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Guidelines for good practices in zeta-potential measurement

Lignes directrices relatives aux bonnes pratiques pour la mesure du potentiel zéta

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Zeta-potential is often used to investigate the isoelectric point (IEP) and surface adsorption for particles in liquid media, and as an indicator in comparing different samples regarding electrostatic-dependent dispersion stability. Zeta-potential is not a directly measurable quantity, but is established using an appropriate theory. Furthermore, zeta-potential is not an intrinsic property of suspended particles; it depends on both particle and medium properties, and how they interact at the interface. Any variation in the liquid chemical and ionic composition affects this interfacial equilibrium and, consequently, zeta-potential. Therefore, sample preparation and measurement procedures can both affect the measurement result. Incorrect conclusions often result from artefacts in sample preparation and issues arising from measurement procedures, or incorrect application of theoretical models for calculating zeta-potential from measurement results.

This document provides general guidelines for sample preparation and measurement procedures for the determination of zeta-potential by optically-based electrophoretic mobility or electroacoustic methods.

Guidelines for good practices in zeta-potential measurement

1 Scope

This document addresses the zeta-potential measurement operation for applications such as new product design, optimization of existing products, quality control during processing and/or during usage of the product. It does not provide a complete procedure for zeta-potential measurements. The instructions and key points addressed in this document are considered useful for performing zeta-potential measurements as specified in ISO 13099-1 and ISO 13099-2.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Symbols

μ electrophoretic mobility

ε_m relative permittivity of the medium

ζ electrokinetic potential, zeta-potential

η_o medium viscosity

5 Principle

Zeta-potential (ζ) is the electric potential at a hypothetical shear plane that separates the mobile solvent from solvent molecules that associate with the particle surface. Zeta-potential is frequently used to predict the stability of a suspension or the adhesion of suspended particles onto macroscopic surfaces (e.g. cellulose fibres, membranes). This is because interaction between particles or between particles and surfaces or between particles and proteins is often governed by the ion distribution in the diffuse layer, which is closely related to zeta-potential. Whenever electrostatic forces dominate interactions between particles or between particles and surfaces, zeta-potential is the principal system parameter to evaluate these interactions[1]. Repulsion requires high surface charges of equal sign, whereas attraction occurs in the absence of surface charge or for oppositely charged or “patchy” surfaces containing both negative and positive domains. High zeta-potential absolute values cause strong repulsion between dispersed particles and, thus, favour the stabilization of colloidal suspensions. This effect is even more pronounced for thick double layers in low electrolyte content. In contrast, low zeta-potential absolute values (+ or −), zeta-potentials of opposite sign (polarity), or high electrolyte concentrations, can promote agglomeration. Hence, zeta-potential can be principally employed to predict the suspension stability, which is frequently determined as a function of the pH and/or the concentration of indifferent electrolytes and surface active ionic species (e.g. ionic surfactants, multi-valent ions and polyelectrolytes)[2][3].

Zeta-potential can be probed by imposing a relative motion between bulk solvent and particle. Zeta-potential measurements on colloidal suspensions are frequently conducted via either electrophoresis or electroacoustics for applications under different sample conditions (e.g. diameter range from nanometre up to tens of micrometres with particle volume fraction from roughly 10^{-4} % to 40 %)[4].

Zeta-potential is not an intrinsic particle property; it depends on the chemical equilibrium between the particle surface and the liquid phase in which it is dispersed. Any variation of the liquid chemical and ionic composition may affect this equilibrium and, consequently, affects zeta-potential[5][6][7].

The perception that there is a universally valid critical zeta-potential value that defines the transition from unstable to stable suspensions has been proven only in limited applications. Zeta-potential values need to be used carefully when evaluating the suspension stability. It is recommended that in formulation work to predict stability, a second measurand (e.g. size distribution, turbidity, viscosity, etc.) can be monitored and correlated to verify the conclusion derived from zeta-potential measurement.

Information on Zeta-potential measurement for particles in non-polar media can be found in Annex A.

6 Sample preparation

6.1 General

As the particle zeta-potential depends on particles as well as the dispersion medium, simple dilution can change the chemical composition of the medium and then affect the particle zeta-potential. Therefore, in addition to general practice in sample preparation for particle systems[8], special measures need to be taken. Dilution can also induce dissolution, which alters both the surface and the medium. The sample preparation needs to follow a procedure such that zeta-potential is not changed from the original system to the diluted sample.

The sample preparation procedure requires that upon dilution not only do particles and their surfaces remain identical between the original system and the diluted system, but also that the medium remains electrochemically identical. Particle surface charge is wholly dependent upon the chemical characteristics of the dispersion medium. Both the pH and specific ion concentration of the dispersing medium are vital characteristics to be controlled if concentrated suspensions are to be diluted for measurement. The conditions that particles undergo within a concentrated suspension need to be entirely matched by the diluent.

This condition is not easy to satisfy if both dilution and surfactant stabilization of the sample are involved. The sample preparation procedures can affect liquid composition tremendously. It is rather difficult to adjust the particle concentration for electrokinetic measurements without impacting the physico-chemical properties of the dispersion medium and the interface. For instance, dispersing amorphous silica in KNO_3 solution results in a suspension that will be different from the same material in de-ionized water with respect to both pH and ionic strength. These differences have a considerable impact on the interfacial properties, such as the zeta-potential.

6.2 Sampling and sample inspection

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

The material to be analysed should be inspected to ensure the particles have been dispersed adequately without any sedimentation occurring upon standing for a period relevant to the measurement time. If particles sediment during measurement, measurement using optical methods may not be appropriate since particles remaining in the laser beam may not represent the whole sample (e.g. with polydisperse samples, large particles will settle differentially resulting in a biased measurement of the smaller size particles).

Considerable care needs to be exercised during sample preparation to avoid changing the electrophoretic mobility[3] of the sample to be tested. Labware that comes into direct contact with the sample, such as a glass beaker or syringe, may adsorb specific ions from the medium or add residual contaminants to the sample that remain from a prior cleaning process or from production of the labware itself. Disposable plastic preparation beakers and pipettes are generally preferred, as long as they are chemically compatible with the sample.

A detailed report describing precisely how the sample was handled and how the diluent was prepared is to accompany the result. Several complete dilutions and measurements of the sample can be made to demonstrate that the method adopted is stable and reproducible.

6.3 Sample dilution procedures

In electroacoustic measurements, little or no sample preparation is typically required and instruments convert the raw measurement data to zeta-potential using a theory and calibration procedure that account for the finite particle concentration[9], as well as for the effect of particle size. In systems relying on electrophoresis with optical detection, particle-particle interactions are minimized by diluting the sample to an appropriate concentration. In this case, care needs to be taken that solvent shock or other dilution effects do not alter the electrokinetic properties of the sample.

Sample dilution can follow the so-called equilibrium dilution approach, wherein the same liquid as that in the original system is utilized as the diluent. When done properly, equilibrium dilution results in a sample where the only parameter modified is the particle concentration. Only sample preparation based on equilibrium dilution yields zeta-potential values that are theoretically identical between the original system and the diluted sample. Simple dilution using deionized water, for example, is a misleading and generally incorrect way to prepare samples for zeta-potential measurement.

There are two approaches to the collection of the liquid used for equilibrium dilution. The first consists of extracting a supernatant using gravitational sedimentation or centrifugation. This supernatant or “mother liquor” can then be used for diluting the initial sample to the degree that is optimal for the chosen measurement technique. This method is suitable for large particles with sufficient density contrast. It is not very convenient for nanoparticles and biological systems with low density contrast. For emulsions, where a third phase (an emulsifier) stabilizes the normally immiscible oil and aqueous phases, dilution into a matched ionic background is typical, due to the difficulty of using centrifugation in this case. Ideally, this preserves the same ionic background in both the concentrated and more dilute forms. This diluent can be obtained by knowledge of the ionic composition (ions, ionic surfactants) in the dispersant phase. However, this will not account for species released by the particle phase itself. A third approach, perhaps more suitable for nano- and bio-colloids is to employ dialysis. Dialysis membranes are required that are permeable for ions and molecules, but not for colloidal particles, and the process needs to be validated to avoid artefacts such as particle or surfactant loss to the membrane.

In some rare cases, there is a need to prepare samples at higher concentrations than the native material. This can be achieved by initially separating particles from the medium and then re-dispersing them into the same medium, but at a higher volume fraction. It may also be possible to gently centrifuge the particles to obtain a more concentrated fraction, after removal of the supernatant phase. This process needs to be optimized to mitigate particle loss or agglomeration effects.

Any medium utilized for dilution or for preparing samples is required to be initially free of particles (at least to the extent that residual particles can impact the zeta-potential measurement). For relatively “clean” media, one can use membrane filters (e.g. syringe filters) with a mean pore size smaller than the smallest particles to be analysed. The hydrophobicity or chemical resistivity of the membrane should also be considered. More complex media may prove more difficult to process. Centrifugation can also be used to achieve this objective.

6.4 Sample stability test

It is advisable to conduct a series of measurements, sequenced in time, to demonstrate that the sample is stable[8]. For example, the disassociation of ionic species from the particles in suspension may result in a change of pH or conductivity over time together with the mobility value. It is recommended that a determination of pH and/or conductivity of the suspension be conducted before and after each measurement, if possible. This can confirm that the sample is not changing. Any observable instability in the sample represents a further challenge for analysis. In such a case, it is recommended that rates of change be reported in addition to absolute values.

It is recommended to conduct a series of measurements at different concentrations if dilution is required. The effect of particle-particle interactions or other dilution effects can thus be observed. Typically, hindered movement resulting from particle-particle interactions reduces the apparent motion, shifting the determined zeta-potential toward the zero-value direction.

7 Measurement uncertainty and sources of error

7.1 General

Normal quality markers for precision (minimum of three consecutive measurements when using optical methods[10] or six consecutive measurements when using acoustic methods[11], under repeatability conditions or different aliquots of the same starting material) and robustness (e.g. dilution effects, voltage effects, etc.) apply. Any specification can be 'just good enough' and 'fit for purpose' noting that a 1 mV change at 80 mV is not comparable to a 1 mV change at or around 0 mV. Trending results are normally an indicator of something changing in the system.

Many manufacturers provide result quality and expert advice assistance that is useful and should not be ignored.

Adequate signal-to-noise ratio is vital. Poor signal-to-noise ratio often results in poor precision. In many cases, the raw signal data can be inspected and expert assistance sought and utilized. Excessively broad or multiple peaks are suspicious and always warrant careful investigation. Concentrations that are too high or too low may cause measurement issues, and thus a concentration series often provides useful insight as to the range within which adequate measurements can be undertaken.

Zeta-potential measurements are particularly sensitive to cleanliness and the presence of small quantities of contaminants such as polyvalent ions or leached materials, which may not significantly affect the conductivity or pH. The quality of any water used for dilution or sample preparation is important. It cannot be assumed that deionized water from a commercial water treatment system meets the requirements for zeta-potential measurements; if possible, this water source should be tested independently from the dispenser (e.g. most commercial zeta-potential instruments allow measurement of conductivity, and pH can be easily measured - see 7.6). The measurement of a certified reference material or secondary measurement materials will confirm instrument performance and should always be performed if doubts exist. Periodic (e.g. annual) instrument performance verification by the instrument manufacturer or their designee is strongly recommended when possible.

7.2 Carryover contamination from previous samples

A residue of the previous sample may remain in the cell due to inadequate flushing, unless the cell is of the disposable or replaceable type. Flushing may not be adequate when switching between very high and very low ion concentrations or distinctly different types of samples. A separate cell kept exclusively for high ion concentration sample measurements is preferred, or disposable cells, if available, may be used. It is also recommended to run measurements, in the case of multiple related samples, from low to high ion concentrations and from low to high pH, in order to avoid carry-over contamination.

7.3 Inappropriate sample preparation procedures

Details regarding sample dilution issues are addressed in 6.2. Any glassware or other vessels used to create a supernatant or equivalent suspending medium need to be clean and free from ionic contamination. Possible adsorption of sample on the measurement cell surface can also alter measurements and result in a biased zeta-potential result. One way to circumvent this problem is to condition the cell with the sample prior to the measurement[12].

7.4 Inappropriate samples for electrophoretic light scattering measurement

Particles should be suspended in the sample cell and remain in the light beam during the entire electrophoretic mobility measurement. Therefore, particle density determines the largest particle that can be measured over a certain time period using a particular method and instrument setup. Capillary cells have very small distances for particles to travel before they settle out of the measurement zone. For sedimenting particles, accessible measurement time using a capillary cell will be much shorter than that using a dip cell, where two plate-electrodes are immersed in a cuvette. Particles can be detected only if sufficient scattering is produced, which then determines the smallest particles at a given concentration that can be measured.

The phase analysis light scattering (PALS) method in electrophoretic light scattering permits the lowest mobility determination for nanoparticles, since the influence of Brownian motion is considerably reduced as the PALS processing largely removes the diffusive term from the result. The influence of Brownian motion can also be empirically removed during processing of data from electrophoretic light scattering measurements[13].

7.5 Inappropriate liquid medium

The medium needs to be clear and non-absorbing at the wavelength of the laser used. The viscosity cannot be too high; preferably viscosity (η_0) is lower than 10 mPa·s. The medium cannot be significantly volatile or evaporative at the measurement temperature, or the cell used for measurement should be capable of preventing significant loss of solvent.

7.6 Incorrect entries of parameters by the operator

Bias will be introduced when parameters such as permittivity, viscosity, refractive index, temperature, conductivity, and electric field are incorrectly entered or determined. It is important to ensure that correct parameters and conditions for the measurement have been entered or selected by the instrument.

It is especially troublesome for non-Newtonian liquids where appropriate values for viscosity become unclear. One approach is to use “micro-viscosity”[14][15]. According to this concept, particles moving in the non-Newtonian liquid experience “micro-viscosity” that differs from the “macro-viscosity” value that can be measured with classical rheological instruments. The value of micro-viscosity would depend on the ratio of the particle size to the mesh size of the additives that constitute the non-Newtonian liquid. It also depends on the frequency of the particle motion if AC electric fields are applied to a specific experiment.

There is only one known way for determining micro-viscosity: measuring a dispersion that contains particles with a known size. The measurement technique needs to be sensitive to the viscosity value. This includes gravitational sedimentation, centrifugation, acoustics, and dynamic light scattering (DLS). The measured raw data can be used to back out a viscosity value that would yield the known particle size. Such a viscosity value can then be used as the “micro-viscosity” input parameter for measuring samples with unknown size and determining their zeta-potential.

There are several conditions to use this micro-viscosity approach. First, the size range of the samples needs to be similar to the size that was used for determining micro-viscosity. This will ensure that the ratio of the particle size to the mesh size does not vary significantly. Another condition is associated with the frequency of the micro-viscosity measurement. Micro-viscosity is frequency-dependent for non-Newtonian liquids. Different zeta-potential measurement methods would require different values of micro-viscosity since they function at different frequencies. For instance, the micro-electrophoresis method and the electrophoretic light scattering (ELS) method require micro-viscosity values measured at zero frequency, while the electroacoustic method requires micro-viscosity determined in the MHz range. Therefore, for micro-electrophoresis and ELS, sedimentation or DLS can be used for micro-viscosity measurements. For electroacoustics, acoustic particle sizing is used for micro-viscosity measurements[16][17].

7.7 Air bubbles

Air bubbles can form during the filling process or during filtration, from dissolved air, or due to electrochemical reactions, e.g. electrolysis occurring at the electrode surface. 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. Gentle tapping is often sufficient to remove bubbles adhered to the electrodes or capillary surface. Identifying the presence of bubbles is the most important step.

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

While zeta-potential is calculated from the measurement of mobility, the appropriate theory and formula to be used for this calculation depends critically upon the nature of particles as well as the suspension environment (see Reference [3] for a more complete explanation). For electrokinetically soft particles, (e.g. hairy particles or emulsion droplets), the zeta-potential derived from the theories included in commercial instruments can be used for the purpose of comparing different samples of the same type but should otherwise be used with caution.

7.9 Carbon dioxide

Atmospheric carbon dioxide (CO_2) dissolves in aqueous solution and forms carbonic acid, which dissociates to form protons (H^+) and bicarbonate ions (HCO_3^-):

$$CO_{2(aq)} + H_2O \Leftrightarrow H_2CO_{3(aq)} \Leftrightarrow H^+ + HCO_3^-$$

The equilibrium of this reaction is affected by pH, but also changes the pH. The system is self-regulating: For deionized water, the pH shifts to about 5,6; this value holds approximately true for any initial pH between 6 and 8. Below pH 6, the initial pH is essentially unaffected by CO_2. Above pH 8, there is a significant downward shift in pH.

This process also affects ionic strength, which is especially important for systems based on distilled and de-ionised water. Based on the limiting ionic equivalent conductivities of hydrogen ion and hydroxide ion and dissociation constant of water, the theoretical value for the electrolytic conductivity of pure water at 25,8 °C is 0,055 µS/cm.

The absorption of ambient CO_2 by water can cause the electrolytic conductivity to increase by a factor of 10 to 30, depending on the level of CO_2 in the atmosphere. For instance, it has been stated[18] that: "... the electrolytic conductivity of pure water equilibrated with atmospheric carbon dioxide can range from 0,7 µS/cm to 1,3 µS/cm depending on atmospheric pressure in the laboratory, giving a standard uncertainty ±0,2 µS/cm. For a 12 µS/cm solution, this amounts to a relative standard uncertainty in the electrolytic conductivity of about ±2 %...".

7.10 Effect of the applied electric field on susceptible samples

There are several potential adverse effects due to the application of the electric field during electrophoresis measurements. One such effect is Joule or resistive heating, an effect that increases with the conductivity of the medium. Joule heating can be mitigated by following the manufacturer's recommendations for an appropriate conductivity range or by using cells designed for high conductivity media. High conductivity typically means aqueous media with an ionic strength significantly above about 0,1 mol/l. Joule heating can produce both temperature increase and a temperature gradient, both of which would impact the electrophoretic and electroosmotic flow during zeta-potential measurement.

Another significant issue related to the passage of current through the sample is the potential denaturation of susceptible samples, such as proteins and protein-like biomolecules (e.g. DNA), including samples (e.g. particles) that have biomolecular coatings or other susceptible coatings on their surface. The redox degradation in the electric field occurs at the electrode surface and may be affected by the electrode material itself; for instance, gold surfaces are highly reactive toward proteins.

Redox related degradation typically exhibits characteristic and observable effects during zeta-potential measurements, which, if present, recommends further investigation. One observable symptom is a time dependent increase in hydrodynamic size. Associated with this increase is a drift toward more negative zeta-potential values (typically), especially for proteinaceous materials. The size increase is due to aggregation induced by degradation of the sample or its surface coating. For instance, the appearance of a visible black precipitate is common for gold nanoparticles that are susceptible to redox induced degradation (e.g. citrate stabilized particles). The appearance of any visible blackening or “growth” at or below the electrode surface is a strong indication of electrolytic degradation.

Several potential solutions to the degradation or denaturation problem can be considered. These include reducing the time that the field is applied, applying a reduced electric field [may only be possible with appropriate cell configuration (e.g. dip cells)], using short bursts of applied voltage to minimize the “rate” of denaturation, using a less reactive electrode material (e.g. palladium vs. gold), or simultaneously monitoring the size and stopping measurements when a significant change/trend is observed. These steps can mitigate or slow the degradation problem.

The only solution that can eliminate the problem involves separating the susceptible analyte from the electrode surface by means of a so-called diffusion barrier. This approach is only viable if used in a closed capillary type cell and does not work in a dip cell. In this case, the capillary is first filled with a buffer matching the analyte medium. Using a syringe needle, a small plug of sample is then injected into the optical measurement zone of the buffer filled capillary. This creates a barrier on either side of the plug that prevents the sample from contacting the electrodes during the typical time necessary for a zeta-potential measurement (minutes). The length of the capillary will impact the amount of time it takes the analyte to diffuse to the electrode surface.

Annex A
(informative)

Zeta-potential measurement for particles in non-polar media

At the time of publication of this document, interpretation of zeta-potential measurements in non-aqueous media, especially in relation to stability, is not well understood. Size and density effects may be much more important than particle-particle interactions in non-polar media.

The characterization procedure, controlling ionic composition, verification procedure, theory and input parameters, when it is applied to samples in non-polar liquids are not the same when comparing with that in aqueous media[19]. For the purpose of this annex, a non-polar liquid is defined as a liquid with relative dielectric permittivity below 5:

$\varepsilon_m < 5$

There are many liquids with even lower dielectric permittivity, around 2, and much smaller conductivity, on the scale of 10^{-10} S/m. The following are examples: diesel, kerosene, chloroform, cyclohexane, decane, hexane, heptane, hexadecane, toluene and a variety of oils.

Low dielectric permittivity results in poor solvation of ions by the non-polar liquid molecules. To properly solvate and stabilize ions one can use a surfactant. Surfactant molecules build up steric solvating layers around ions preventing them from re-aggregating into neutral molecules. Application of both ionic and non-ionic surfactants allows control of the ionic strength in non-polar liquids. Non-ionic surfactants are preferable because they minimize water contamination[20][21][22].

Low dielectric permittivity also affects the electrophoretic mobility of the particles. It is known from Smoluchwski theory[3] that electrophoretic mobility is proportional to the dielectric constant of the media. Consequently, electrophoretic mobility of equally charged particles is at least 40 times smaller in nonpolar liquid compared with aqueous medium.

Low electrophoretic mobility presents a challenge to the measuring techniques, both electrophoretic and electroacoustic. The electrophoretic method would require a sufficiently strong field for moving particles in non-polar media. On the other hand, a strong electric field causes nonlinear effects. The window for optimum electric field strength is narrow and additional efforts are required to define this window for a particular sample. In the case of electroacoustics, low electrophoretic mobility reduces the amplitude of the colloid vibration current. This complicates the measurement, causing higher variation in measured signal.

Another factor that should be considered is viscosity. The viscosity of many non-polar media is higher than that in aqueous samples, which in turn further reduces electrophoretic mobility.

Finally, an adequate conversion of the electrophoretic mobility into zeta-potential requires knowledge of both the dielectric constant and the viscosity. These parameters might be unknown for many non-polar liquids and their mixtures, which would require an additional measurement. A trace amount of water, even in the range of parts per million, affects the measurement in non-polar media.

