Next Generation Electrophoretic Light Scattering (NG-ELS)

confidence at high ionic strength

John F. Miller, Ph.D

ENLIGHTEN SCIENTIFIC

john@enlightenscientific.com
http://www.enlightenscientific.com

Hello – my name is John Miller and I'm the owner of Enlighten Scientific.

30 years ago, I invented Phase Analysis Light Scattering. This has become the *de facto* method for measuring zeta potential at moderately high ionic strengths. It's because of this that various versions of it are sold by most manufacturers of commercial instruments.

Today, I'm going to talk about the Next Generation Electrophoretic Light Scattering system that I have developed that improves the confidence of such measurements and extends the measurement of zeta potential to significantly higher ionic strengths.

Key acknowledgement

David Fairhurst, PhD
Colloid Consultants Ltd
Congers NY

https://www.linkedin.com/in/david-fairhurst-a31a1523/

I must acknowledge David Fairhurst of Colloid Consultants Limited for his ongoing support and many hours of discussions that have helped me tremendously in my work. He has had a very long and influential career in this area. In fact, he's been doing this since before I was even born and almost since before the invention of the laser itself!

Introduction

- Why current generation of ELS instruments fail to make measurements >10mM ionic strength with confidence

- What's needed to overcome this?

- Next Generation Electrophoretic Light Scattering

- Strategy for maximizing confidence

- Compare NG-ELS with commercial instrument up to 4M ionic strength

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I'm going to explain why the current generation of instruments fails to make measurements at greater than 10mM ionic strength with confidence and I'll explain what is needed to overcome this failure and how my patent-pending Next Generation Electrophoretic Light Scattering system achieves this.

I'll describe a strategy for maximizing the confidence in the measurements in high ionic strength media using the Next Generation system.

I'll conclude the presentation with a comparison of measurements made with the Next Generation system and a commercial instrument with samples up to 4M ionic strength.
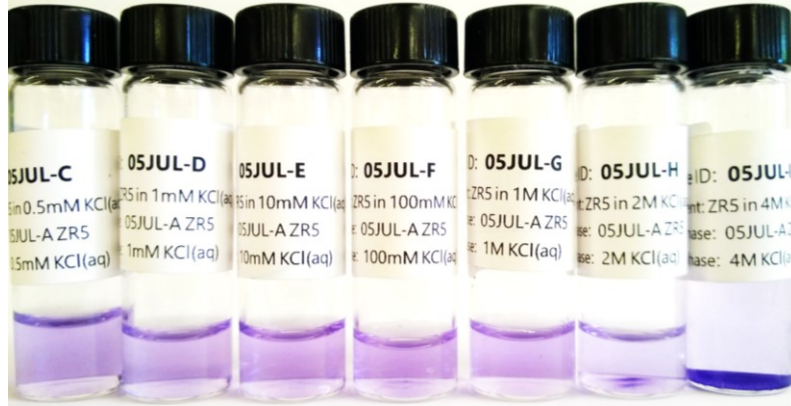
Here you see dispersions of Brookhaven Instruments' zeta reference standard in increasing concentrations of KCl from left to right, starting with 0.5mM on the left and ending with 4M on the right. They stood undisturbed for seven days.

The samples up to and including 1M KCl appear stable, there's evidence of weak instability at 2M and considerable instability at 4M. With this visual observation in mind, you can imagine what the zeta potential as a function of KCl concentration should look like.

compensated　　　　　　　　original

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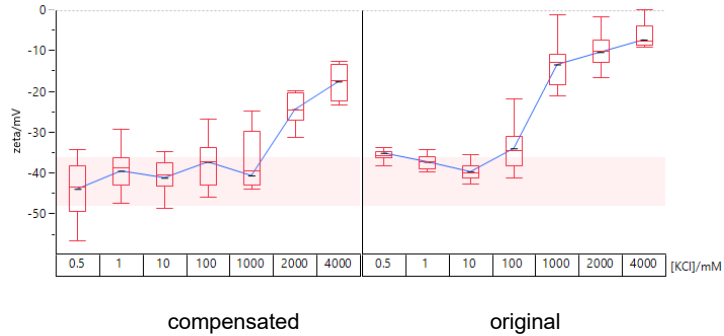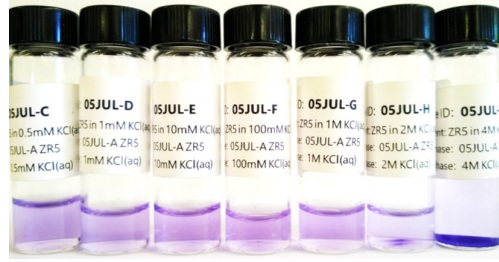
It is the data set on the left that you should expect to obtain. The measurements were made using the Next Generation system. The data set on the right was also obtained using the Next Generation system, and both sets were calculated using a common set of raw light scattering data.

So, why are they different?

The Next Generation system can identify and compensate for effects that result in measured zeta potentials that are lower than their true values. The data on the left have been compensated and the data on the right have not. They were collected using experimental parameters similar to those used by commercial instruments. This implies that commercial instruments will underestimate zeta potential measurements beyond 10mM by an unknown amount and I'll show evidence of this later. I'll explain the effects causing the underestimation in a moment after a brief overview of Electrophoretic Light Scattering.

$$E = V/d$$

$$v = \mu E$$

$$\mu \propto \zeta$$

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LASER

DET

Laser-Doppler Electrophoresis
(early 1970s)

Phase Analysis Light Scattering
(late 1980s)

100mM+
Waste water
Desalination
Isotonic i.v.
etc

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For an electrophoresis experiment, a well-defined electric field, E, is applied across the sample. The electric field is simply the voltage applied divided by the separation between the electrodes. The resultant electrophoretic velocity of the particles is measured using light scattering. Each moving particle will cause a Doppler shift of the incident light that can be measured. Because the detected signal contains contributions from every particle, it can be converted via a Fourier transform into a distribution of Doppler shifts and, hence, to a distribution of zeta potentials.

The velocity is proportional to the electric field and the proportionality constant is the electrophoretic mobility which is proportional to the zeta potential. Throughout the 1970s, this method of Laser-Doppler Electrophoresis was the standard form of Electrophoretic Light Scattering. My invention of Phase Analysis Light Scattering in the late 1980s overcame some of the limitations of the laser-Doppler technique, notably for measuring small mobilities and mobilities at ionic strengths of the order of 10mM.

The state-of-the-art is now 30 years old! The fundamental ways that the measurements are done and the calculations that are used remain the same today. This presents a growing problem to extending the technique to ionic strengths significantly higher than 10mM. Industrially important applications include wastewater treatment, desalination, and the development of intravenous and topical therapies. The current generation of commercial instruments has hit a proverbial brick wall.
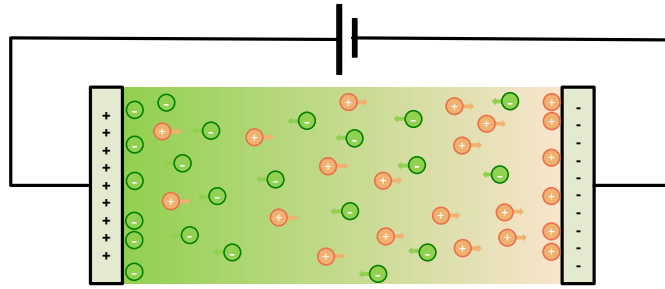
$$E < V/d$$

Electrode polarization	Worse at **low frequency** and low voltage
	Difficult to avoid at high ionic strength. Underestimates electrophoretic mobility.

Electrolysis	Worse at **low frequency** and high voltage
	Moderately easy to avoid by using higher frequencies

| Heating	Worse at **low frequency** and high voltage
	Avoided by using higher frequencies

$\therefore$ increase frequency

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The reason for this brick wall is due to the presence of the electrolyte. Irrespective of the concentration, electrical double layers form at the interface between the electrode surfaces and the electrolyte solution which leads to polarization. A potential drop occurs across each double layer such that the voltage presented to the electrolyte solution is less than that applied to the electrodes. As a result, the strength of the electric field in the sample is also lower. This invalidates the assumption that states that the electric field is the externally-applied voltage divided by the distance between the electrodes. Consequently, the measured zeta potential is lower than the true value. This is the cause of the difference between the two sets of data shown earlier.

This slide shows a DC voltage applied to the electrodes. In practice, an alternating electric field is used at a frequency of the order of 1Hz for reasons I'll explain shortly.

There are three significant effects that must be taken into account at higher salt concentrations. The first is electrode polarization that I have just described. This becomes worse at low frequency and also at low voltage. Electrolysis – which is the chemical reaction between the sample and the electrode – becomes worse at low frequency and high voltage. At high ionic strengths, significant Joule heating of the sample occurs due to the lower electrical resistance of the electrolyte solution leading to a higher current at a given voltage. This creates turbulence that interferes with the electrophoresis. In all three cases, increasing the frequency of the electric field will reduce their impact and improve the quality of the measurements.

α-Al$_2$O$_3$

Why 1Hz?

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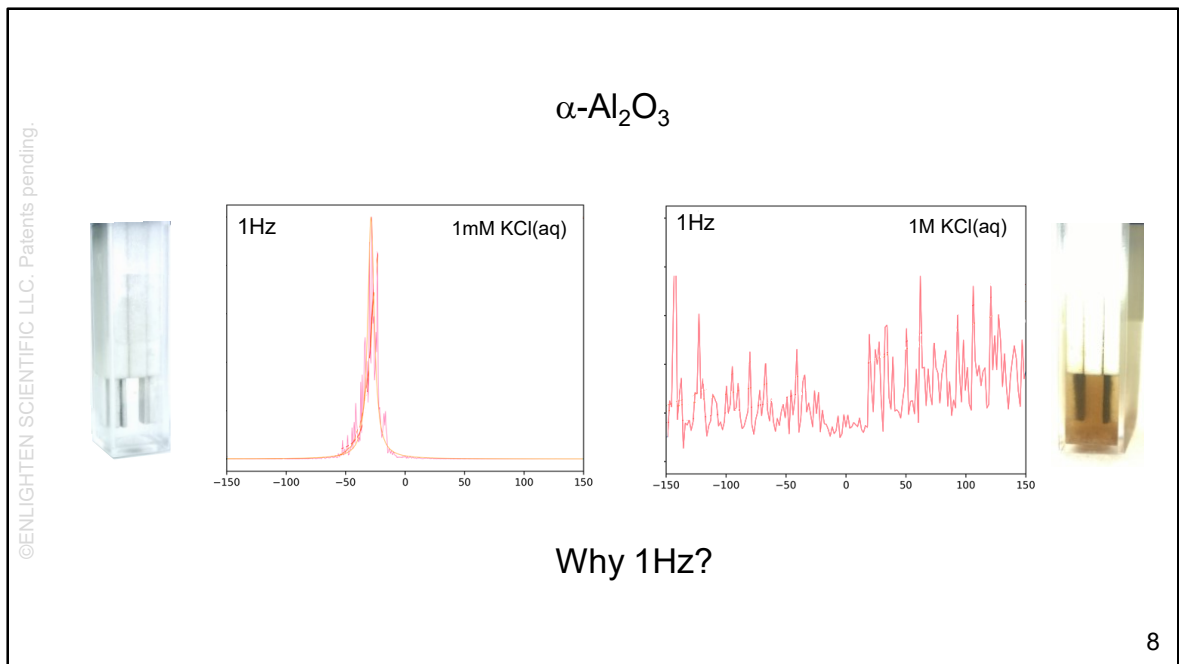
These are zeta potential distributions for alpha-alumina dispersed in alkaline aqueous KCl measured using a 1Hz electric field. The distribution on the left was obtained for a sample at 1mM KCl and the one on the right was for a sample at 1M KCl. The difference in the quality of the distributions is significant and primarily due to convection or turbulence caused by high Joule heating of the 1M sample. In these examples, the "dip" electrodes were made from palladium and are similar to palladium dip electrodes provided by some manufacturers. Now, I should mention that I've seen similar effects with gold electrodes and – in my opinion – see little benefit of one over the other.

The photographs show the appearance of the electrodes after measurement. At low ionic strength the electrodes have maintained their greyish metallic appearance and the sample is colorless. At high ionic strength, the electrodes have turned black and the sample has turned brown.

So, just why are these measurements performed at 1Hz?

Why not just increase the frequency to avoid adverse electrode effects at higher ionic strengths?
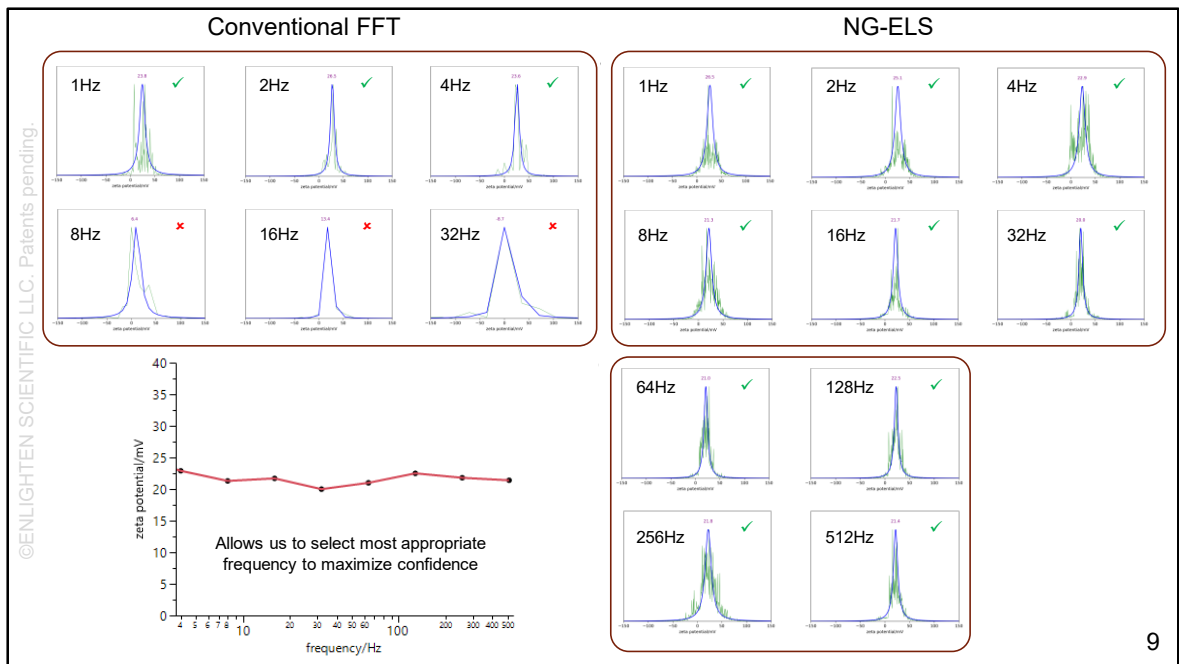
There is a fundamental mathematical limitation to how the distribution is calculated. Typical Doppler shift frequencies for Electrophoretic Light Scattering experiments may be of the order of tens of hertz, depending on the implementation. The maximum resolution attainable in the frequency distribution is twice the frequency of the applied electric field. At 1Hz, the maximum resolution is 2Hz.

The group of distributions on the left illustrates the increasing loss of resolution at higher frequencies. The distributions were calculated using the same conventional Fourier transform method as the current generation of instruments. As you can see, accurate determination of the distribution is not possible above 4Hz. The Next Generation system calculates the distribution in a different way such that there is no limitation imposed by the electric field frequency. To-date, the Next Generation system has measured zeta potential distributions using electric field frequencies up to 1kHz. The distributions shown on the right were calculated using the Next Generation system. The same raw light scattering data at a given electric field frequency were used to perform both the conventional and Next Generation calculations.

The Next Generation system yields distributions at frequencies exceeding 500Hz that appear indistinguishable from those obtained at the lowest end of the frequency range. The graph shows that the calculated zeta potentials are independent of frequency. This allows us to select the most appropriate frequency to maximize confidence.

Phase analysis can also be performed at the same frequencies so why is it necessary to obtain a distribution, as well?

Because phase analysis calculates an average zeta potential it is not possible to confirm if the distribution is truly monomodal which phase analysis requires.

Typically, this isn't a concern at low ionic strengths but the presence of significant turbulence or convection at higher ionic strengths as shown earlier will likely invalidate the phase analysis results. Therefore, it's important to obtain the distribution as well.

Once you approach 100mM ionic strength, commercial instruments cannot do this which makes it impossible to confirm phase analysis results. The Next Generation system calculates the distribution and phase analysis results simultaneously using the same raw light scattering data.

All of this provides a tool to go to much higher frequencies than commercial instruments can in order to avoid electrode effects and enable measurements to be made at very high ionic strengths.

Deploying NG-ELS

Application
- Determination of zeta potential as CQA
- Method development
- Specification setting
- Registration/QC/Release

Robustness essential
- Create response surface (voltage, frequency, electrode etc)
- Identify flat region for routine measurement
- Determine compensation
- Translate to traditional operating parameters if possible

Electrode condition
- Overlooked but critical
- System suitability/ performance check

10mM   100mM   1M
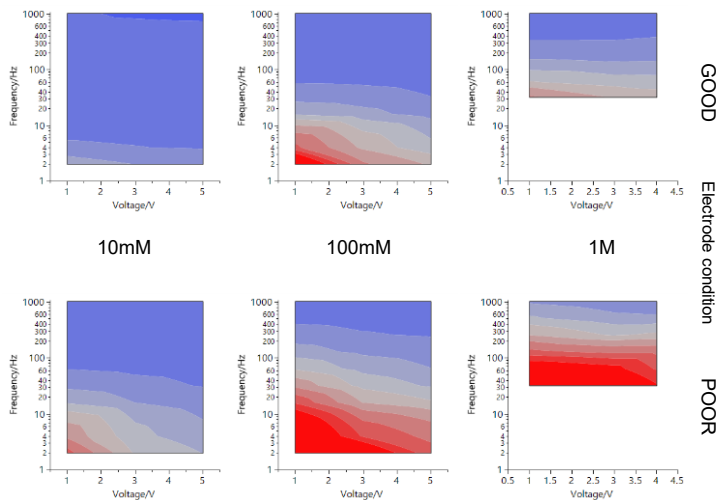
GOOD   Electrode condition   POOR

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So how are the optimum frequency and voltage determined for a given class of dispersion?

A preferred way is to generate a response surface with frequency and voltage as variable parameters and responses that include measured zeta potential, experimental compensation information gathered during measurement, variability, reproducibility, and so on. The combination of responses can be used to quantify the confidence level of the measurement.

In the examples shown, blue indicates high confidence in the measurement and red shows where the electrode effects are too great to allow meaningful compensation of the measured values and, therefore, indicate very low confidence, if any.

The top row of response surfaces shows that at lower ionic strength – 10mM – high confidence will be obtained for measurements above 5Hz.

At 100mM, a red region is present in the low frequency and low voltage range primarily because of the polarization effects. The polarization is so high that it is not possible to apply a compensation to the measured zeta potential. In this case, the response surface indicates that measurements should be made using electric field frequencies greater than 60Hz.
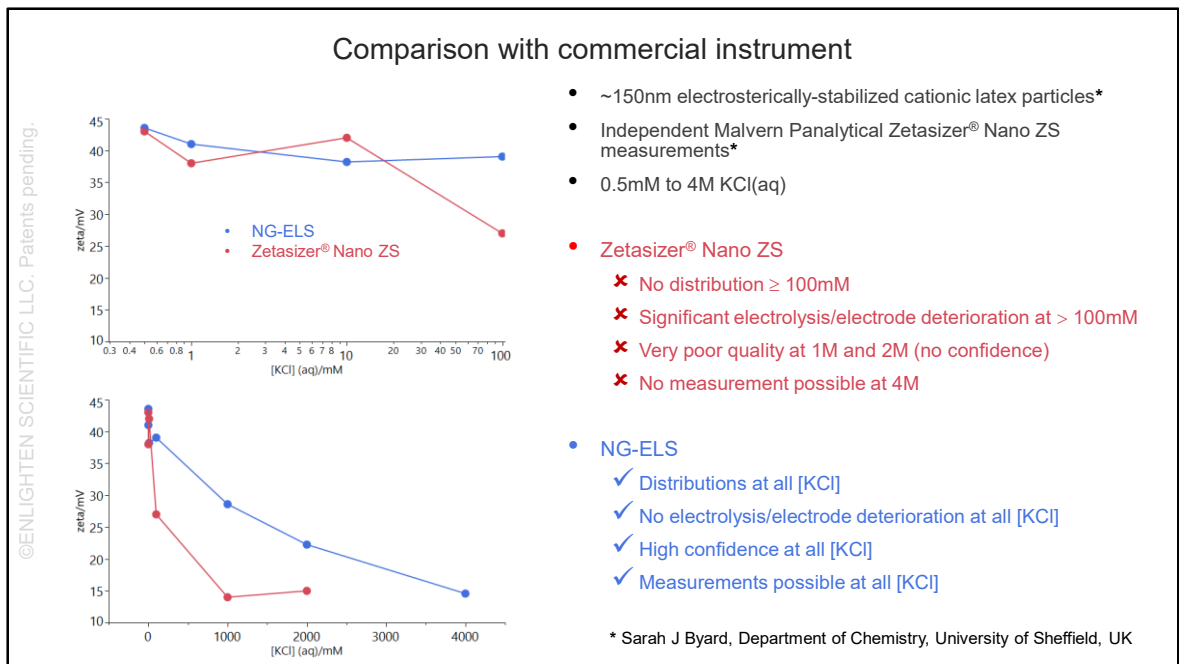
At 1M, the electrode effects are so great it's not possible to generate the entire response surface and, in this case, measurements need to be made above 300Hz to have maximum confidence in the results.

The red zone – particularly for the 100mM case – is where commercial instruments generally operate. Remember, these instruments can only perform phase analysis at this concentration adding further uncertainty to the measurement.

The bottom row is a repeat of the same response surfaces but with electrodes that have begun to deteriorate in some way due to lack of cleaning, mishandling or other causes of damage to their surfaces.

Historically, this has been COMPLETELY ignored.

Current instruments do not monitor the condition of the electrodes to determine if they are fit for purpose. This is important if you are trying to develop a validated method in a regulated industry.

Comparison with commercial instrument

- ~150nm electrosterically-stabilized cationic latex particles*
- Independent Malvern Panalytical Zetasizer® Nano ZS measurements*
- 0.5mM to 4M KCl(aq)

- Zetasizer® Nano ZS
  - ✘ No distribution ≥ 100mM
  - ✘ Significant electrolysis/electrode deterioration at > 100mM
  - ✘ Very poor quality at 1M and 2M (no confidence)
  - ✘ No measurement possible at 4M

- NG-ELS
  - ✓ Distributions at all [KCl]
  - ✓ No electrolysis/electrode deterioration at all [KCl]
  - ✓ High confidence at all [KCl]
  - ✓ Measurements possible at all [KCl]

* Sarah J Byard, Department of Chemistry, University of Sheffield, UK

Here are results from a comparison of measurements with the Next Generation system and the state-of-the-art Malvern Panalytical Zetasizer Nano ZS.

Sub-200 nm electrosterically-stabilized cationic latex particles were supplied by Sarah Byard at the University of Sheffield in the UK who also measured the same particles with the Zetasizer.

Samples were measured as a function of ionic strength ranging from 0.5mM to 4M KCl. The graphs clearly show that the Zetasizer significantly underestimates the zeta potential even at physiological ionic strengths.

It fails to measure with any confidence at 100mM or higher.

The instrument reported that it could not measure a distribution at 100mM or higher.

Significant electrolysis, electrode deterioration, sample discoloration were reported by the operator at higher ionic strengths.

The quality of the measurements at 1M and 2M – according to the instrument's reports – are very poor and the instrument didn't even attempt to make a measurement at 4M.

In contrast, the Next Generation system was able to provide distributions at all of the salt concentrations.

There was no electrolysis or electrode deterioration at any of the salt concentrations.

There's high confidence in the measurements at all salt concentrations and the instrument was able to make measurements at all of the salt concentrations.

Final comments

- Commercial instruments at physiological ionic strengths – or higher – will almost certainly give incorrect results for two reasons:

  - Cannot measure distribution $\therefore$ default to phase analysis

  - Assumes monomodal – if you don't know this then result is meaningless

  - Electrode effects etc. lead to underestimation of zeta potential by unknown amount

  - Can only give a minimum value

- All you can say is –

  "My sample has a zeta potential of at least X mV but only if it has a monomodal zeta potential distribution – which I don't know!"

- The Next Generation Electrophoretic Light Scattering system solves these issues and can accurately and confidently measure zeta potential up to supermolar ionic strengths

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So, to summarize, commercial instruments at physiological ionic strengths – or higher – will almost certainly give incorrect results for two reasons:

They cannot measure the zeta potential distribution and so default to phase analysis. This assumes that the zeta potential distribution is monomodal – if you don't know this then result is meaningless.

The electrode effects lead to underestimation of zeta potential by unknown amount so you can only get a minimum value.

All you can say is – "My sample has a zeta potential of at least X mV but only if it has a monomodal zeta potential distribution – which I don't know!"

The Next Generation Electrophoretic Light Scattering system solves these issues and can accurately and confidently measure the zeta potential up to supermolar ionic strengths.

Services available

Detailed characterization

Routine measurement

Workshops
and
Seminars

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Consultation

john@enlightenscientific.com
http://www.enlightenscientific.com
(presentation available online)

I've only given you a small example of the extensive and wide-ranging measurements I have done. David Fairhurst and I are in the process of submitting a paper for peer-reviewed publication that will describe and demonstrate the major benefits of the Next Generation system in greater detail.

Thank you for your attention.