Measure and Modify Colloidal Stability
Featuring the SZ-100 Nanoparticle Analyzer

Mark Bumiller
mark.bumiller@horiba.com
www.horiba.com/particle
Colloids Definition

Two phases:
- **Dispersed phase** (particles)
- **Continuous phase** (dispersion medium, solvent)

May be solid, liquid, or gaseous
Size range 1 nm – 1 micron
High surface area creates unique properties

<table>
<thead>
<tr>
<th>Continuous Medium</th>
<th>Dispersed Medium</th>
<th>Gas</th>
<th>Liquid</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>NONE (All gases are mutually miscible)</td>
<td>Liquid Aerosol Examples: fog, mist, clouds</td>
<td>Solid Aerosol Examples: smoke, air particulates</td>
<td></td>
</tr>
<tr>
<td>Liquid</td>
<td>Foam Examples: whipped cream</td>
<td>Emulsion Examples: milk, mayonnaise, hand cream</td>
<td>Sol (suspension) Examples: paint, pigmented ink</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>Solid Foam Examples: aerogel, styrofoam, pumice</td>
<td>Gel Examples: gelatin, jelly, cheese, opal</td>
<td>Solid Sol Examples: cranberry glass, ruby glass</td>
<td></td>
</tr>
</tbody>
</table>
Colloid or Nanoparticle?

- Colloid: 1 nm – 1 µm
- Nanoparticle: 1 – 100 nm
- So a suspension of 50 nm gold particles in water: colloid or nanoparticle?
- Both, mostly terminology
- Also hear “nanocolloid, nanoparticle colloid…” (<100 nm)
Nanoparticle or Not?

SSA = \( \frac{6}{\rho D} \)

D from SEM \( \sim 50 \text{ nm} \)
D from SSA \( \sim 60-70 \text{ nm} \)
D from DLS \( \sim 250 \text{ nm} \)
So: is this a nanoparticle?

Used ultrasound to disperse to primary particles or use weak acid to break bonds
D from DLS \( \sim 50 \text{ nm} \)
Stability

- Want stable dispersion
- Either suspensions or emulsions
- Suspensions sediment & flocculate
- Emulsions phase separate, creaming or coalescence
Measuring Stability

(a) (b) (c) (d) (e) (f) (g) (h)

Scattered Light

Light Source

Transmitted Light

Transmitted Light

Scattered Light

Oil

Cream

Serum

H_{Released}

H_C
Stabilization

- Steric stabilization: coat surface with polymers
  - Particles can’t touch so they don’t interact

- Electrostatic stabilization: alter surface chemistry to put charge on particle surface
  - Repel like magnets
Zeta Potential

- If surface has + charge, then - ions attracted to surface
- + ions attracted to – ions, builds electric double layer
- Slipping plane: distance from particle surface where ions move with particle
- $ZP = \text{potential (mV) at slipping plane}$
Zeta Potential

- Stern layer
- Negatively charged diffuse layer
- Bulk of the liquid
- Positively charged particle
- Electric potential
- Slipping plane
- Stern plane
- Debye length
- $\Psi_s$
- $\Psi_d$
- $\zeta$
- $\kappa^{-1}$
Stability Theory

Electrostatic Stability (DVLO)

- Approaching particles undergo two forces
  - Van der Waals attraction \((V_{vdw})\)
  - Electrostatic repulsion \((V_{er})\)
- Total energy = balance of two
  - \(V_{total} = V_{vdw} + V_{er}\)

Steric Stability

- Approaching particles undergo two forces
  - Van der Waals attraction \((V_{vdw})\)
  - Forces from adsorbed polymers \((V_{ster})\)
- Total energy = balance of two
  - \(V_{total} = V_{vdw} + V_{ster}\)
Total Interaction Energy Curve: DVLO

Important parameters:

- $1/K$ Debye Length, double layer thickness: depends on concentration
- $a$ particle size
- $\zeta$ surface charge
- $A$ Hamaker constant, nature of particle
Zeta Potential: Measurement

- Apply electric field
- Measure particle motion
- Direction tells + or –
  - + particles move to –
  - - particles move to +
- Speed tells amplitude
  - Get speed from frequency shift from motion of particles
Zeta Potential Measurement

Particle motion causes Doppler shift
Frequency → mobility
Mobility → zeta potential

Mobility

\[ U = \frac{\lambda \Delta \nu_d}{2En \sin(\theta/2)} \]

Zeta potential

\[ \zeta = \frac{3U \cdot \eta}{2\varepsilon \cdot f(ka)} \]
Measurement Results

- Polystyrene Polymer Microspheres: 500nm (100ppm)

<table>
<thead>
<tr>
<th></th>
<th>SZ-100</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>f_d</td>
<td>-41.1 mV</td>
<td>-39.5 mV</td>
</tr>
</tbody>
</table>

Average value of zeta potential from three times measurements
Measurement Details

- First measure conductivity
- Then decide applied electric field
  - Auto or manually
- Reverse electric field to avoid polarization & electroosmosis
- To avoid electroosmotic effect near cell walls
  - “Uzgiris” type cells avoid this problem
Zeta Potential Cells

Gold coated electrodes (ruined)  Carbon coated electrodes

IEP 3.4 nm protein  800 measurements with one cell
Thin vs. Thick Double Layer

\[ \zeta = \frac{3U \cdot \eta}{2\varepsilon \cdot f(ka)} \]

Debye length = \( \kappa^{-1} \)

EDL thickness

**Huckel**
\[ \kappa a = 1 \]

**Smoluchowski**
\[ \kappa a >> 1 \]

\[ \kappa a << 1 \]
Default is Smoluchowski
Selection for Huckel
Or enter manually for other model
Zeta Potential Predicts Stability

Different guidelines

- Positive zp
  - +30 mV: stable
  - 0 mV: not stable
  - -30 mV: stable

- Negative zp

Sample Dependency

- Oil/water emulsions > 10 mV
- Polymer latices > 15 mV
- Oxides > 30 mV
- Metal sols > 40 mV
Zeta Potential: Emulsion Isoelectric Point (IEP)

Isoelectric point:
\[ \text{pH where zeta potential} = 0 \]

Automate IEP studies with auto titrator
IEP of Some Materials

- Another use of IEP is to characterize the surface of complex particles.
- TiO$_2$ coated with alumina will have the IEP of alumina.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha aluminium oxide Al$_2$O$_3$</td>
<td>8-9</td>
</tr>
<tr>
<td>alpha iron (III) oxide (hematite) Fe$_2$O$_3$</td>
<td>8.4-8.5</td>
</tr>
<tr>
<td>antimony(V) oxide Sb$_2$O$_5$</td>
<td>&lt;0.4 to 1.9</td>
</tr>
<tr>
<td>cerium(IV) oxide (ceria) CeO$_2$</td>
<td>6.7-8.6</td>
</tr>
<tr>
<td>chromium(III) oxide (chromia) Cr$_2$O$_3$</td>
<td>6.2-8.1</td>
</tr>
<tr>
<td>copper(II) oxide CuO</td>
<td>9.5</td>
</tr>
<tr>
<td>delta-MnO$_2$ 1.5, beta-MnO$_2$</td>
<td>7.3[5]</td>
</tr>
<tr>
<td>gamma aluminium oxide Al$_2$O$_3$</td>
<td>7-8</td>
</tr>
<tr>
<td>gamma iron (III) oxide (maghemite) Fe$_2$O$_3$</td>
<td>3.3-6.7</td>
</tr>
<tr>
<td>iron (II, III) oxide (magnetite) Fe$_3$O$_4$</td>
<td>6.5-6.8</td>
</tr>
<tr>
<td>lanthanum(III) oxide La$_2$O$_3$</td>
<td>10</td>
</tr>
<tr>
<td>lead(II) oxide PbO</td>
<td>10.7-11.6</td>
</tr>
<tr>
<td>magnesium oxide (magnesia) MgO</td>
<td>9.8-12.7</td>
</tr>
<tr>
<td>manganese(IV) oxide MnO$_2$</td>
<td>4-5</td>
</tr>
<tr>
<td>nickel(II) oxide NiO</td>
<td>9.9-11.3</td>
</tr>
<tr>
<td>silicon carbide (alpha) SiC</td>
<td>2-3.5</td>
</tr>
<tr>
<td>silicon dioxide (silica) SiO$_2$</td>
<td>1.7-3.5</td>
</tr>
<tr>
<td>silicon nitride Si$_3$N$_4$</td>
<td>6-7</td>
</tr>
<tr>
<td>silicon nitride Si$_3$N$_4$</td>
<td>9</td>
</tr>
<tr>
<td>tantalum(V) oxide, Ta$_2$O$_5$</td>
<td>2.7-3.0</td>
</tr>
<tr>
<td>thallium(I) oxide Tl$_2$O</td>
<td>8</td>
</tr>
<tr>
<td>tin(IV) oxide SnO$_2$</td>
<td>4-5.5</td>
</tr>
<tr>
<td>titanium(IV) oxide (rutile or anatase) TiO$_2$</td>
<td>3.9-8.2</td>
</tr>
<tr>
<td>tungsten(VI) oxide WO$_3$</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>vanadium(V) oxide (vanadia) V$_2$O$_5$</td>
<td>1 to 2</td>
</tr>
<tr>
<td>yttrium(III) oxide (ytria) Y$_2$O$_3$</td>
<td>7.2-8.9</td>
</tr>
<tr>
<td>zinc oxide ZnO</td>
<td>8.7-10.3</td>
</tr>
<tr>
<td>zirconium(IV) oxide (zirconia) ZrO$_2$</td>
<td>4-11</td>
</tr>
</tbody>
</table>
Salt Concentration Effect

The effect of electrolytes (ppm) on \(\zeta\)-potential of a colloidal dispersion
**Surfactant Concentration Effects**

Surfactant concentration vs. $\zeta$

Spherical glass particles

Alter the surface chemistry – alter the zeta potential

$\zeta$ vs. $c_{15}$ (mM)
De-stabilization

- Can also use zeta potential to study how to cause instability
- Example: water treatment
- Add chemicals to IEP
- Particles flocculate
- Easier to filter
Applications: Colloidal Gold

- Stable base particle
- Used in drug delivery
- Attach proteins, DNA, etc. to surface
Colloidal Gold: Drug Delivery

Particle size and zeta potential for colloidal gold base particles (average size 51 nm prior to modification) after immobilizing a prodrug activating enzyme onto the surface at different concentrations.

<table>
<thead>
<tr>
<th>Molar ratio of enzyme to gold colloid</th>
<th>90:1</th>
<th>180:1</th>
<th>270:1</th>
<th>360:1</th>
<th>450:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>His-NfnB-gold colloid</td>
<td>53.5</td>
<td>57.5</td>
<td>82.6</td>
<td>69.7</td>
<td>75.4</td>
</tr>
<tr>
<td>Zeta-potential (mV)</td>
<td>-43</td>
<td>-31.7</td>
<td>-30.7</td>
<td>-33.3</td>
<td>-30.4</td>
</tr>
<tr>
<td>Cys-NfnB-gold colloid</td>
<td>56.3</td>
<td>59.8</td>
<td>61.1</td>
<td>69.8</td>
<td>69.7</td>
</tr>
<tr>
<td>Zeta-potential (mV)</td>
<td>-23.4</td>
<td>-25.3</td>
<td>-26.0</td>
<td>-27.7</td>
<td>-34.2</td>
</tr>
</tbody>
</table>

Data generated on SZ-100

Colloidal Gold Modified with a Genetically Engineered Nitroreductase: Toward a Novel Enzyme Delivery System for Cancer Prodrug Therapy
Vanessa V. Gwenin, Chris D. Gwenin, and Maher Kalaji
### SZ-100 Results

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic Force Microscopy</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Differential Mobility Analysis</td>
<td>11.3 ± 0.1</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>Small-Angle X-ray Scattering</td>
<td>9.1 ± 1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic Force Microscopy</td>
<td>24.9 ± 1.1</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>26.9 ± 0.1</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>27.6 ± 2.1</td>
</tr>
<tr>
<td>Differential Mobility Analysis</td>
<td>28.4 ± 1.1</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td>28.6 ± 0.9</td>
</tr>
<tr>
<td>173° scattering angle</td>
<td>26.5 ± 3.6</td>
</tr>
<tr>
<td>90° scattering angle</td>
<td></td>
</tr>
<tr>
<td>Small-Angle X-ray Scattering</td>
<td>24.9 ± 1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic Force Microscopy</td>
<td>55.4 ± 0.3</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>54.9 ± 0.4</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>56.0 ± 0.5</td>
</tr>
<tr>
<td>Differential Mobility Analysis</td>
<td>56.3 ± 1.5</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td>56.6 ± 1.4</td>
</tr>
<tr>
<td>173° scattering angle</td>
<td>55.3 ± 8.3</td>
</tr>
<tr>
<td>90° scattering angle</td>
<td></td>
</tr>
<tr>
<td>Small-Angle X-ray Scattering</td>
<td>53.2 ± 5.3</td>
</tr>
</tbody>
</table>

### NIST Certificates

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 HORIBA</td>
<td>13.4 nm</td>
</tr>
<tr>
<td>Sample 2 HORIBA</td>
<td>12.6 nm</td>
</tr>
<tr>
<td>ASTM Average</td>
<td>15.8 nm</td>
</tr>
<tr>
<td>Combined HORIBA</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 HORIBA</td>
<td>31.5 nm</td>
</tr>
<tr>
<td>Sample 2 HORIBA</td>
<td>32.4 nm</td>
</tr>
<tr>
<td>ASTM Average</td>
<td>31.2 nm</td>
</tr>
<tr>
<td>Combined HORIBA</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 HORIBA</td>
<td>57.6 nm</td>
</tr>
<tr>
<td>Sample 2 HORIBA</td>
<td>58.4 nm</td>
</tr>
<tr>
<td>ASTM Average</td>
<td>59.8 nm</td>
</tr>
<tr>
<td>Combined HORIBA</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Other Colloidal Metals

- **Colloidal silver**: number mean = 2.4 nm
- **Copper**: number mean = 9.6 nm
- **Platinum**: number mean = 3.4 nm
- **Palladium**: number mean = 23.9 nm
- **Nickel**: number mean = 4.2 nm
- **Germanium**: number mean = 6.0 nm
Colloidal Silica

- Possible reference material for both size and zeta potential
- Ludox TM size analyzed on both SZ-100 and LA-950
- Ludox zeta potential can be used to verify zeta potential
- IRMM has issued silica colloidal reference materials ERM-FD100 (20 nm) & ERM-FD304 (40 nm) w/zeta values
New* ISO Standards

- ISO/CD 13099-1 Methods for zeta potential determination — part 1: General Principle
- ISO/CD 13099-2 Methods for zeta potential determination — part 2: Optical Methods
- ISO/NWI 13099-3 Methods for zeta potential determination — part 3: Acoustic Methods
ISO Guidelines: Dilution

- Try to avoid dilution
- 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 0.01 M KCl solution
ISO Guidelines: Verification

- No accepted standards, each vendor supplies reference samples
- Measure three times, mean value within 10% of published electrophoretic mobility value
- Repeatability; CV <10%
- Note: expect most customers to use zeta potential values
- If system is within 12%, don’t lose sleep
Summary

- Particle size, zeta potential, chemistry all related for colloidal suspensions
- Use zeta potential as a predictive tool for stability
- Alter surface chemistry, does zeta potential improve?
  - pH, salt, surfactant, etc.
- IEP useful for both stability and surface definition
Resources: www.horiba.com/particle

Receive news of updates

View application notes, webinars, etc.

Thank-you
For More Details

Visit [www.horiba.com/particle](http://www.horiba.com/particle)

Contact us directly at [labinfo@horiba.com](mailto:labinfo@horiba.com)

Visit the [Download Center](http://www.horiba.com) to find this recorded presentation and many more on other topics

Thank-you
Thank you

Danke
Grazie
Grazias
Thank you

Merci
Obrigado

Tacka dig

Danke

Grazie

Thank you