Trusting Your Zeta Potential Results

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What we'll talk about

- Intro to zeta potential
- Factors which build trust/distrust
- What to look for before measuring
- Evaluating measurements
- Q&A

Starting point

- Instrument is fully functional
- Suitable sample for technology
  - Counters are classic example
- Stay current with free webinars
  - Particle size essentials, method development, sampling & dispersion advice, DLS and zeta potential technology reviews

General concerns

- What is zeta potential? (webinar TE013)
- Dilution, DILUTION, **DILUTION!**
- Zeta potential result interpretation
- Assess data quality
- How can z.p. be used in conjunction with particle size
  (larger topic, IEP, etc.)

Featured technologies

- **LA-950**
  Laser Diffraction
- **SZ-100**
  Dynamic Light Scattering & Zeta Potential
- **CAMSIZER & CAMSIZER XT**
  Dynamic Image Analysis
- **PSA300**
  Static Image Analysis
- **SA-9600**
  Flowing Gas BET Surface Area

SZ-100: Dynamic Light Scattering

- Particle size: 0.3 nm – 8 µm
- Zeta potential: -200 – +200 mV
- Molecular weight: $1x10^3$ – $2x10^7$ Da
- Patented ultra long-life graphite electrodes
- Lowest total cost of ownership
- Optional autotitrator

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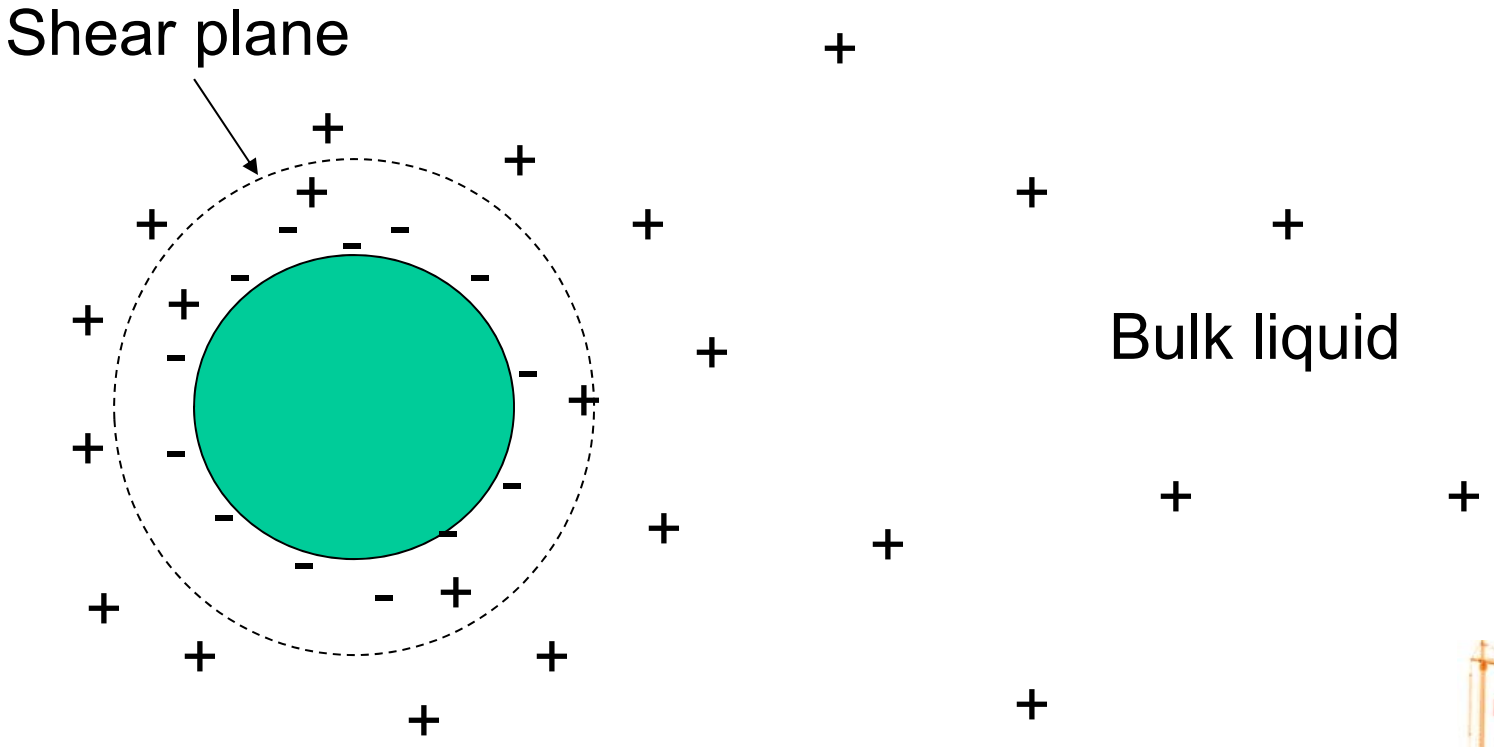
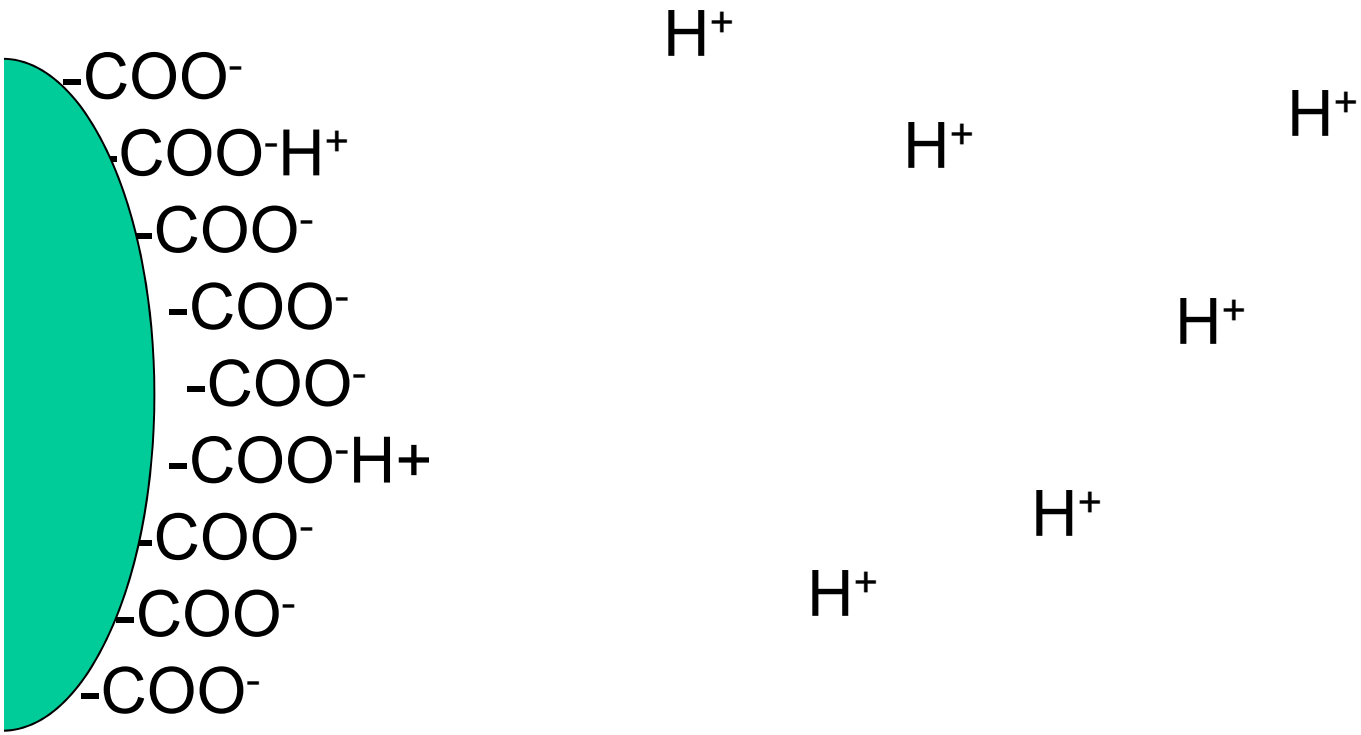
What is Zeta Potential?

- Zeta potential is the charge on a particle at the shear plane.



How do Surfaces Acquire Charge?

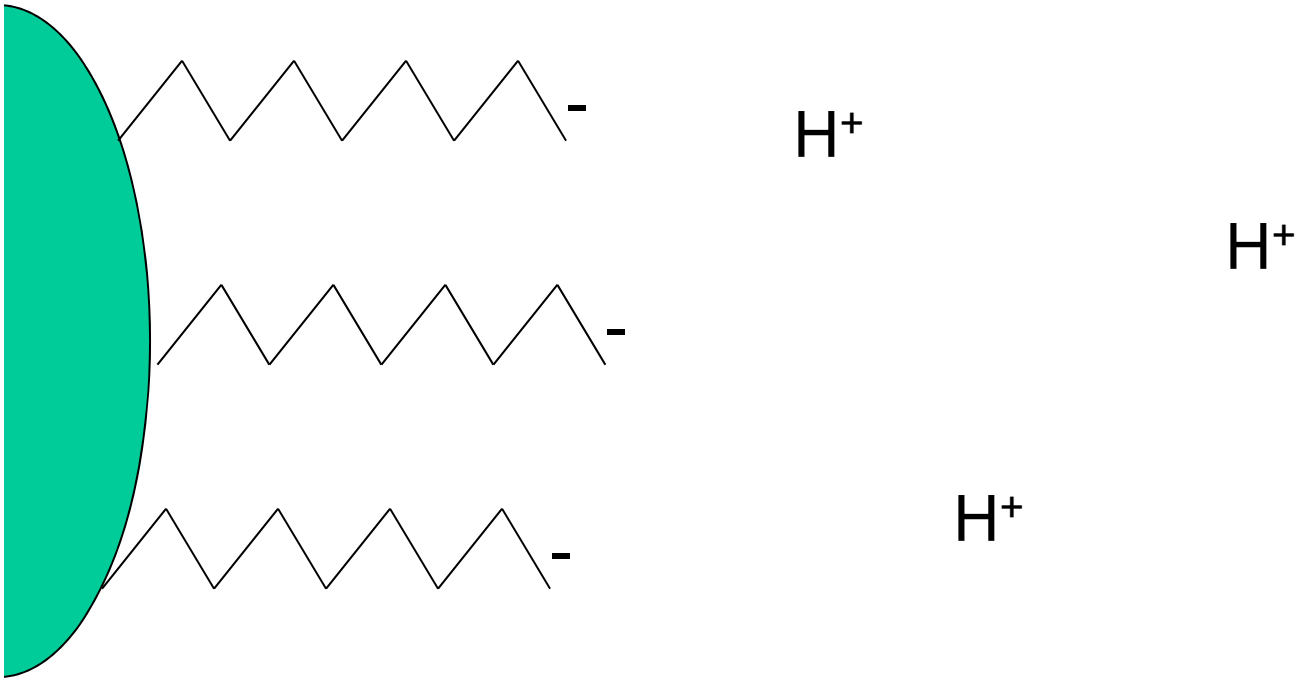
Ionization of surface groups



How do Surfaces Acquire Charge?

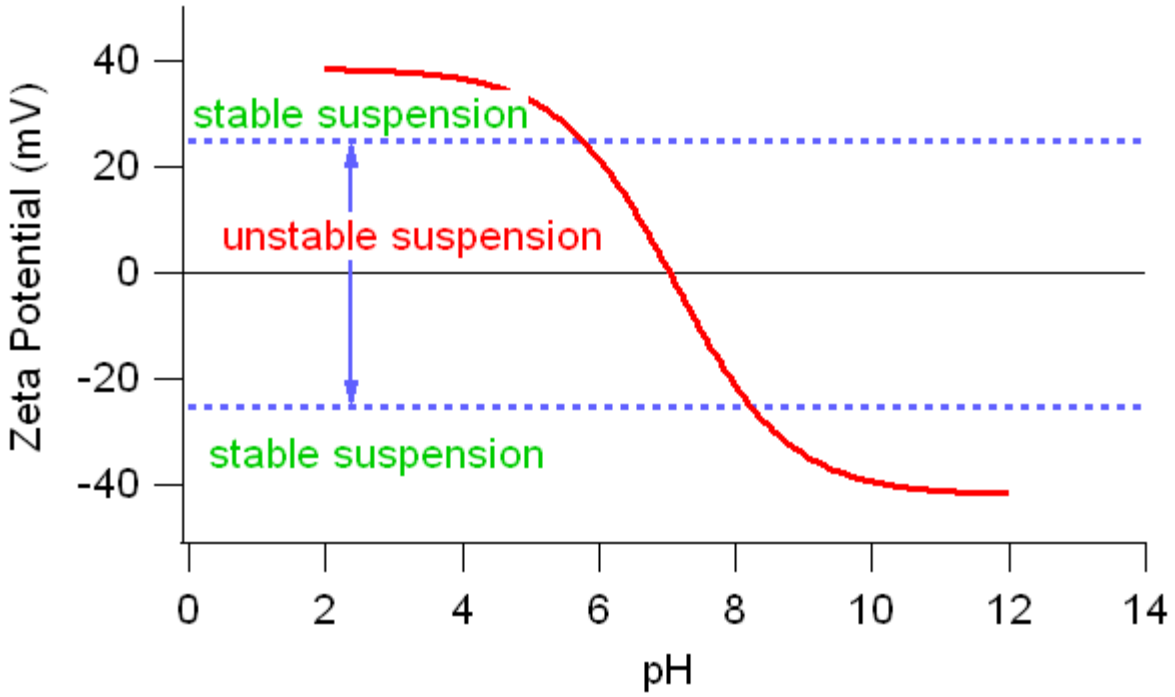
Specific adsorption of ions, e.g. ionic surfactants

Anionic surfactant

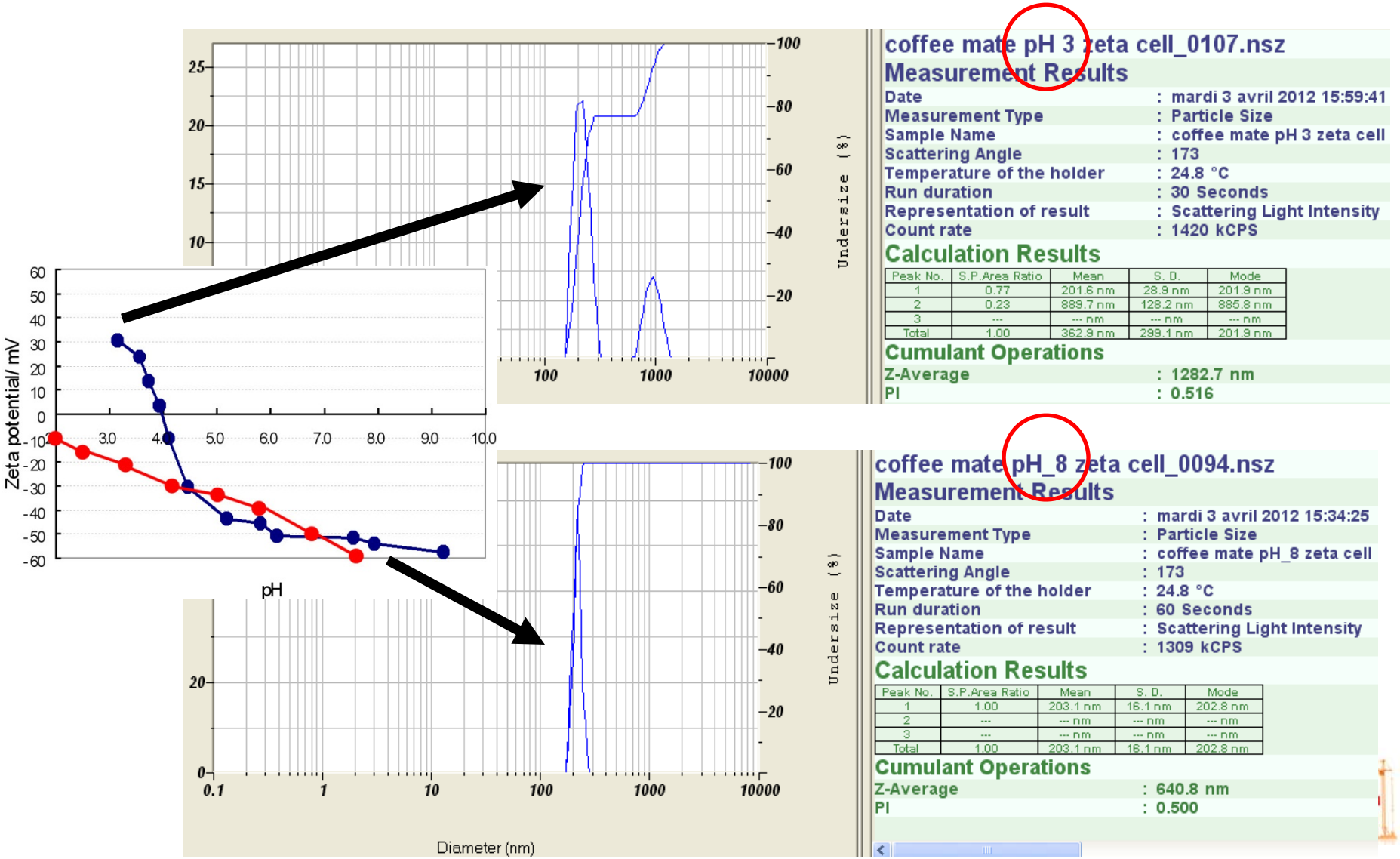


Why Zeta Potential?

- Good way of evaluating electrostatic stabilization of suspensions
- Can use to predict interactions



Emulsion IEP Study: Stability



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Factors creating trust

- Excellent repeatability, reproducibility
- Stable results over time
- Long lifetime parts
- Robust method (avoid finicky results)

- A bit of training to know what results to expect when conditions change

Precision = Confidence

- Real life situation: historic, reproducible data is trusted data… though it may not be accurate data
- Is the data actionable?

Factors creating distrust

- Poor precision
  - Resort to “cherry pick” data
  - Is it a regular practice to discard 10%+ of z.p. results?
- Trending results
  - Indicates changing conditions
- Replacing cell too often
  - Ten measurements?
- Lack of technical support, customer care

Question

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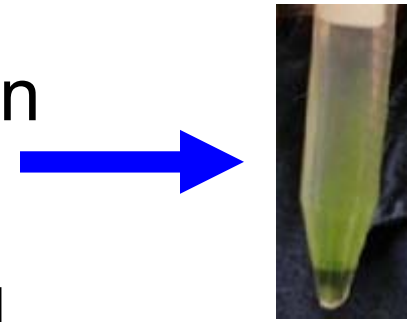
Practical Tips

- Visual inspection
  - Floaters?
- Minimum concentration
  - Concentrate with a centrifuge
- Maximum concentration
  - Dilute using supernatant (or similarly close)
  - Take care to match:
    - Salt concentration
    - pH
    - Surfactant concentration

Dilution

- Avoid dilution if possible ← simplest approach
- Don't dilute with DI water
  - No ions, changes surface chemistry & ZP
- Best: equilibrium dilution with same liquid as sample, but with no particles
  - Use supernatant after sedimentation or centrifugation

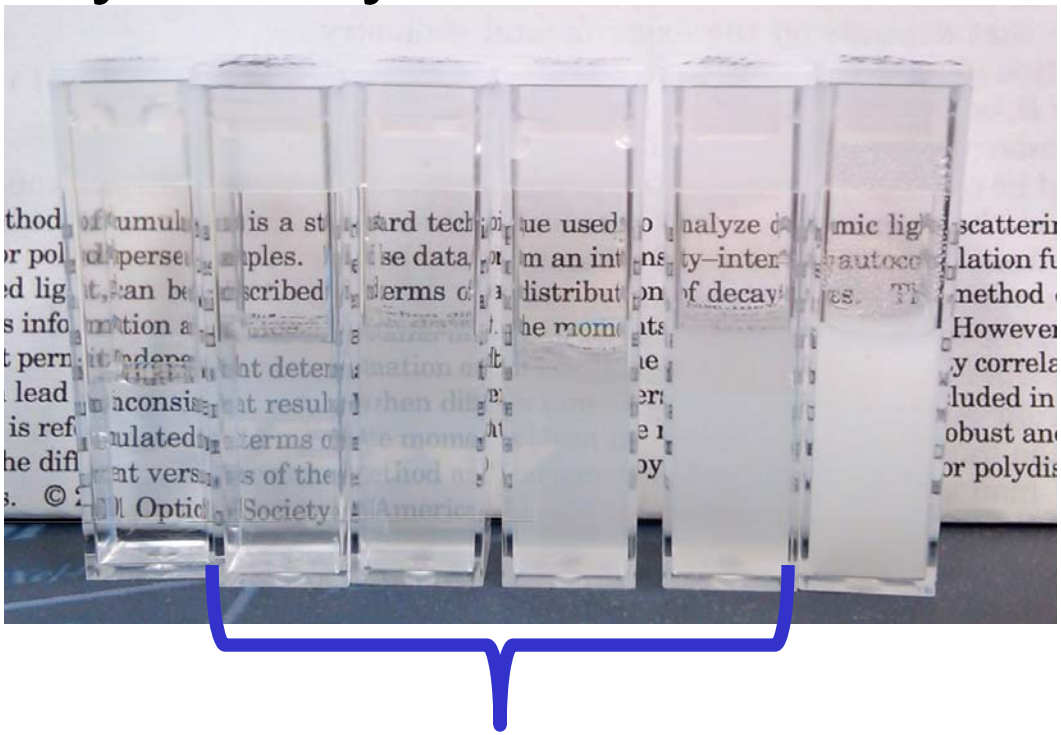


- Otherwise, dilute with 10 mM KCL solution

Question

Eyeballing it

- Samples up to little bit cloudy are OK.
- Pick samples that are clear or just slightly cloudy.



Best range for this material

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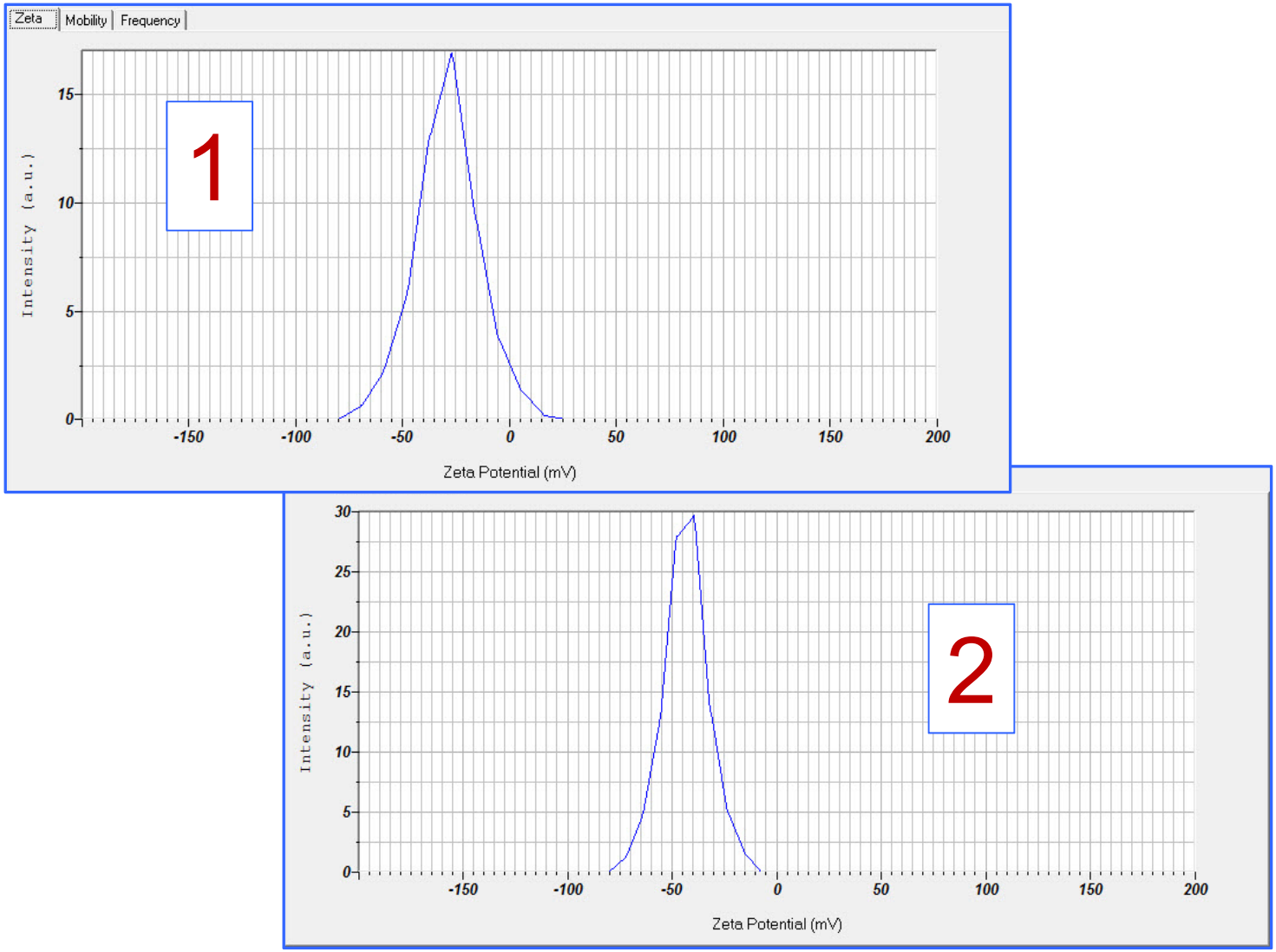
Method validation

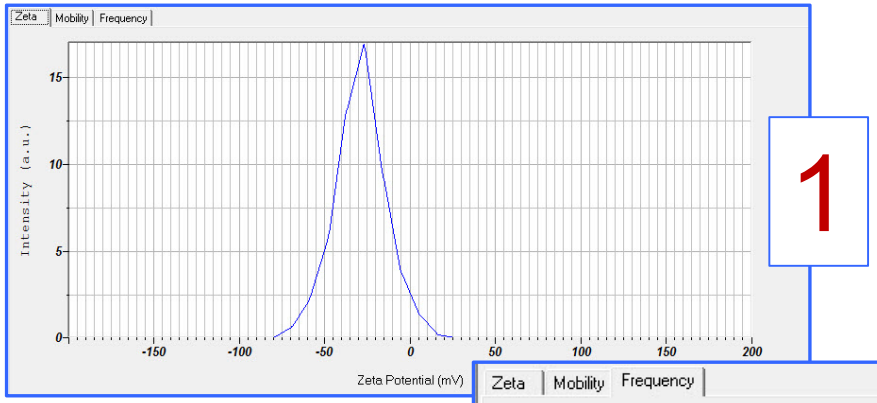
- Have existing method, does it produce "good" data?
- Stress the method
  - Measure at multiple pH values
  - Measure at multiple concentrations
- Always! collect multiple samplings
  - Reproducibility > challenge than repeatability
  - Evaluate with COV of chosen metric(s)

Question

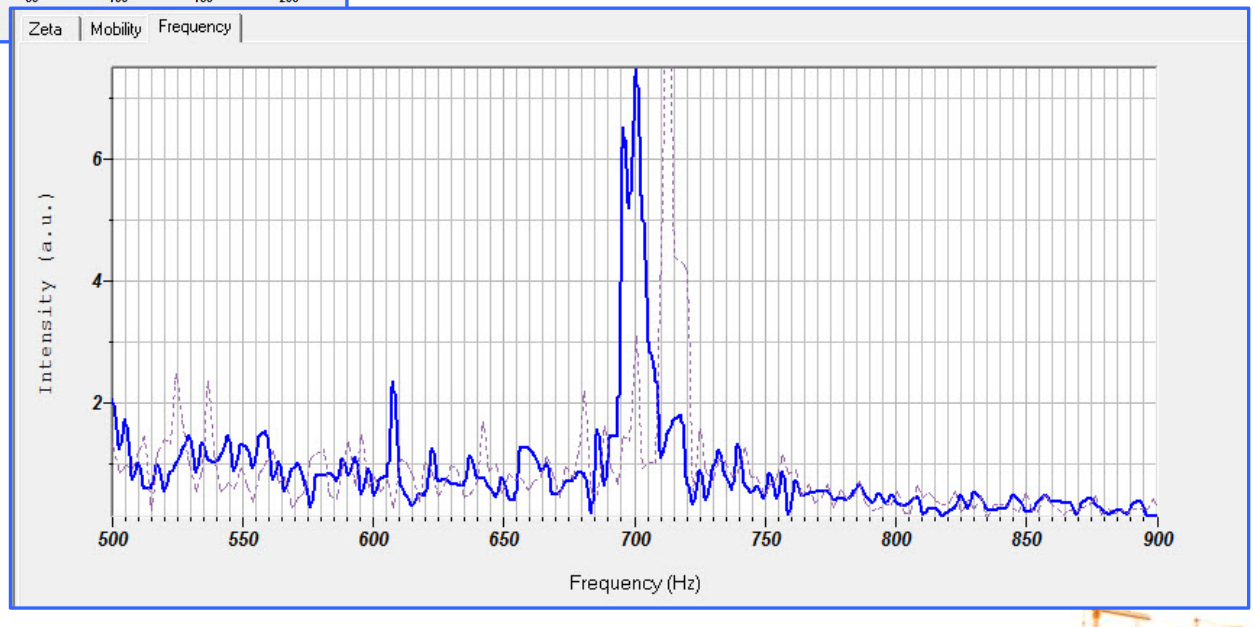
Good zeta potential data?



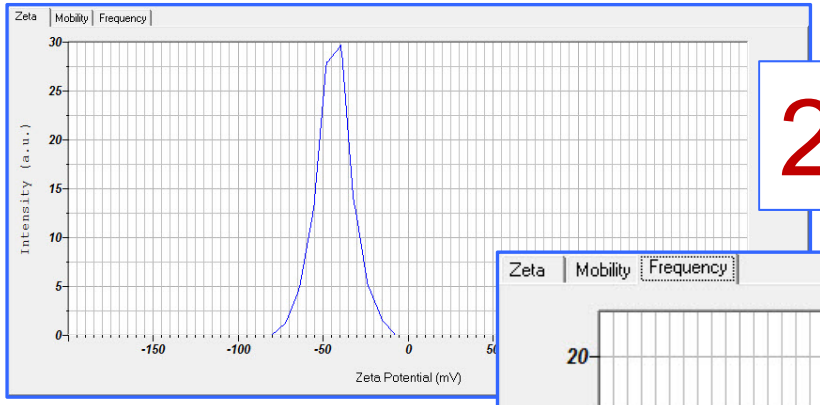
Look at frequency data



1



Look at frequency data



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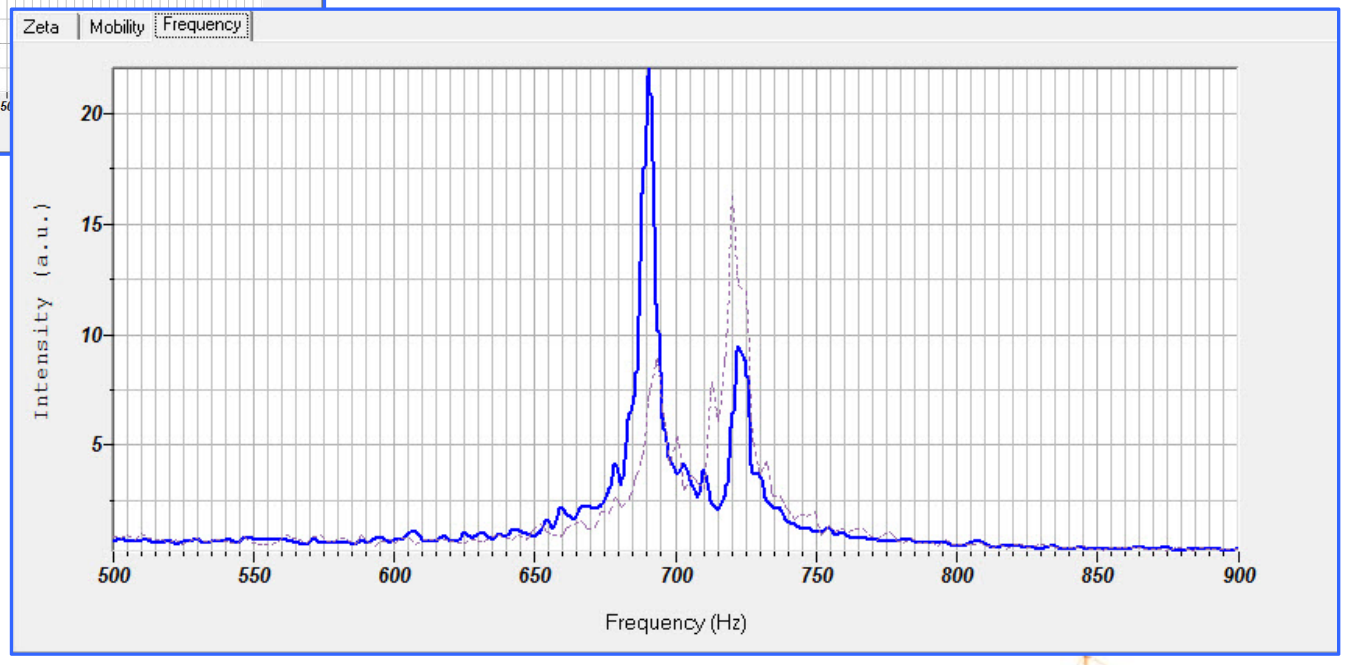
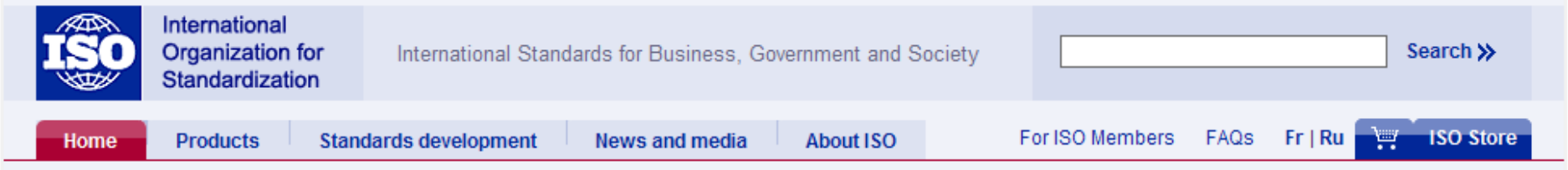


New ISO Standards

www.iso.org



ISO 13099-1:2012
Colloidal systems -- Methods for zeta-potential determination -- Part 1: Electroacoustic and electrokinetic phenomena

ISO 13099-2:2012
Colloidal systems -- Methods for zeta-potential determination -- Part 2: Optical methods

ISO/NP 13099-3
Methods for zeta potential determination -- Part 3: Acoustic methods

Standards under development

ISO13099: Verification

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

ISO13099: Verification

- 8.2.2 **Repeatability**
- Prepare sample following provided procedure
- Measure same portion three times
- Pass if mean value CV <10%
  - Assuming 2 × 10−8 $m^2/V•s$
- Note: expect most customers to use zeta potential values

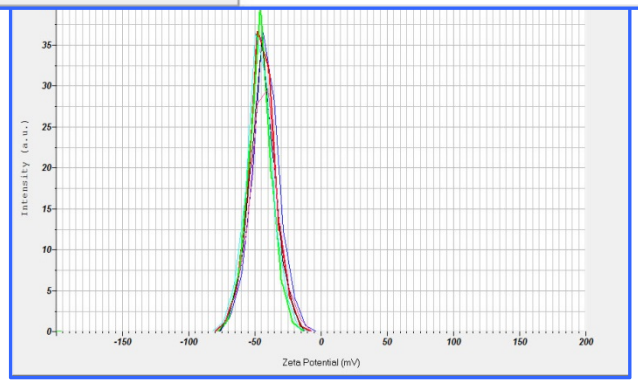
Calculate COV

- Liposome sample
- 6 repeats, 30 sec measurement, 5 sec delay

Zeta Potential

Legend	No.	Date	Measurement Type	Sample Name	Zeta Potential (Mean)(mV)
	1	Friday, December 14, 2012 8:51:04 AM	Zeta Potential	FSS Liposome	-43.4
	2	Friday, December 14, 2012 8:52:20 AM	Zeta Potential	FSS Liposome	-44.2
	3	Friday, December 14, 2012 8:53:35 AM	Zeta Potential	FSS Liposome	-46.3
	4	Friday, December 14, 2012 8:54:51 AM	Zeta Potential	FSS Liposome	-46.1
	5	Friday, December 14, 2012 8:56:06 AM	Zeta Potential	FSS Liposome	-44.4
	6	Friday, December 14, 2012 8:57:39 AM	Zeta Potential	FSS Liposome	-41.7
Average					-44.3
S. D.					1.7

COV = 3.8%



ISO13099: Verification

- 8.2.3 **Intermediate Precision**
- Same procedure as 8.2.2 but using <u>different portions</u> of sample
- Pass if Repeatability; CV <15%
  - Assuming 2 × 10−8 $m^2/V•s$
- Doubt this is done often
- In pharmaceutical industry intermediate precision implies multiple systems, multiple operators, different days

Calculate COV

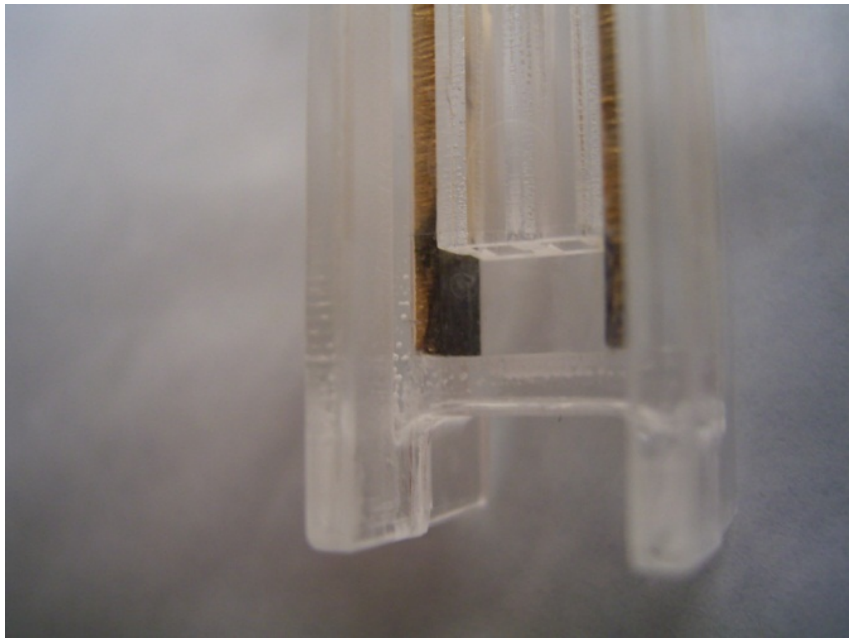
- Liposome sample, two samplings
- 12 repeats, 30 sec measurement, 5 sec delay

Zeta Potential

Legend	No.	Date	Measurement Type	Sample Name	Zeta Potential (Mean)(mV)
	1	Friday, December 14, 2012 8:51:04 AM	Zeta Potential	FSS Liposome	-43.4
	2	Friday, December 14, 2012 8:52:20 AM	Zeta Potential	FSS Liposome	-44.2
	3	Friday, December 14, 2012 8:53:35 AM	Zeta Potential	FSS Liposome	-46.3
	4	Friday, December 14, 2012 8:54:51 AM	Zeta Potential	FSS Liposome	-46.1
	5	Friday, December 14, 2012 8:56:06 AM	Zeta Potential	FSS Liposome	-44.4
	6	Friday, December 14, 2012 8:57:39 AM	Zeta Potential	FSS Liposome	-41.7
	7	Friday, December 14, 2012 9:45:24 AM	Zeta Potential	FSS Liposome	-49.1
	8	Friday, December 14, 2012 9:46:41 AM	Zeta Potential	FSS Liposome	-50.2
	9	Friday, December 14, 2012 9:47:58 AM	Zeta Potential	FSS Liposome	-51.3
	10	Friday, December 14, 2012 9:49:15 AM	Zeta Potential	FSS Liposome	-48.7
	11	Friday, December 14, 2012 9:50:33 AM	Zeta Potential	FSS Liposome	-51.5
	12	Friday, December 14, 2012 9:52:08 AM	Zeta Potential	FSS Liposome	-48.6
Average					-47.1
S. D.					3.2

COV = 6.8%

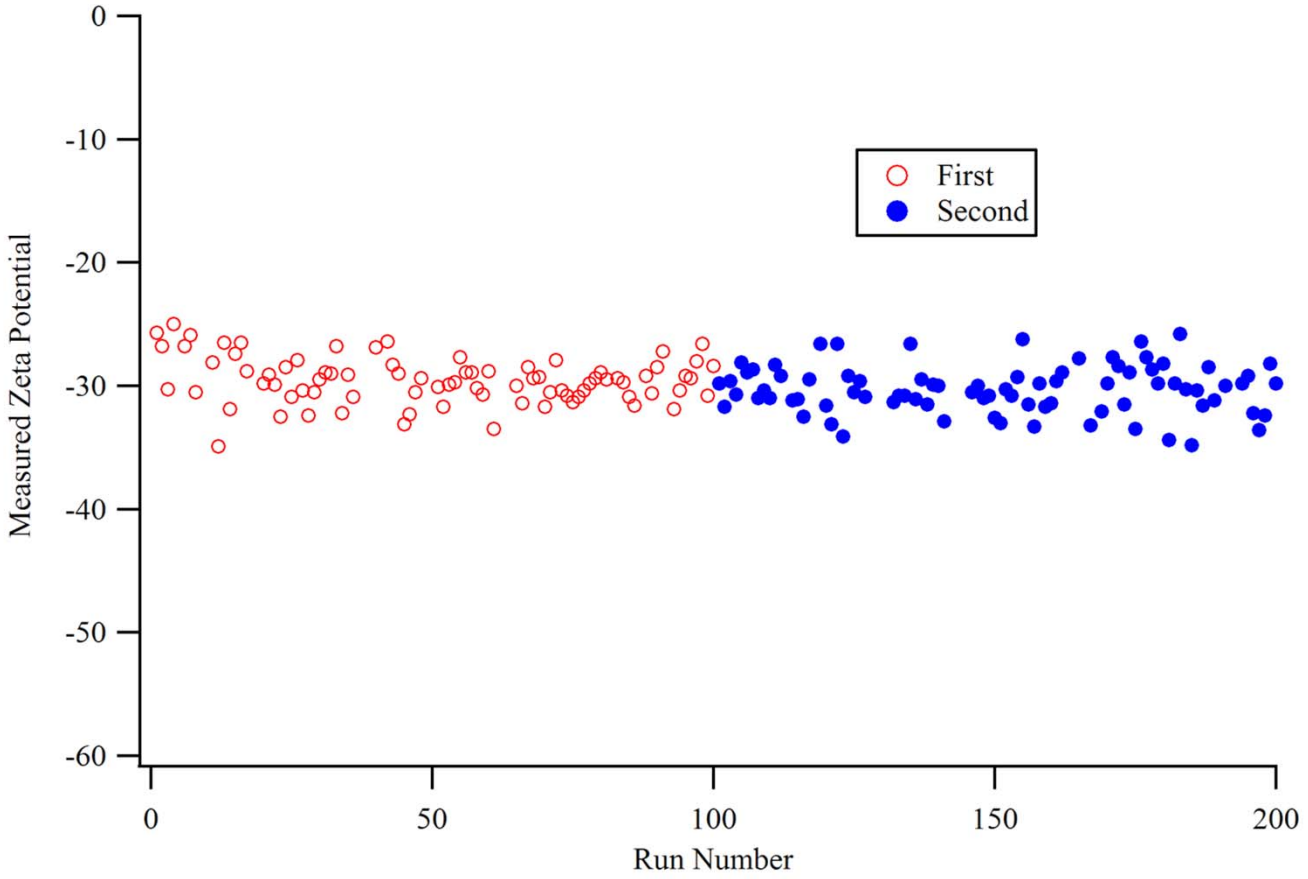
Zeta Potential Cells



Gold coated electrodes (ruined)

Carbon coated electrodes

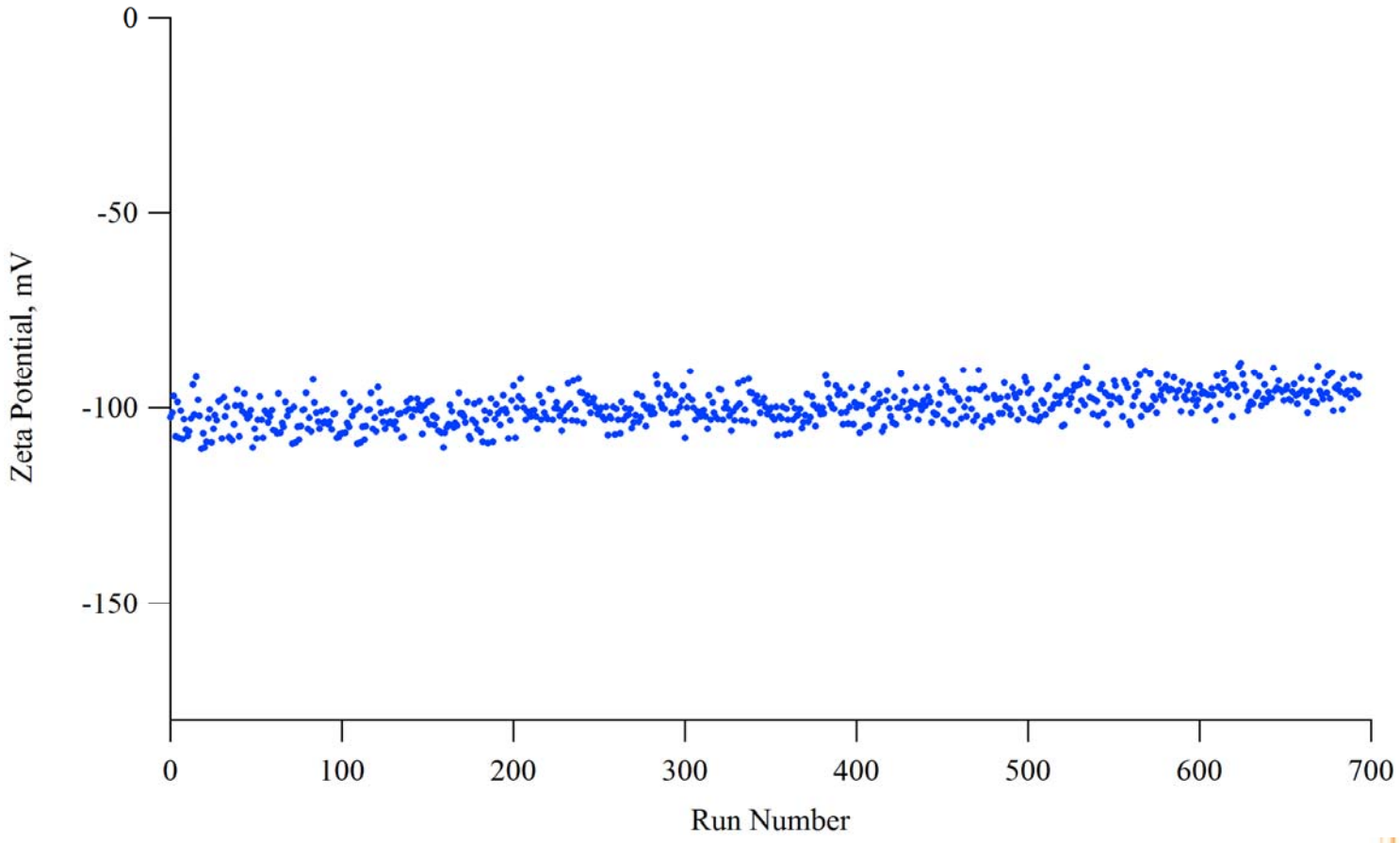
Degradation Test with BSA



Rinse cell between 1st and 2nd series.

Data taken from poster submission to Techconnect Nanotech 2013

Soft Nanoparticle



Data taken from Technical Note 167 available at www.horiba.com/particle

ISO13099: Verification

- 8.2.4 **Trueness** (accuracy)
- Prepare, measure same portion 3 times
- Pass if mean value within 10% of published electrophoretic mobility value, assuming > 2 × 10−8 m2/V•s.
- Note: when calculating pass/fail criteria it is OK to include the uncertainty of the sample
- Note: most customers use the zeta potential value, not the mobility

Instrument validation

- Ideally, run traceable standard reference material(s)

- NIST SRM 1980 is candidate, but not terribly popular
- Can run internal reference and/or transfer “standard”

NIST SRM1980 on SZ-100

Pass criteria:
Mean within 10% of reference value
COV<10%

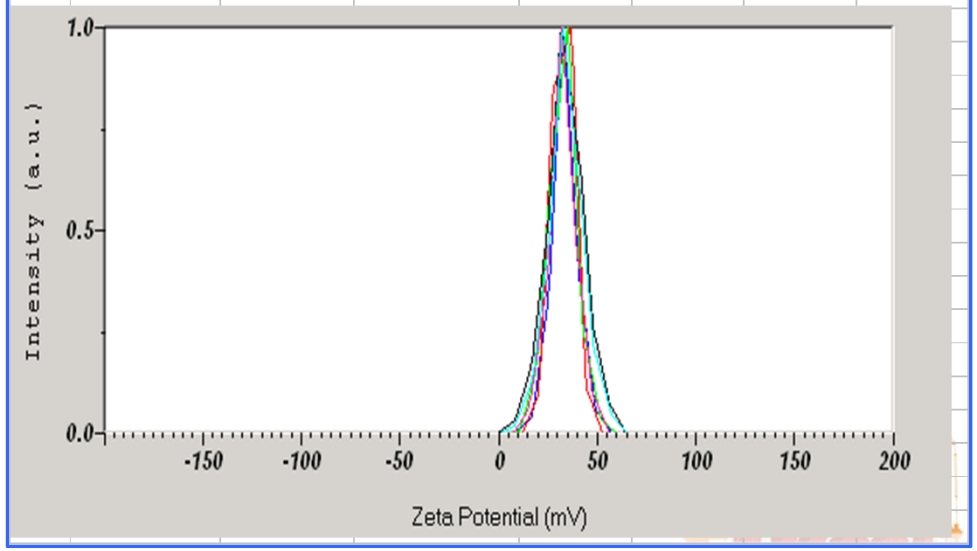
Reference value
2.53 μm·cm/V·s
± 0.12 μm·cm/V·s

Upper limit:
(2.53 + 0.12)*1.1 = 2.92

Lower limit:
(2.53 – 0.12)* 0.9 = 2.17

Pass

No.	Sample Name	Zeta Potential(mV)	Electrophoretic Mobility(cm2/Vs)
17	NIST SRM1980	33,4	0,000257
18	NIST SRM1980	32,7	0,000252
19	NIST SRM1980	32,5	0,00025
20	NIST SRM1980	33,9	0,000261
21	NIST SRM1980	32,7	0,000252
22	NIST SRM1980	34,2	0,000263
Mean		33,2	0,000256
S. D.		0,7	0,000005
COV		2,11	1,95



Sources of Error

- Contamination from previous sample
- Poor sample preparation
- Inappropriate sample
- Inappropriate liquid medium
- Poor temperature stabilization
- Condensation on the illuminated surfaces
- Too large a potential applied

Sources of Error

- Particles, fingerprints or scratches on the optical surfaces
- Incorrect entry of parameters by the operator
- Air bubbles
- Cell coating damage
- Inappropriate theory for calculating zeta-potential from the measured electrophoretic mobility

Summary

- Zeta potential is less well understood
- Look at the sample before measurement
- (If possible) Know about the sample's chemistry before measurement
- Try to maintain sample integrity
- Look beyond the final z.p. result
- When all else fails… minimize the sample amount ☺

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Thank you

ありがとうございました

ขอบคุณครับ

谢谢

اشكُرْ

Gracias

Grazie

Σας ευχαριστούμε

धन्यवाद

நன்றி

Tacka dig

Danke

Merci

Obrigado

감사합니다

Большое спасибо

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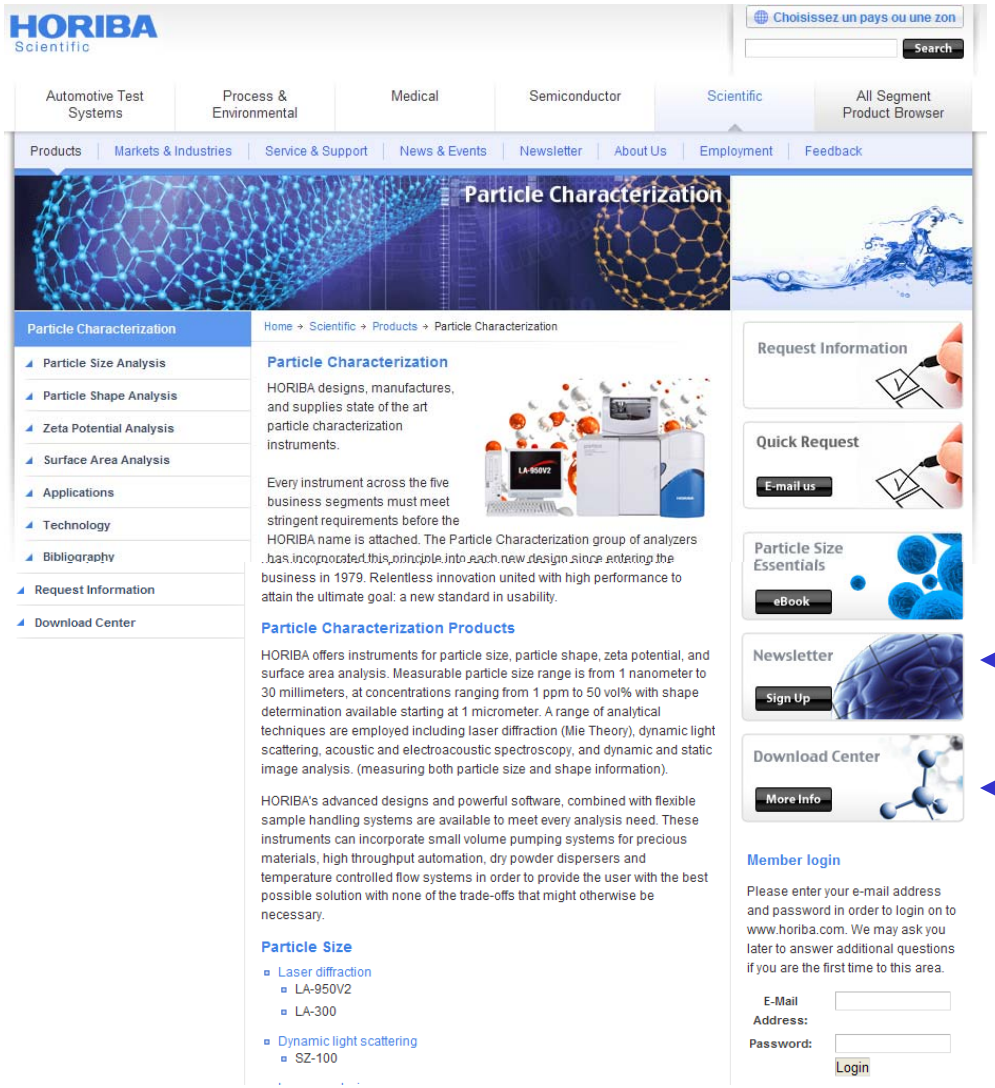

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