Characterizing Nanoparticles Used in Bio Applications

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Outline

- Define nanoparticle
- Particle size analysis techniques
- Making nanoparticles
- Applications
  - Micelles, liposomes, engineered nanoparticles for drug delivery
- Other analytical techniques
  - Fluorescence
- Zeta potential
What is a Nanoparticle?

- Size range from approximately from 1-100 nm

Particle size answer: “it depends..”
Size Measurements

- **Dynamic Light Scattering (DLS)**
  - Particle size 0.3 nm – several µm
    - Suspensions only
  - Zeta potential
  - MW, A2

- **Laser diffraction**
  - Particle size 30 nm – 3000 µm
    - Suspensions
    - Powders
DLS Optics

- Laser: 532nm, 10mW
- Attenuator
- Particles moving due to Brownian motion
- 90° for size and MW, A2
- Backscatter (173°) (High conc.)
- PD
- For T%
- Zeta potential
- Modulator
DLS Measurement Principle

\[ \frac{1}{\tau_R} = -\lim_{\tau \to 0} \left\{ \frac{\partial \ln[g(q, r)]}{\partial \tau} \right\} \]

\[ \langle |\gamma(t) - \gamma(t + \tau)|^2 \rangle \approx 6D\tau \]

Particle's moving distance

Diffusion constant

Relaxation time

\[ D = \frac{1}{q^2 \tau_R} = \frac{k_B T}{6\pi\eta a} \]

q: Scattering vector
\( \eta \): Viscosity
\( k_B \): Boltzmann constant

Autocorrelation Function

\( g(q, r) \)

Relaxation time

Particle radius

Particle's moving distance

Diffusion constant

Relaxation time

Particle radius

Autocorrelation Function

\( g(q, r) \)
Laser Diffraction

- Converts scattered light to particle size distribution
- Quick, repeatable
- Most common technique
- Low end: 30 nm
Making Nanoparticles

- **Top Down**
  - Make particles smaller

- **Bottom Up**
  - Build from atomic or molecular level up

Self assembly of micelles
Top Down: Elan NanoCrystal® Technology
Top Down: Elan NanoMill
Size Reduction Measured on LA-950

NanoMill-10 Particle Size vs. Mill Residence Time

<table>
<thead>
<tr>
<th>Size (nm)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48,400</td>
<td>0</td>
</tr>
<tr>
<td>5,240</td>
<td>0.82</td>
</tr>
<tr>
<td>406</td>
<td>5.6</td>
</tr>
<tr>
<td>240</td>
<td>18.1</td>
</tr>
<tr>
<td>155</td>
<td>26.3</td>
</tr>
</tbody>
</table>
Top Down: Microfluidizer*

* See http://www.microfluidicscorp.com/
Ceria: Before, After Processing

<table>
<thead>
<tr>
<th>Data Name</th>
<th>Graph Type</th>
<th>Median Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>201006021603291</td>
<td></td>
<td>1.29844(μm)</td>
</tr>
<tr>
<td>201006021603292</td>
<td></td>
<td>0.06020(μm)</td>
</tr>
<tr>
<td>201006021603293</td>
<td></td>
<td>0.06786(μm)</td>
</tr>
</tbody>
</table>

Laser diffraction required for before sample

\[ \begin{align*}
D(v,0.1) & : 0.10395(μm) \\
D(v,0.5) & : 1.29844(μm) \\
D(v,0.9) & : 4.05885(μm)
\end{align*} \]

\[ \begin{align*}
D(v,0.1) & : 0.05406(μm) \\
D(v,0.5) & : 0.06822(μm) \\
D(v,0.9) & : 0.08535(μm)
\end{align*} \]
Bottom-up Self Assembly: Micelles

Hydrophobic tail

Hydrophilic head

-c-h-c-h-c-h-

non polar

R

+/-
polar

Hydrophilic head

Aqueous solution

Hydrophobic tail

Hydrophilic head

Organic solvent

Hydrophobic tail
Critical Micelle Concentration

Triton X-100

Experiment
1. Determine weight a drop from pipette
2. 10 mMol NaCl soln prepared in beaker w/stir bar
3. Drops Triton X-100 were added, mixed 10 minutes
4. Remove small amount, measure by DLS

<table>
<thead>
<tr>
<th>Triton x-100</th>
<th>Conc. wt%</th>
<th>Intensity</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mMol NaCl</td>
<td>0</td>
<td>0.94</td>
<td>-</td>
</tr>
<tr>
<td>1 drop</td>
<td>0.0017</td>
<td>1.78</td>
<td>-</td>
</tr>
<tr>
<td>5 drops</td>
<td>0.0086</td>
<td>2.35</td>
<td>-</td>
</tr>
<tr>
<td>10 drops</td>
<td>0.0172</td>
<td>3.18</td>
<td>-</td>
</tr>
<tr>
<td>15 drops</td>
<td>0.0255</td>
<td>4.78</td>
<td>9</td>
</tr>
</tbody>
</table>
Curcumin- Casein Micelles*

Particle size by DLS (insert), SEM (left), and AFM (right)

*Sahu, et al., Fluorescence Study of the Curcumin-Casein Micelle Complexation and Its Application as a Drug Nanocarrier To Cancer Cells, Biomacromolecules 2008, 9, 2905–2912
Curcumin- Casein Micelles*

Binding constant $k_b = 1.48 \times 10^4 \text{ M}^{-1}$

\[
\frac{1}{\Delta F} = \frac{1}{\Delta F_{\text{max}}} + \frac{1}{K_b \Delta F_{\text{max}}[CM]}
\]

*Sahu, et al., Fluorescence Study of the Curcumin-Casein Micelle Complexation and Its Application as a Drug Nanocarrier To Cancer Cells, *Biomacromolecules* 2008, 9, 2905–2912
Liposomes

- Liposome
- Micelle
- Bilayer sheet
- Hydrophilic head
- Aqueous solution
- Hydrophobic tail

100 nm
Liposome Size Reduction: Filter Membrane

Liposome particle size after 5 passes through a 100 nm membrane ~ 250 nm

Size reduced by passing through 100 nm filter membrane Measured by DLS

Liposome particle size after 20 passes through a 100 nm membrane ~ 150 nm
Liposome Size Reduction: Microfluidizer

Laser diffraction

Unprocessed

\[ D(v, 0.1) : 0.09712(\mu m) \]
\[ D(v, 0.5) : 0.16934(\mu m) \]
\[ D(v, 0.9) : 0.29075(\mu m) \]
Liposome Size Reduction: Microfluidizer

Processed

D(\text{v,0.1}) : 0.06364(\mu m)
D(\text{v,0.5}) : 0.09296(\mu m)
D(\text{v,0.9}) : 0.15176(\mu m)

Laser diffraction
Liposome: Before, After

<table>
<thead>
<tr>
<th>Data Name</th>
<th>Graph Type</th>
<th>Median Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>201006221640296</td>
<td></td>
<td>0.09296(μm)</td>
</tr>
<tr>
<td>201006221635795</td>
<td></td>
<td>0.16934(μm)</td>
</tr>
</tbody>
</table>

- $D_{v,0.1} = 0.09712(\mu m)$
- $D_{v,0.5} = 0.16934(\mu m)$
- $D_{v,0.9} = 0.29075(\mu m)$

- $D_{v,0.1} = 0.06364(\mu m)$
- $D_{v,0.5} = 0.09296(\mu m)$
- $D_{v,0.9} = 0.15176(\mu m)$
Nanoparticles for Drug Delivery: Bottom Up
Nanoparticles for Drug Delivery

Targeting ligand provides recognition, enabling targeted nanoparticles to identify and bind to their intended target site. Surface functionalization shields targeted nanoparticles from the immune system. Polymer matrix encapsulates payload molecules in a matrix of biodegradable polymers. Therapeutic payloads include small molecules, peptides, proteins, etc.
Nanoparticles for Drug Delivery

\[ D(v, 0.1) : 0.08201(\mu m) \]
\[ D(v, 0.5) : 0.10530(\mu m) \]
\[ D(v, 0.9) : 0.13110(\mu m) \]

Mean Size : 0.12502(\mu m)
Median Size : 0.10530(\mu m)
Mode Size : 0.1067(\mu m)
<table>
<thead>
<tr>
<th>Size</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10</td>
<td>10% below</td>
</tr>
<tr>
<td>D50</td>
<td>50% below</td>
</tr>
<tr>
<td>D90</td>
<td>90% below</td>
</tr>
<tr>
<td>D100</td>
<td>100% below</td>
</tr>
</tbody>
</table>

Never use D100 from laser diffraction
Diffraction Results: D10, D50, D90

Symmetric distribution: mean = median = mode

Central value the same but D10 & D90 significantly different
Asymmetric Distribution

Mode < Median < Mean
if skewed to larger sizes

Note: D4,3 sensitive to large particles
Volume Mean Diameter

- D[4,3] which is often referred to as the Volume Mean Diameter [VMD]

\[ D[4,3] = \frac{\sum D_i n_i}{\sum D_i n_i} \]

Setting a D[4,3] specification will emphasize the presence of large particles.

Mean Size

The frequency distribution is found using the arithmetical mean diameter, as shown in the formula below.

\[ \text{Mean Diameter} = \frac{\sum q(J) \times X(J)}{\sum q(J)} \]

- J: Particle Diameter Division Number
- q(J): Frequency Distribution Value (%)
- X(J): Jth Particle Diameter Range’s Representative Diameter (μm).
PLA Nanoparticles for Drug Delivery

- $D(v,0.1): 0.06752(\mu m)$
- $D(v,0.5): 0.08108(\mu m)$
- $D(v,0.9): 0.09753(\mu m)$

- Mean Size: 0.08172(\mu m)
- Median Size: 0.08108(\mu m)
- Mode Size: 0.0812(\mu m)

- $D(v,0.1): 0.06746(\mu m)$
- $D(v,0.5): 0.08112(\mu m)$
- $D(v,0.9): 0.09825(\mu m)$

- Mean Size: 0.73105(\mu m)
- Median Size: 0.08112(\mu m)
- Mode Size: 0.0811(\mu m)

9 fold increase
PLA Nanoparticles for Drug Delivery

Pure

Spiked with 1 µm PSL

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>D(0.1) (µm)</th>
<th>D(0.5) (µm)</th>
<th>D(0.9) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50928-6-1</td>
<td>0.06541</td>
<td>0.06222</td>
<td>0.13786</td>
</tr>
<tr>
<td>50928-6-1</td>
<td>0.06541</td>
<td>0.06222</td>
<td>0.13786</td>
</tr>
<tr>
<td>50928-6-1</td>
<td>0.06540</td>
<td>0.06221</td>
<td>0.13787</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>D(0.1) (µm)</th>
<th>D(0.5) (µm)</th>
<th>D(0.9) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50926-6-2</td>
<td>0.07348</td>
<td>0.13035</td>
<td>1.21951</td>
</tr>
<tr>
<td>50926-6-2</td>
<td>0.07345</td>
<td>0.13085</td>
<td>1.20702</td>
</tr>
<tr>
<td>50926-6-2</td>
<td>0.07360</td>
<td>0.13155</td>
<td>1.25225</td>
</tr>
</tbody>
</table>

Multimodal Size Distribution
Proteins

Molecular surface of several proteins showing their comparative sizes.

- Immunoglobulin G (IgG)
- Hemoglobin
- Adenylate kinase
- Insulin
- Glutamine synthetase

Fig. 2. Comparison of molecular size between chignolin (left, with 16 amino acid residues) and human hemoglobin, one of representative protein (right, with 574 residues). (1 nm = 1 / 1,000,000,000 m)
Protein: Lysozyme

Protein; Lysozyme

<table>
<thead>
<tr>
<th>Lysozyme</th>
<th>from egg white, Molecular weight; 14,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Preparation</td>
<td>pH 4.3, 0.1 mg / mL, 0.1 M Sodium-Acetate buffer</td>
</tr>
</tbody>
</table>

Conditions
Temperature; 25 C degree
Solvent; Water
Refractive Index; 1.333
Distribution base; Mass

<table>
<thead>
<tr>
<th>Results</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Dia. (nm)</td>
<td></td>
</tr>
</tbody>
</table>
Ferritin

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Preparation</td>
<td>10 %, pH 6.0</td>
</tr>
</tbody>
</table>

**Conditions**
- Temperature: 25°C
- Solvent: Water
- Refractive Index: 1.333
- Distribution base: Mass

**Results**
- Z ave. (nm): 19.7
Protein Reproducibility

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Measurement Type</th>
<th>Sample Name</th>
<th>Scattering Angle</th>
<th>T% before meas</th>
<th>T% after meas</th>
<th>Z-Average (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>mercredi 22 septembre</td>
<td>Particle Size</td>
<td>bsa protein</td>
<td>90</td>
<td>31931</td>
<td>31459</td>
<td>0.2</td>
</tr>
<tr>
<td>76</td>
<td>mercredi 22 septembre</td>
<td>Particle Size</td>
<td>bsa protein</td>
<td>90</td>
<td>31931</td>
<td>31428</td>
<td>7.9</td>
</tr>
<tr>
<td>77</td>
<td>mercredi 22 septembre</td>
<td>Particle Size</td>
<td>bsa protein dilute</td>
<td>90</td>
<td>32118</td>
<td>32096</td>
<td>8.1</td>
</tr>
<tr>
<td>78</td>
<td>mercredi 22 septembre</td>
<td>Particle Size</td>
<td>bsa protein dilute</td>
<td>90</td>
<td>32118</td>
<td>32096</td>
<td>8.1</td>
</tr>
<tr>
<td>79</td>
<td>mardi 12 octobre 2010</td>
<td>Particle Size</td>
<td>LYZ 10 F100nm NC AA StdM</td>
<td>90</td>
<td>32109</td>
<td>32293</td>
<td>4.0</td>
</tr>
<tr>
<td>80</td>
<td>mardi 12 octobre 2010</td>
<td>Particle Size</td>
<td>LYZ 10 F100nm NC AA StdM</td>
<td>90</td>
<td>32109</td>
<td>32293</td>
<td>3.7</td>
</tr>
<tr>
<td>81</td>
<td>mardi 12 octobre 2010</td>
<td>Particle Size</td>
<td>LYZ 10 F100nm NC AA StdM</td>
<td>90</td>
<td>32109</td>
<td>32293</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Average:
T% before meas: 32109
T% after meas: 32293
Z-Average (nm): 3.8

**Calculation Results**
- Sample Name: LYZ 10 F100nm NC AA StdM
- Scattering Angle: 90°
- Temperature of the holder: 25.0 °C
- T% before meas: 32109
- Viscosity of the dispersion medium: 0.896 mPa·s
- Form Of Distribution: Standard
- Form Of Distribution (Dispersity): ---
- Representation of result: Scattering Light Intensity
- Count rate: 397 KCPs
- Z-Average: 4.0 nm
Protein Size

Melting Point

Lysozyme Aggregation

Temperature (C)

T_m

Size in nm

Hours

[Graphs showing the relationship between temperature and protein size]
## Virus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenzavirus</td>
<td>1% Sodium-Acetate buffer</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results</th>
<th>Z ave. (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>138.1</td>
</tr>
</tbody>
</table>

![Image of Virus](image1.png)

![Graph showing Diameter (nm) vs. Percentage](image2.png)
Dendrimers

Repeatedly branched, roughly spherical large molecules

- Core
- Inner shell
- Outer shell
Dendrimers

- Applications: typically involve conjugating other chemical species to the dendrimer surface that can function as detecting agents (such as a dye molecule), affinity ligands, targeting components, imaging agents, or pharmaceutically active compounds
  - Drug & gene delivery
  - Sensors
Dendrimers

- DDS materials; Dendrimer

<table>
<thead>
<tr>
<th>PAMAM Dendrimer</th>
<th>DNT-107 (7.2 nm); 1,4-Diaminobutan Core, Amidoamine surface, Gen 6.0, 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Methanol</td>
<td>DNT-189 (9 nm); 1,4-Diaminobutan Core, Amidoethanol surface, Gen 6.0, 10%</td>
</tr>
</tbody>
</table>

Conditions
Temperature: 25 °C degree
Solvent: Methanol
Refractive Index: 1.329
Distribution base: Scattering light

<table>
<thead>
<tr>
<th></th>
<th>Z ave. (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNT-107</td>
<td>7.6</td>
</tr>
<tr>
<td>DNT-189</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Graphs of DNT-107 and DNT-189 showing size distribution.
Detection Limit

Bio-sample; Vitamin B1

<table>
<thead>
<tr>
<th>Thiamine</th>
<th>Vitamin B1 Molecular weight; 337.27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Preparation</td>
<td>300 mg / mL</td>
</tr>
</tbody>
</table>

**Conditions**
- Temperature: 25°C
- Solvent: Water
- Refractive Index: 1.333
- Viscosity: 1.68
- Distribution base: Mass

| Mean Dia. (nm) | 0.4 |

![Graph showing detection limit](image.png)
Molecular Weight Measurement Principle

Multi angle scattered light intensity measurement preparing diluted solutions of known concentration.

\[ \lim_{\theta \to 0} \frac{KC}{\Delta R_\theta} = \frac{1}{M} + 2A_2C + 3A_3C^2 \ldots \]

KC/R_\theta parameter is calculated by scattered light and sample concentration.

\[ \theta \quad \text{Radius of gyration} \]

\[ \sin \theta + kC \]

e.g. Polystyrene sample (Mw=5 \times 10^5)

\[ \rightarrow \text{Mw} = 6.35 \times 10^5 \]

Optical Constant

\[ K = \frac{4\pi^2 n^2}{N_A \lambda_0^4} \left( \frac{\partial n}{\partial c} \right) \]

Reduced Scattered light Intensity

\[ R_\theta = \frac{I_0 r^2}{I_0 V} \]

M: Molecular weight
A_2: Second virial coefficient
A_3: Third virial coefficient

C = Concentration
MW and A2 using One Angle

- Light scattering independent of angle at very small sizes (< 60-100 nm)
- Measure several concentrations at one angle to create Debye plot
MW Data
Zeta Potential

Measure mobility $\mu$
Calculate zeta potential

$$\mu = \frac{\Delta \omega \lambda_0}{4\pi n E \sin\left(\frac{\theta}{2}\right) \sin\left(\frac{\theta}{2} + \xi\right)}$$
$$\mu = \frac{2\xi \epsilon}{3\eta_o} f(\kappa r)$$
Zeta Potential: Emulsion

Isoelectric point: pH where zeta potential = 0

The graph shows the change in zeta potential (mV) with pH for an emulsion, indicating the point of zero charge (pI) where the emulsion is stable.
Zeta Potential: Lysozyme (4 nm, 10 mg/mL)

Isoelectric point: pH where zeta potential = 0
Summary

- Both laser diffraction (LA-950) and DLS can measure nanoparticles
- Use diffraction when some samples > 1 μm, but all > 30 nm
  - Check lower limit of system used
- Use DLS when all < 1 μm
- DLS + zeta potential + molecule wt + A2
Acknowledgements

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- Unnamed (at their request) customers
Thank you
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Q&A

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