Optimization of Particle Size for PLGA Nanoparticles Prepared by the Ouzo Effect

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Why Nanoparticles?

- Nanoparticles can be the right tool for the job

- Advantages
  - Enhanced bioavailability and controlled release
  - Penetration across biological barriers
  - Increased drug targeting efficiency
Methods for Nanoparticle Production

Materials

- Polymers
- Metals
- Ceramics
- Biological materials
  - Liposomes
  - Peptides

- Polymeric Nanoparticles
  - Nanoparticles formed using monomers as the starting point
  - Nanoparticles prepared using preformed polymers as the starting point
Methods Employing Preformed Polymers

- Emulsion Diffusion
  - Interfacial Precipitation
- Emulsion Evaporation
  - Double Emulsion Evaporation
- Salting Out
- Solvent Displacement
  - Nanoprecipitation, Solvent Diffusion, Spontaneous Precipitation, Ouzo Effect
Solvent Displacement

- Polymer and drug are dissolved in a water-miscible organic solvent
Solvent Displacement

- Polymer and drug are dissolved in a water-miscible organic solvent
- This solution is added to an aqueous solution
Solvent Displacement

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- Rapid solvent diffusion leads to instantaneous nanoparticle formation
Solvent Displacement

- Polymer and drug are dissolved in a water-miscible organic solvent
- This solution is added to an aqueous solution
- Rapid solvent diffusion leads to instantaneous nanoparticle formation
- Solvent is removed by evaporation
Solvent Displacement

**Advantages**
- Relatively simple process
- Nontoxic solvents can be used
- Does not require high shear stress
- Does not require surfactants or stabilizers

**Disadvantages**
- Solvent evaporation can be time consuming
- Poor encapsulation of hydrophilic drugs
Nanoparticle Characterization

- Size
- Surface Characteristics
- Encapsulation efficiency
- Drug Release
- Degradation
- Biocompatibility
Nanoparticle Characterization

- Size
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The Importance of Size
The Importance of Size

- Basketball

Average Player Height/40 minutes

- Air Force
- Kansas
- Florida

2005-2006 Season
## The Importance of Size

<table>
<thead>
<tr>
<th>NCAA Div. I Rank</th>
<th>Air Force</th>
<th>Kansas</th>
<th>Florida</th>
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<td>20/326</td>
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<td>281/326</td>
<td>20/326</td>
<td>22/326</td>
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</table>
The Importance of Size

• Nanoparticle Size
  – Endothelial Permeability
    • Particle escape from vasculature
      – Liver sinus endothelium (up to 150 nm)
      – Tumor capillaries (up to 300 nm)
    • Blood-brain barrier
  – Splenic Filtration
  – Macrophage Clearance
  – Cellular Uptake
  – Particle Degradation and Drug Release Rates
Ouzo Effect

• Solvent displacement is also referred to as the Ouzo effect
  – Ouzo is a mixture of anethol, ethanol, and water (from anis seeds)
  – Additional water leads to micro/nanoparticle formation
  – Solution proportions fall into a thermodynamically metastable region
    • Homogeneous nucleation occurs
    • Leads to a uniform dispersion (milliseconds) followed by Ostwald ripening (seconds)
Ouzo Region

Ouzo Region for RG502H/Acetone

![Ouzo Region Graph](image-url)
Ouzo Region for RG502H/Acetone

- Weight fraction polymer
- Weight fraction acetone

- Ouzo region
- No Particles
- Stable Nanoparticles
- Unstable Particles

Graph showing the regions of stability and instability for different weight fractions of polymer and acetone.
Particle Size Distribution

![Particle Size Distribution Graph]

![Particle Size Distribution Image]
Surfactant Concentrations

![Bar chart showing nanoparticle size (nm) in relation to poloxamer concentration (%).]
Different Solvents

Particle Size (nm)

- Acetone
- Acetonitrile
- THF
Different Solvents

- Particle Size (nm)
  - Acetone
  - Acetonitrile
  - THF

- Solvent Viscosity (cP)
  - Acetone
  - Acetonitrile
  - THF
## PLGAs of Increasing MW

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (kDa)</th>
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<tr>
<td>RG502H</td>
<td>12</td>
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<tr>
<td>RG503H</td>
<td>23</td>
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<tr>
<td>RG504H</td>
<td>48</td>
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</table>

![Graph showing the relationship between polymer concentration and kinematic viscosity.](image)
Relative Viscosity

$$RV = \frac{\text{Viscosity of 10 mg/mL polymer}}{\text{Viscosity of acetone alone}}$$
4.0 mg/mL RG 502 H in acetone added to 0.1% Poloxamer.
Surfactants – Fast Injection

![Graph showing nanoparticle size vs poloxamer concentration.](image-url)
Surfactants – Slow Injection

The graph shows the relationship between Poloxamer Concentration (%) and Nanoparticle Size (nm). As the Poloxamer Concentration increases from 0 to 0.3%, the Nanoparticle Size increases from approximately 100 nm to 140 nm.
Relative Viscosity

$$RV = \frac{\text{Viscosity of 10 mg/mL polymer}}{\text{Viscosity of acetone alone}}$$
Viscosity – Slow Injection

[Graph showing the relationship between Particle Size (nm) and [Polymer in Acetone] (mg/mL) for RG502H and RG504H.]
## Importance of Size

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<td>Scoring Defense</td>
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<td>22/326</td>
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<td>Personal Fouls/Game</td>
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<td>140/326</td>
<td>29/326</td>
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<tr>
<td>3-Pointers Made/Game</td>
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<td>70/326</td>
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<tr>
<td>3-Point Shooting %</td>
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<td>44/326</td>
<td>15/326</td>
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<tr>
<td>Free Throw Shooting %</td>
<td>29/326</td>
<td>226/326</td>
<td>34/326</td>
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Improved Drug Delivery

• Nanoparticle Size
Improved Drug Delivery

- Nanoparticle Size
- Precision/Accuracy
Improved Drug Delivery

- Nanoparticle Size
- Precision/Accuracy/Targeting
Improved Drug Delivery

- Nanoparticle Size
- Precision/Accuracy/Targeting
- Other Strategies
How can one control Basketball player size?

- Nutrition
- Exercise
- Genetics
Summary

• How can one control Nanoparticle size?
  – Solvent viscosity
  – Injection speed
  – Polymer concentration
Acknowledgments

• Co-authors: Tobias Lebhardt & Thomas Kissel
• Others
  – Claudia Packhäuser
  – Theresa Haas
  – Ulrike Nierste
References

- Bala et al., *Critical Rev Ther Drug Carrier Sys*, 2004, 21, 387-422.
Poloxamer 188 NF (Pluronic F68)

Surface Charge

![Graph showing nanoparticle size and zeta potential as a function of Poloxamer concentration.](image-url)
Fluorescent Nanoparticles

Nanoparticles prepared with 10 µg Coumarin-6 per 10 mg RG502H in 1 mL acetone, $Z_{\text{ave}} = 117 \pm 3$ nm. Fluorescence Microscopy settings: $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 515$ nm. Suspension of particles in water after washing in a Sephadex G-50 column.