Nanoparticle Size Analysis: A Survey and Review

Marc Wolfgang
Vice Chair – Nanomedicines Alliance

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Introduction

In 2014 the Nanomedicines Alliance conducted a blinded survey of its member companies on the topic of particle size analysis. The survey was followed by an expert panel discussion at the NMA’s annual meeting in Washington D.C.
Our Mission is to promote and facilitate the scientific advancement, regulatory approval, safe use and public appreciation of nanotechnology-based medicines world-wide for the diagnosis, treatment and prevention of disease.

| The objective of the Alliance is to provide a forum for pharmaceutical, biotechnology and medical device companies with an interest in the development and approval of nanotechnology-based medicines | Define priority issues of science, regulation, and policy and work with government partners and other stakeholders to realize the potential of nanomaterials and nanotechnology for medical applications | Provide government agencies and other stakeholders with practical, science-driven information on nanotechnology as it is used in the development and manufacturing of nanotechnology-based medicines and medical devices. |
Survey

Purpose and Scope

◦ to share information on particle sizing technologies and their use in the characterization and quality control of nanomedicine based nanoparticle formulations
◦ to set the stage for a follow up discussion on the application of particle sizing technologies for product characterization and control with a panel of experts
Survey Overview

- Seven (7) companies participated
- Thirteen (13) formulations were presented, representing five (5) types of nanoparticle formulations
  - Polymeric nanoparticles – 7
  - Liposomes – 2
  - Micelles – 2
  - Metallic nanoparticles – 1
  - Nano-suspension (API) – 1
- Six (6) particle sizing technologies employed

<table>
<thead>
<tr>
<th>Technology</th>
<th>Characterization</th>
<th>QC</th>
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<tbody>
<tr>
<td>Dynamic light scattering (DLS)</td>
<td>X</td>
<td>X</td>
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<td>Disc centrifugation</td>
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<td>X</td>
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<tr>
<td>Electron microscopy (SEM, TEM, cryo-TEM)</td>
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<td>Nanoparticle tracking analysis (NTA)</td>
<td>X</td>
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<td>Size exclusion chromatography w/ DLS detection</td>
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<tr>
<td>Static light scattering (SLS)</td>
<td>X</td>
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Survey Results

- **Dynamic Light Scattering (DLS)** most frequently cited/used particle sizing technology
  - Over 85% (6/7) of participating companies use for QC and/or characterization
  - Used for analysis of over 90% (12/13) of nanoparticle formulations (20 to 1,000 nm)
  - Primary measurements included Zavg (avg particle diameter) and PDI (polydispersity)
  - Multiple instruments/vendors cited

- **Electron microscopy** was the next most frequently cited technology
  - Employed for characterization only
  - Used by ~60% (4/7) of participating companies for characterization of ~60% (8/13) of the nanoparticle formulations
  - Confirmation of particle size and morphology
  - Sample preparation challenges and incompatibilities cited
Survey Results

- Limited use of two other techniques for QC testing
  - **Disc Centrifugation** – one company for QC of metallic nanoparticles, mean particle size and polydispersity
  - **Static light scattering (SLS)** – one company for QC to supplement DLS analysis, mean particle size, polydispersity and fractionation profiling (D90, D50 and D10)

- Limited use of three other techniques for characterization testing
  - **Size exclusion chromatography coupled to DLS detection** – two companies to generated size distribution profiles
  - **SLS** – two companies to generate size distribution profiles
  - **Nanoparticle tracking analysis** – one company to generate particle size and concentration measurements
Expert Panel Discussion

- **Purpose and Scope**
  - to discuss the strengths, weaknesses and limitations of different particle sizing technologies with a panel of particle measurement experts
  - to discuss the application of particle sizing for control and characterization of nanomedicine formulations
Panel

Jeff Clogston, Ph.D.
Nanotechnology Characterization Laboratory
Senior Scientist

Kevin Mattison, Ph.D.
Malvern Instruments
Principal Scientist – Bioanalytics

Dariusz Stramski, Ph.D.
University of California, San Diego
Professor of Oceanography, Scripps Institution of Oceanography

Hans van der Voorn
Chief Executive Officer
Izon Science Ltd.
Key Points – Dynamic Light Scattering

- Provides average particle size diameter (Zavg), polydispersity and size distribution
Key Points – Dynamic Light Scattering

- **Advantages**
  - Ease of use and fast analysis
  - Broad dynamic range, ~ 1 to 1,000 nm
  - High sensitivity and reproducibility for monodisperse, homogenous samples

- **Limitations**
  - Low resolution for polydisperse, heterogeneous samples
  - Requires transformative calculations with assumptions that must be considered when interpreting data – particularly with polydisperse samples
  - Assumes spherical shaped particles
Key Points – Dynamic Light Scattering

- Issue with resolution and accuracy of polydisperse, heterogeneous samples
  - Zavg a function of light scattering intensity
  - Intensity increases exponentially w/ particle size, large particles contribute more to the Zavg than the smaller particles – overestimation of average particle size
  - Can detect small number of larger particles in a polydisperse sample, but difficult to detect populations of small particles in polydisperse samples
  - Limitation can be addressed by coupling to a separation technique (e.g. SEC or FFF)
Key Points – Dynamic Light Scattering

- Application
  - Good quality control tool for
    - measuring batch to batch consistency
    - changes overtime (stability)
    - resolution limitation must be considered for polydisperse samples
  - Excellent diagnosis tool for detecting aggregation
    - due to sensitivity to large particles
    - post stress exposure
Key Points – SEC coupled to DLS

- Powerful characterization tool if you can get it to work
  - Can be lengthy and difficult
  - Low sample recoveries are common
  - Resins often interact with the nanoparticles
    - SEC columns designed for separating soluble proteins not nanoparticles
  - Reproducibility between resin lots can be problematic
  - Often run into flow rate issues
Key Points – Static Light Scattering

- Technique is more applicable to determining molecular weight than particle size
- Suffers from same limitation as dynamic light scattering
Key Points – Electron Microscopy

› **Advantage**
  ◦ visual size measurement
  ◦ visual of surface properties
  ◦ works very well with electron dense nanoparticles (e.g. gold)

› **Limitations**
  ◦ labor intensive and time consuming
  ◦ sample prep and staining may affect particle character and produce artifacts
    • required for “less” electron dense particles
  ◦ doesn’t work well with “soft”, polymer based nanoparticle – cryo TEM often required
  ◦ field of view small, many analyses required to be “representative”

› **Application**
  ◦ characterization and proof of concept work
  ◦ not a practical QC tool
Key Points – Recent Developments

- Tunable Resistive Pulse Sensing (TRPS)
  - Impedance based measurement where voltage is applied across a pore that is filled with electrolyte, resulting in an ionic current. As particles cross the pore they briefly increase electrical resistance, creating a resistive pulse proportional to particle volume.
  - provides particle size, charge, distribution, and concentration measurements
Key Points – Recent Developments

- **Nanoparticle Tracking Analysis (NTA)**
  - microscopy-based video tracking of particles under Brownian motion utilizing laser light scattering
  - provides particle size, distribution and concentration measurements
Key Points – TRPS and NTA

- Less mature but rapidly developing and improving
- Currently a bit more labor and time intensive than DLS, but provide more information
- Higher resolution and more suited to analysis of heterogeneous, polydisperse samples
- Well suited for characterization
- High potential for quality control analysis
Conclusions

- Properties of the nanoparticle formulation must be considered when selecting a particle sizing technology.
- DLS appears to be the current industry standard but limitations must be carefully considered for data interpretation (particularly with heterogeneous, polydisperse samples).
- EM is a powerful characterization tool, but difficult to use for “less” electron dense nanoparticle formulations.
- New technologies are currently under development and offer promise for improved and more comprehensive particle size analysis.
QUESTIONS

Marc Wolfgang  
Vice Chair, Nanomedicines Alliance  
mwolfgang@ceruleanrx.com  
(617) 551–9620