How to Choose a Zeta Potential Analyzer: General Guidelines

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I. Introduction

Your life-saving drug, which was supposed to look clear before injection, has got large particles floating in it. The can of paint you bought to spruce up your living room is caked, and no amount of stirring makes it homogeneous again. And, adding insult to injury, the Béarnaise sauce for the broccoli is lumpy and your tap water is hazy. What do all these problems have in common? The answer is zeta potential represented by the Greek letter "ζ".

Zeta potential is related to the charge density on small particles[i]. In most, but not all cases, the repulsive force that keeps particles from aggregating is a result of this charge density. It can't be measured directly, but it can be inferred from a determination of zeta potential. The square of this potential is proportional to the force of electrostatic repulsion. As this potential goes to zero, repulsion goes to zero, and instability will occur unless there is steric repulsion.

There are different ways to determine zeta potential, but all have this in common: zeta potential is not measured directly; it's calculated from the electrophoretic mobility that, in turn, is determined from the electrophoretic velocity. Putting a charged particle in an electric field causes it to move: electrophoresis. The most well-known type is gel electrophoresis, widely used in protein and DNA separations. The medium is a sponge-like gel. Particle or micro-electrophoresis does not use a gel; charged particles move in a liquid. Remember this when searching the web for information. If you search for electrophoresis, you will get many more hits related to gel electrophoresis. Search instead for "zeta potential".

So what do you do if you want to purchase a zeta potential analyzer? Start with some very general questions. Here are the general questions to ask:

- How do I classify the various techniques?
- How do I set specifications: quantitative and qualitative?
- And then, which techniques have the best chance of solving my problems?

Let us now consider the answers to these questions in more detail.

II. Classifying Techniques

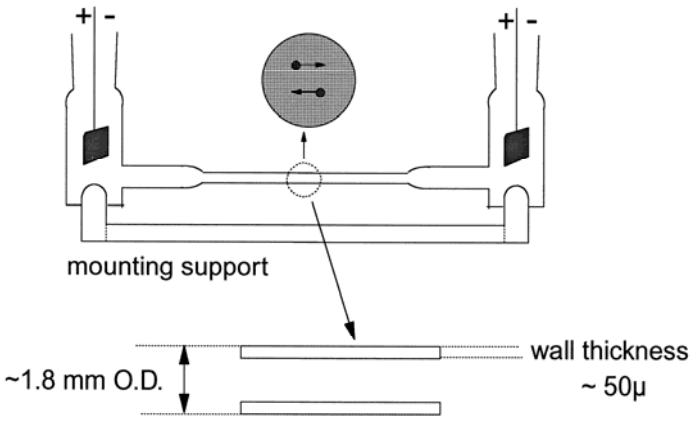
Start by reviewing some common ways to classify the commercially available techniques. Techniques can be classified in any of three ways:

- Imaging: Single Particle Observations
- Concentrates: Acoustical Methods
- Ensemble Averaging: Laser Light Scattering

II.1 Classification: Imaging, Single Particle Observations

The classical method used cells like the one shown in Figure 1.

Figure 1: Van Gils' classical micro-electrophoresis cell



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An applied voltage leads to an electrical field, $\vec{E}$. Charged particles move toward the electrode of opposite sign. Using a stop watch, individual particles are timed as they move a distance determined by the reticle in the microscope's objective. The electrophoretic velocity, $\overrightarrow{V_{ep}}$, is determined. But if the voltage is increased so is the velocity. To get around this, an intrinsic property is defined, the electrophoretic mobility, μ_{ep}.

$$\overrightarrow{V_{ep}} = \mu_{ep} \cdot \vec{E} \qquad \text{Equation 1}$$

However, because of the cell design, there is a counter flow due to charged entities bonded to or adsorbed on the cell walls. This gives rise to an interfering electro-osmotic velocity, V_{eo}. If this is not avoided, the velocity observed is the sum of electro-osmosis and electrophoresis. Measurements must be made close to the cell walls, at the so-called stationary planes, where the electro-osmosis cancels out. This condition is a limitation of such techniques.

While the original image analyzers for zeta potential looked at one particle at a time, newer versions use CCD cameras and automate the tracking of particles in the field. Still, as single particle devices, they do not average a lot of values to obtain average μ_{ep} from which average ζ potential is calculated. In addition, there is a lower limit of particle size associated with any technique where an image is required. Depending on the optics this limitation is somewhere around 100 nm and so many types of important nanoparticles are precluded.

There are different relationships between ζ potential and μ_{ep}, but the most common historic model is that of Smoluchowski as embodied in the default equation used in all instruments:

$$\mu_{ep} = \frac{\varepsilon\varepsilon_o}{\eta}\zeta \qquad \text{Equation 2}$$

Where ε and η are the liquid's relative dielectric constant (permittivity) and bulk viscosity, and ε_o is a constant.[ii] Strictly speaking this equation only applies for large particles (greater than, very approximately, a few tens of nanometers) and high salt concentration (greater than, very approximately, a few tens of millimolar). Yet for historic reasons, if not otherwise mentioned, it is this relationship that is used to calculate zeta potential.

II.2 Classification: Concentrates, Acoustic Methods

A 1% standard of 993 nm polystyrene latex suspended in water appears like slightly diluted milk. Unless the path length through which you look is less than a millimeter or two, the sample is too dense for projecting the image of particles moving in an electric field. And it is too dense for any current form of commercially available light scattering to determine zeta potential. The light is multiply scattered because, before it exits, it is likely to encounter and scatter off another particle. This is where acoustic techniques can help, from about 1% to 50% volume fraction.[iii]

Now it is easy to dilute a 1% latex suspension and still preserve the charge density around the particle. But with many industrial samples that is not always the case. The charge density changes unless special precautions are taken. In some cases, normal emulsions, for example, it may be difficult to preserve the charge density upon dilution, but if measurements are made quickly after dilution, good results should obtain. In addition, in such cases, dilute with the same concentration of dispersing agents that were in original sample. The same holds for microemulsions.

When an oscillating electric field is applied to electrodes in a suspension of charged particles, whose density is different from than that of the surrounding medium, the particles oscillate producing a very weak sound wave. Detecting this weak signal in the presence of other effects (cross talk) requires a delay rod and a well-reasoned approach to the applied frequency of the oscillating field. It may depend on several physical properties of the particle and the liquid. This technique is called ESA, electrokinetic sonic amplitude.

An alternate approach uses the sonic transducer to drive the charged particles producing an alternating current that can be picked up by electrodes surrounding the suspension. This is called CVI, colloid vibration current, or CVP, colloid vibration potential.

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Together these alternate techniques ---each having advantages and disadvantages depending on the sample, its concentration, and the liquid--- are called EAA, electroacoustic attenuation. One or the other is the preferred approach if you must measure in concentrates.

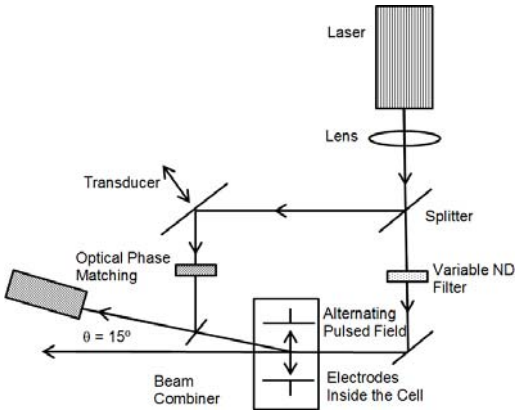
The mathematics that relates measured acoustic properties (impedance, attenuation, speed) or electroacoustic property (vibration potential, current) and the relationship to particle size distribution, charge density (leading to zeta potential), and concentration is complicated. Not fully understanding this is a weakness of the technique, especially for novices.

Sizing can be done with acoustics and zeta potential with electroacoustic attenuation, but only for sizes small enough. How small also depends on particle density. A further complication is that size can also be obtained from electroacoustic attenuation by looking at the frequency dependence of the phase. But other particle and liquid properties are involved.

II.3 Classification: Ensemble Averaging, Laser Light Scattering

The most popular zeta potential analyzers are based on laser light scattering: either electrophoretic (ELS) or phase analysis (PALS). In ELS, a Doppler shifted frequency is measured from which the electrophoretic velocity can be calculated. In PALS, a phase difference is measured from which the electrophoretic velocity can be calculated.

Figure II. Laser light scattering optical configuration for ζ potential determination



In both cases, a small portion of the original beam (the reference beam) is re-routed and a fixed frequency imparted such that when recombined with the scattered beam, it is the difference that is observed. This allows the frequency and phase shift to be analyzed for positive and negative mobility and ζ potential. See Figure II.

Before the electrodes are inserted, if the reference beam is blocked, and a digital autocorrelator used to analyze the scattered light signal, this same optical configuration can be used as a dynamic light scattering particle sizer. (A photon counter is part of a digital autocorrelator; thus, the intensity of the scattered light can also be measured. Such information also yields the molecular weight if the particles are dissolved proteins or polymers.) Note, however, that the determination of size and zeta potential are two separate measurements, not interdependent.

Unlike the classical micro-electrophoresis instruments that are looking at relatively few particles, the number of particles in the light scattering volume is very large, usually much above several thousand. Thus, these instruments are not single particle counters and the average results are averaged over a very large number of scattering particles.

Yet, to interpret the scattering correctly, samples are dilute; otherwise, multiple scattering leads to a mismatch between the internal scattering angle and the angle at which the detector is positioned. This compromise between a lot of particles in the scattering volume yet a dilute sample is easy to accomplish with colloids, nanoparticles and proteins. The samples look clear to slightly turbid.

III Specifications & Definitions

III.1 Quantitative Specifications

Specifications of this type can be listed as follows:

- Electrophoretic mobility range
- Throughput
- Accuracy
- Precision
- Reproducibility
- Resolution

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Since all the techniques cover about the same ζ potential range (-100 mV to +100 mV), you can't decide based on this specification. Electrophoretic mobility is another matter. Look at Equation 2. In a nonpolar liquid (ε small) and/or in a viscous liquid (η large), while the ζ potential may be substantial, the electrophoretic mobility may be small. This leads to a small electrophoretic velocity unless the electric field is set very high, a bad idea that may lead to undesirable secondary effects. Better to use a sensitive detection technique like PALS. In water at room temperature, one mobility unit (10^{-4} $cm^2/V{\cdot}s$) corresponds to 12.8 mV. The classical techniques are limited to a few millivolts as is ELS. But PALS has the ability to measure down to 0.001 mobility units. In water at room temperature this would amount to 0.0128 mV, impossible to measure using ELS. But in oleic acid, with a ratio of dielectric constant to viscosity at room temperature 1,000 times smaller than in water, it would amount to 12.8 mV, easily measureable with PALS. See Table I for examples.

Table I. Mobility Ratios, ε/η normalized to water			
Water	0.89 cP	78	1.0
Toluene	0.56 cP	2.38	0.05
Ethylene Glycol	17 cP	40	0.03
Oleic Acid	26 cP	2.46	0.001

Throughput is the sum over all of the following times:

- Sample preparation time
- Analysis time
- Data reduction/printout/interpretation time
- Cleanup time

Sample preparation may be as short as a few minutes or require overnight wetting with a roller or orbital shaker. The measurement or analysis time is generally quite short with the newer instruments; yet, for older, single particle techniques, it can take longer. Statistical bias is possible if too few particles are measured. This is not a problem with the acoustic or light scattering methods. Likewise, data reduction and printout are fast given modern computers. The time to interpret the data depends on the individual analyst and what criteria have been set. Cleanup time is often underestimated as samples may become contaminated with previous ones if care is not taken in flow systems and in concentrates. Disposable cells are convenient in this regard.

Ask yourself this: How many measurements per hour or per day do you need to make? Are you looking for single point characterization (same solution conditions each time) or for the IEP, isoelectric point where the zeta potential is zero at a particular pH or additive concentration? Such measurements require either an autotitrator or manual measurements after the pH or the additive concentration changes have been made.

III.2 Definitions

Accuracy is a measure of how close an experimental result is to the "true" value. For techniques that cannot be calibrated, or for any other set of conditions where a "true" value is either unknown or not well defined, then accuracy has no meaning. However, where more than one technique is applicable, by comparison between several techniques, different instruments, and institutions, accuracy can be established. The NIST Standard Reference Material SRM 1980 consists of goethite at 500 mg/L, prepared under very stringent phosphate and sodium perchlorate solution conditions and at pH 2.5. Under such circumstances the electrophoretic mobility, μ_{ep} = (+2.53 ± 0.12) μ·cm/V·s with 95% confidence level. That is almost +/-5%. Compared to particle sizing of spheres, electrophoretic mobility measurements are only modestly accurate in general.

Precision is a measure of the variation in repeated measurements under the same conditions including instrument, sample, and operator. Accuracy (associated with systematic error) and precision (associated with random error) are related: The results of many measurements may group tightly together (high precision, low random error) but the mean of the group may be far from the true value (low accuracy, high systematic error). However, if a measurement is highly accurate, then repeated measurements must have grouped around the true value. Still, accurate mean values may consist of either high or low precision. In such cases, precision limits accuracy. Precision for most zeta potential measurements is no better than 5%, depending on the conductivity. At

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high conductivity (greater than say 100 mM ionic strength), thermal effects result in lower precision.

Resolution is a measure of the minimum detectable differences between distinct features in a distribution. It is rare to find a broad or multimodal zeta potential distribution and so resolution is not a major concern in zeta potential determinations. Mixtures are the exception.

Reproducibility is a measure of the variation between different machines, operators, sample preparations, etc. It becomes most important when comparing the results produced on two different machines of supposedly the same type. Such a situation is quite common in industry where multiple machines from one manufacturer and one model have been purchased for use in different labs and/or locations around the world. It is surprising how often the resolution, expressed as a range of values, exceeds the basic precision for any one of the machines. In such cases, it is useful to have round-robin tests done on the same sample and under the same set of prescribed conditions to isolate any machine-to-machine variations.

III.3 Qualitative Specifications

In addition to quantitative specifications, there are qualitative ones that are important for the purchase of any analytical instrument. These include the following:

- **Support**: Is training, service, and application assistance available during the installation, during the warranty period and for as long as the instrument is still serviceable? Instruments are now available from many parts of the world at half price, but they do not include the type of support most users expect.
- **Ease-of-Use**: Will the instrument be used by experts trained in its use or by inexperienced or temporary users? Although the goal of a “one button” device is admirable, it is rarely achieved if for no other reason than sampling and sample preparation is not always one-button amenable.
- **Versatility**: Does the instrument handle aqueous samples in low, medium and high salt concentrations? Does the instrument work in polar as well as nonpolar liquids? Does the instrument work with dilute samples or concentrates or both?
- **Life-Cycle Cost**: The instrument cost is only one factor to consider. If labor intensive, the life-cycle cost can be quite high. Are cleanup costs involved? The life-cycle cost may be higher than alternate choices.

Of all these considerations, support is, perhaps, the most important. When choosing between vendors of the same or similar equipment, the one with better support may tip the scale in its favor. Too often customers assume the largest vendor or the one with the biggest website provides the best support. Often, these are the vendors with the largest overheads who may not find that the sales of analytical instruments for zeta potential determination produce enough profits to stay the course. In the past 25 years, the leaders in zeta potential instrumentation have come from smaller, dedicated firms.

IV. Narrow Down the Possibilities and Make a Choice

Now ask yourself how much information you really want from the measurement. Are you after just the sign of the zeta potential? Are you monitoring changes with time and additive? Specifically, do you need to find the IEP, the isoelectric point, the pH or concentration of the additive that renders the zeta potential zero? Do you need to automate that with a dedicated autotitrator whose operation is integrated with that of the main instrument?

If you intend to add particle sizing and/or molecular weight, the ELS and PALS are the techniques of choice.

If price is the main consideration, the classical optical choices are generally, but not always, the least expensive, if you ignore labor costs. They are generally slower and subject to operator bias more than the other techniques. The acoustic instruments can be the most expensive with the light scattering choices somewhere in between.

If you have to make measurements without dilution, clearly the acoustic instruments are the way to go. Cleanup and cross contamination are a concern. They are also, generally, considerably more complicated to operate.

Get some preliminary measurements made but see the Appendix for useful suggestions before doing so.

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Now carefully consider the quantitative and qualitative specifications, giving the most weight to those aspects that pertain to your situation.

Appendix: Sampling and Sample Preparation

You have narrowed down the choice to one or two techniques and one to three vendors in each category. You are about to send samples to each, requesting results. By comparing the results, you hope to answer the question about mobility range. In addition, you can ascertain the type of support you might expect from the timeliness of the vendors questions and results. In fact, the vendor who asks lots of pertinent questions about the sample properties is often the one who can support you best in the future.

To determine the electrophoretic mobility range of interest, have preliminary measurements made, taking special care to ensure the sampling and sample preparation are equal for all samples sent out for analysis. Initially, if the sample is not a common one, assume that there may be more than one round of sampling, sample preparation and range determination. For common samples, consult references. It is all too easy to believe that an instrument with the largest, claimed-range of application is the most effective. While it might work for the sample at hand, it may not be the optimum choice.

The first problem is to ensure your sample is representative of the whole and is divided without bias amongst the various samples to be sent. There is an entire literature on sampling that should be consulted, but if it has to be reduced to a few ideas, here they are:

If the sample is a dry powder, always sample from a moving stream of dry particles and always across the entire stream to avoid segregation by size. Once the sample is reduced to a manageable size, say one kilogram or less, use a spinning riffler to make smaller, equal portions that are evenly distributed with respect to size distribution. (This is more important for particle sizing, less important for zeta potential.)

If the sample is a suspension in a liquid, mix it gently and thoroughly using a roller (larger sizes) or an orbital shaker (colloidal and nanoparticle dispersions) before separation into smaller lots is made. For large, dense particles, pay particular attention to segregation by sedimentation during the separation process.

The second problem is to ensure that each of the vendors is given the same instructions for sample preparation. Otherwise, the variety of results you get back says more about the different sample preparations than it does about the different instrumental techniques or the different vendors. This is especially true if different wetting, dispersing, or pH agents are used as these will change the surface charge density. If you are not sure how to prepare the sample properly, consult the literature and consult the vendors asking for their advice. Here are the questions any good vendor should ask and the information you should be prepared to give them.

- Are the samples powders, dilute dispersions, dissolved polymers or proteins, or concentrates?
  - If concentrates, are they dilutable without changing the zeta potential. If yes, what is the diluent?
- What additives, if any, are required? Additives that preferentially adsorb onto the surface ***definitely*** affect zeta potential.
  - Wetting agents decrease the contact angle between particles and air allowing liquid to wet the surface.
  - Dispersing agents add repulsive forces between particles long enough to make zeta potential measurements.
  - Stabilizing agents add long-term repulsive forces between particles and are primarily used for long-term product performance.
  - NOTE: Any single additive can provide two but not all three of these properties. AND it is worth repeating: Additives (wetting, dispersing, stabilizing agents) going to the surface change the zeta potential. Ask yourself if this is what you want to measure.
- How much energy, if any, and for how long is it required to disperse the particles? If you create new surface, you almost certainly change the zeta potential.
  - The least energy is best such as manual swirling, gentle rolling or orbital shaking. A low power ultrasonic bath is better than a strong sonic probe that may generate new surface area and change the size

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distribution and zeta potential. It depends on the sample.

In order to properly interpret the measurements, one of more of the following bits of information about the particle and liquid properties is either required or helpful in making estimates:

Particle Properties: Most important are any surface treatments

- Type: latex, clay, organic pigment, drugs, oil/water emulsion, etc. Knowledgeable analysts can estimate many particle properties just from knowing the type of chemistry they are dealing with.
- Surface Treatments: The major determinant of zeta potential prior to the addition of wetting and dispersing agents.

Medium Properties

- Type: water or chemical name of organic solvent
- Viscosity, η_o is required in calculations as is the temperature.
- Dielectric constant (relative permittivity), ε_o is required in calculations as is the temperature.
- pH of aqueous suspensions can affect zeta potential of electrostatically stabilized particles, so it is helpful to know what the starting point is before further steps taken.
- Ionic Strength, I, is related to the conductivity of an aqueous-based sample and is a function of the concentration of free ions such as salts, acids/basis, and ionic additives such as wetting and dispersing agents.

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[i] Small here means mostly below one micron: colloids, nanoparticles, proteins, etc. While all macroscopic surfaces when wetted may develop charge, and while such a charge density may have a profound influence on what adheres to that surface, it is the zeta potential of small particles that determines dispersion stability.
[ii] The permittivity of free space, $\varepsilon_o = 8.854 \times 10^{-12}$ Farad/m.
[iii] D. Fairhurst, American Pharmaceutical Review, vol. 16, April, 2013.

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