the stationary layer. Bubbles adhering to electrode surfaces can result in an incorrect measurement of conductivity.

8.3.11 Cell coating damage

Some implementations employ a coated capillary cell. This coating neutralizes the surface charge of the capillary wall, enabling measurements to be made at any point across the capillary. These coatings can be fragile and readily destroyed by using suspensions, chemically unsuitable for this technique.

8.3.12 Inappropriate theory for calculating zeta-potential from the measured electrophoretic mobility

Zeta-potential is calculated from the measurement of mobility. The appropriate theory and equation to be used for this calculation critically depends upon the suspension environment. See ISO 13099-1 for a more complete explanation.

8.3.13 Sample stability consideration

It is always advisable to conduct a series of measurements, sequenced in time, to demonstrate that the sample is stable. Alternatively, any instability may be the focus of the experiment. For example, the dissociation of ion species from the particles in suspension can result in a change of pH or conductivity over time together with the mobility value. It is recommended that if the apparatus permits, a determination of pH or conductivity of the suspension be conducted before and after each measurement. This either shows that the sample remains stable or demonstrates instability. Any rapid instability represents a further challenge in as much as the sample shall be prepared and presented to the instrument for measurement. Any variation in the time steps of this procedure results in variation of the final result. If faced with this problem, then it is recommended that rates of change be reported and not absolute values.

8.4 Test report

The average values of zeta-potential and electrophoretic mobility as well as their distributions if the instrument provides such information, should be reported. The following details shall also be provided:

a) a title and a unique identification on each page;

b) complete sample identification and date sample received;

c) instrument model and serial number;

d) original sample conditions:

- nature of particles,
- dispersing liquid,
- concentration of particulate material,
- additives, if any,
- ion types and concentrations if known,
- pH and conductivity;

e) sample preparation procedures:

- method of dilution in detail,
- sonication conditions if used: frequency and applied power,
- additional information including other reference procedures if any;

f) measurement conditions:

- actual concentrations investigated,
- measurement temperature,
- viscosity, permittivity and refractive index of the dispersion liquid,
- electric field voltage and field strength applied;

g) analyst identification:

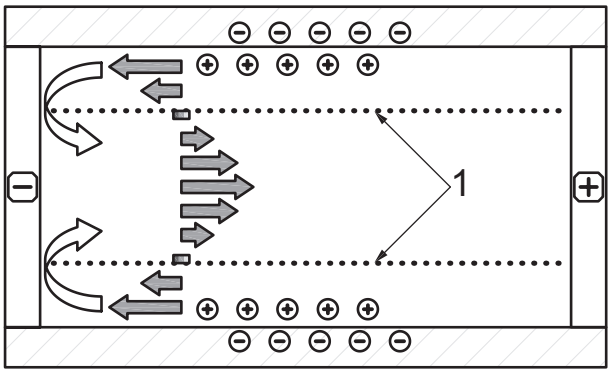
- name and place of laboratory,
- operator’s name or initials,
- date;

h) a clear identification of the end of the report.

Annex A
(informative)

Electroosmosis within capillary cells

Electroosmosis is the motion of liquid relative to a fixed, charged surface. When an electric field is applied to a capillary electrophoresis cell, the liquid near the capillary surface moves along the applied field. When the entire capillary is a closed system, the moving liquid near the wall has to reverse its motion at the end of the capillary and thus pushes the liquid in the central part of the capillary in the other direction forming a parabolic liquid flow profile as illustrated in Figure A.1. For a given capillary shape and dimension of simple geometry, the profile can be theoretically predicted if all walls have the same surface charge condition.



Key
1 stationary layers

Figure 6 — Liquid flow profile in a capillary

For cylindrical capillaries, the electroosmotic fluid mobility profile is

$$\mu_{\text{eo}}(r) = \mu_{0,\text{eo}}\left(\frac{2r^2}{R_{\text{cap}}^2} - 1\right) \tag{A.1}$$

where

$\mu_{0,\text{eo}}$ is the electroosmotic fluid mobility at the slip plane of the wall;

r is the distance from the axis;

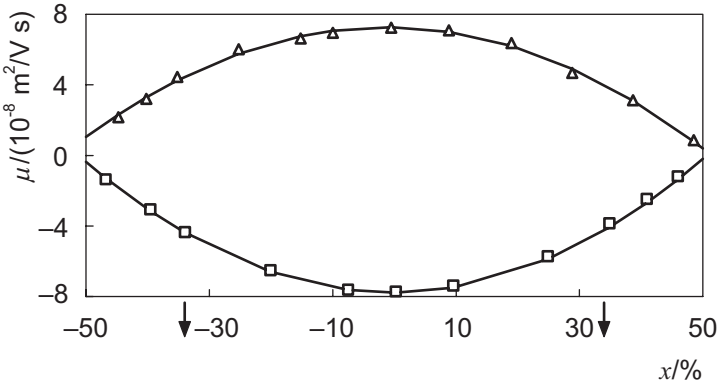
R_{cap} is the capillary radius.

Similar formulations have been derived for rectangular capillaries (Reference [14]). The shape of the parabolic flow profile varies with $\mu_{0,\text{eo}}$ which depends on surface condition. Because of the effect on liquid motion from electroosmosis, the observed Doppler frequency shift is no longer purely from particle motion, but is the net result of electrophoresis and Brownian motions of particles and liquid electroosmosis. In order to correctly access the particle motion, the measurement has to be performed at the locations where liquid is stationary, i.e. $\mu_{\text{eo}} = 0$. These locations are called the stationary layer. For circular capillaries, this layer is a circle with $r = 0{,}707 R_{\text{cap}}$. For rectangular capillaries, the stationary layer is a rectangle with the locations, the distance from the walls, dependent on the ratio of width to height of the capillary. For a width to height ratio of 3, the upper and lower stationary layers are located at 84 % and 16 % of the capillary height, respectively.

Figure A.2 shows apparent electrophoretic mobility values obtained at different cell positions in a rectangular capillary cell. The squares are from a polystyrene latex (PSL) sample (a = 155 nm) with a mobility of $-4{,}2 \times$

10^{-8} $m^2/V•s$. The triangles are from a PSL sample (a = 45 nm) with positive dodecyltrimethylammonium ions adsorbed in a 1 mmol/l solution of NaBr. The symmetric shape of the profiles indicates that the upper cell surface and lower cell surface have the same zeta-potential. Two arrows indicate the two stationary layers where electroosmotic flow is zero and true electrophoretic mobility can be obtained.

In practice, when an incident beam is placed at the stationary layer location, the effect of liquid electroosmosis can be mostly eliminated. Obviously, since the beam has a certain thickness, particles to be measured are actually seen in a layer of non-zero thickness around the stationary layer. For rectangular capillaries, it can be expected that liquid above and below the stationary layer experiencing only a small amount of electroosmosis in opposite directions has on the average net zero electroosmotic velocity. For circular capillaries, since the stationary layer is not a plane, a deviation from zero liquid velocity can result even when the beam centre is located exactly at the stationary layer.



Key

μ electrophoretic mobility

x position

Figure 7 — Apparent electrophoretic mobility values obtained at different cell positions (Reference [8])

If affected by adsorption of ions and other species from the sample and air or gas bubbles, the surface potential of different sides of the capillary can become inhomogeneous. Then an asymmetric liquid profile is produced. This profile can be used to determine the surface potential of both upper surface and lower surface of rectangular capillaries (References [15]).

Another effect of electroosmosis is the broadening in the reported mobility distribution. Any light beam has a finite thickness. When the beam centre is located at the stationary layer, part of the beam illuminates the liquid flowing toward the right side and part of the beam illuminates the liquid flowing toward the left side. Even for particles having ideally monodisperse mobility, the reported mobility is a distribution instead of a single value, although the mean may still be correct. At the capillary centre where the flow profile has a much flatter slope, liquid and particles experience similar flow. Thus distribution broadening is minimal. Therefore, one way to minimize the effect of electroosmosis is to make two measurements, the first at the capillary centre and the second at one of the stationary layers. The mobility distribution obtained at the centre has the correct shape, but a shifted absolute value that can then be corrected by the mean value obtained at the stationary layer.

One way to reduce electroosmosis is by coating the internal surface of the capillary by a material that reduces zeta-potential of the wall. In particular, poly(ethylene glycol)–poly(ethylene imine) (PEG–PEI) coating or grafting significantly reduce electroosmosis over a wide range of pH and ionic strength. However, the coating stability and ease of coating need to be further improved in order to be adopted as a routine procedure.

Another method to avoid liquid electroosmosis is to use a d.c. field with a changing polarity of high frequency (>10 Hz). The main idea is that it takes a much longer time for liquid to reach the terminal velocity than that of particles. The acceleration time for non-moving particles to reach their electrophoretic velocity is in the range of nanoseconds to microseconds, but for liquids it is in the range of sub-seconds. If the electric field polarity changes rapidly, the liquid is static and electroosmosis is eliminated (References [16][17]). Therefore, measurements can be performed at any location in the capillary. However, when using a high frequency field, spectrum resolution is inevitably reduced and harmonic sidebands are produced due to field modulation. This

complicates the spectrum, especially for materials of polydisperse mobility. One way to take the advantages of both ordinary d.c. measurement and high frequency field reversal measurement is to perform a high frequency field reversal measurement to obtain the mean mobility value of the sample, followed by a d.c. measurement that provides an electrophoretogram without sidebands, but with incorrect values of electrophoretic mobility because of electroosmosis of the liquid. The entire electrophoretogram is then shifted to yield the correct mean value obtained from the high frequency measurement (Reference [12]).

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