Week #7 Novel Pharmaceutical Particles

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Advanced Drug Delivery Systems

Computation Simulation and Optimization

Optimized Strategy for Cancer Therapy

Patient Specific Treatment

Novel Fabrication of Nano-/Micro-particles
Novel Fabrication of Pharmaceutical Particles

**Electrohydrodynamic atomization applications**

- Micro- and nanofibers.
- Thin films.
- Micro- and nanoparticles.
- Microencapsulation of living cells, DNA etc.

**Paclitaxel**

Chemotherapy & radiotherapy for brain tumors.

Penetrates the blood-brain barrier poorly.


Using electric field to break liquid drops into fine droplets

- **Vn**: high voltage used to break liquid drops;
- **Vr**: high voltage applied to metal hoop;
- **$N_2$**: the trace of protective Nitrogen;
- ****: solvent with polymer & drugs;
- ****: volatilized solvent;
- ****: collected polymer & drug particles;
- **Vacuum pump**
- **Filter**
- **Particles**
- **Syringe**
Electrohydrodynamic atomization is an atomization method based on the application of an electrical stress on the fluid that emerges from the tip of the nozzle. A Taylor cone, which is formed due to the acceleration of the fluid by the applied electrical stress, reduces the diameter of the jet, so that a thin jet is formed at the tip of the Taylor cone. Depending on the solution properties, either discrete particles or strands of fibers can be formed using the same equipment.
Droplet Size Variation with Different Ring Electrical Potential ($V_r$)

- $V_n=5\text{kV}, 3\text{ml/h}$
- $V_n=8\text{kV}, 3\text{ml/h}$

I: changes in droplet size when $V_n$ is kept at 5kV.
II: changes in droplet size when $V_n$ is kept at 8kV.

Particle Size $\rightarrow$ Mass balance $\rightarrow$ Droplet Size $\rightarrow$ Modulation $\rightarrow$ Varying $V_r$
The Front Tracking/Finite Difference CFD simulation was able to replicate all observable phenomenon of the EHDA process, including the Taylor Cone, Jet and the droplet formation process. Since the electrical charge resides on the surface of the liquid, the electrical field accelerates the surface of the liquid, and results in the formation of the circulating fluid inside the Taylor cone.

Fluid: Dichloromethane; Flowrate: 6ml/h; Vn=8kV, Vr=8.9kV
Nozzle inner radius: 110 micron; Nozzle outer radius: 170 micron
Nozzle to Ring: 10mm; Nozzle to Ground: 100mm; Ring Diameter: 40mm
Taylor Cone, jet and droplet formation process

A transient CFD simulation was done, so that the formation of the Taylor Cone, jet and droplet was captured with time. Droplet formation was also simulated and the simulated droplet size can be obtained directly from the simulation data.

Fluid: Dichloromethane; Flowrate: 6ml/h; Vn=8kV, Vr=8.9kV
Nozzle inner radius: 110 micron; Nozzle outer radius: 170 micron
Nozzle to Ring: 10mm; Nozzle to Ground: 100mm;
Ring Diameter: 40mm

Nozzle Diameter ~ 340micron
Droplet Diameter ~ 50micron

B: CFD simulation results;
The Taylor Cone is an important phenomenon in the EHDA process, enabling the formation of thin jet that is at least one order of magnitude smaller than the inner diameter of the nozzle, resulting in droplets that is smaller than the inner diameter of the nozzle. The Taylor cone angle is also a useful parameter for comparison between the experimental and the simulation results.

Fluid: Dichloromethane; Flowrate: 6ml/h; Vn=8kV, Vr=8.9kV
Nozzle inner radius: 110 micron; Nozzle outer radius: 170 micron
Nozzle to Ring: 10mm; Nozzle to Ground: 100mm;
Ring Diameter: 40mm
Electrohydrodynamic Atomization

Different polymer solution flow rates.

a: 3.0 ml/h size: 11±0.8μm;
b: 1.0 ml/h size: 6.5±0.8μm;
c: 0.5 ml/h size: 4.9±0.8μm.
d: 3ml/h size 17μm;
e: 10ml/h size 26μm;
f: 15ml/h size 32μm.

\[ I \propto (\gamma K Q)^{1/2} \]
\[ d = c \left( \frac{\rho \varepsilon_0 Q^4}{I^2} \right)^{1/6} \propto Q^{1/2} \]

K: Conductivity
Q: flow rate
d: diameter of droplets
\( \gamma \): surface tension
I: current

Xie J, Jan CMM, Wang CH. Microparticles developed by electrohydrodynamic atomization for the local delivery of anticancer drug to treat C6 glioma in vitro. Biomaterials 2006; 27: 3321-3332
Variation of operating parameters results in controllable size and morphology of microparticles.

Characterization for samples S1 – S5.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Drug loading (%)</th>
<th>Encapsulation Efficiency (%)</th>
<th>Particle size (μm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>8.1</td>
<td>81.3</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>S2</td>
<td>7.9</td>
<td>82.3</td>
<td>15.2 ± 1.7</td>
</tr>
<tr>
<td>S3</td>
<td>8.4</td>
<td>84.1</td>
<td>14.2 ± 2.2</td>
</tr>
<tr>
<td>S4</td>
<td>15.8</td>
<td>78.1</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>S5</td>
<td>0</td>
<td>-</td>
<td>12.6 ± 0.8</td>
</tr>
</tbody>
</table>

S1 - Paclitaxel-loaded PCL microspheres
S2 - Paclitaxel-loaded PLGA microspheres
S3 - Paclitaxel-loaded PLGA particles of biconcave shape
S4 – Paclitaxel-loaded PLGA microspheres
S5 – Blank PLGA microspheres

EHDA Microparticles

In vitro release

Paclitaxel-loaded PLGA microparticles could release faster than paclitaxel-loaded PCL microparticles. Biconcave-shaped PLGA microparticles may release slightly faster than PLGA microspheres. 20% paclitaxel-loaded PLGA microparticles could release slightly faster than 10% paclitaxel-loaded samples. The total amount of paclitaxel released seemed to be less than 60% of the total amount of drug in the microparticles.

Representative optical images of C6 glioma cells after being treated by paclitaxel-loaded PLGA microparticles (≈50μm). a, b, c: 250μg/ml 1, 2, 5 day; d, e, f: 1250μg/ml 1, 2, 5 day; g, h, i: 2000μg/ml 1, 2, 5 day.
**Cell viability**


The values of IC50 for Taxol and paclitaxel-loaded microspheres after 5 days are around 14\(\mu\)g/ml and 160\(\mu\)g/ml, respectively. (Actual amount of paclitaxel: 160\(\times\)10\%\(\times\)0.8 = 12.8 \(\mu\)g/ml). IC50 (inhibitory concentration 50\%) represents the concentration of a drug that is required for 50\% inhibition in vitro.
Electrospinning

Schematic of electrospinning setup

Representative image of electrospinning jet with the exposure time of 10ms.
Polymer concentration

- 15%
- 10%
- 5%
- 2%

Tetrabutylammonium tetraphenylborate (TATPB)

Increasing conductivity

Ionic surfactant
Electrospun Micro- and Nano-fibers

SEM images of paclitaxel-loaded PLGA Fibers

Characterization of paclitaxel-loaded fibers

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fibers mean diameter</th>
<th>Drug loading (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA MF (s1)</td>
<td>2.5 ± 0.32 µm</td>
<td>9.9 ± 0.1</td>
<td>99.0 ± 1.0</td>
</tr>
<tr>
<td>PLGA NF (s2)</td>
<td>770 ± 13 nm</td>
<td>9.2 ± 0.03</td>
<td>92.0 ± 0.3</td>
</tr>
</tbody>
</table>

http://www.drugs.com/PDR/Gliadel_Wafer.html
Laser scanning confocal microscopy images
Cell morphology after 72h incubation with different formulations

Blank PLGA nanofibers

10% Paclitaxel-loaded PLGA nanofibers 500µg/ml

10% Paclitaxel-loaded PLGA nanofibers 1000µg/ml

10% Paclitaxel-loaded PLGA nanofibers 2000µg/ml

10× green colour indicates living cells stained with FDA

Electrospinning Micro- and Nano-fibers

Cell viability after 72h incubation with different formulations.

The IC50 value of paclitaxel-loaded PLGA nanofibers was around 1200µg/well.

IC50 of paclitaxel of this formulation was about 36µg/ml, which was comparable to the commercial paclitaxel formulation Taxol® (30µg/ml).

PLGA NF500: 500µg/ml; PLGA NF 1000: 1000µg/ml; PLGA NF 2000: 2000µg/ml.
In-Vovo Experiment Overview: Tumor Volume Response

Balb/c Nude mice

Subcutaneous Inoculation with 1x 10^6 C6 glioma cells

Disc (Surgical Implantation)

Microspheres (Surgical Implantation)

Microparticles (Intra-tumor injection)

After period of tumor growth, Microparticles, Discs or Microspheres are implanted

\[ Tumor\_Vol = \frac{1}{6} \pi ab^2 \]
In-Vivo Experiment Overview: Tumor Volume Response

Representative pictures of tumours after 21 days of C6 glioma cells implantation

Blank Microspheres

10% Taxol loading EHDA Microparticles

Taxol®

20% Taxol loading EHDA Microparticles
Tumor volume response: Paclitaxel EHDA Microparticles

Tumor was allowed to grow for a period of 14 days before 1st injection of microparticles and commercial taxol (for control were administered) directly into the tumor mass.

20% Taxol Loading group had 1 mg taxol administered in two doses of 0.5 mg as injection directly into the tumor on Day 14 & 21.

Significant tumor growth suppression for at least 7 days after first injection (30% in vitro release reached).

Tumor volume is among lowest of treatment (see next slide).

No significant variation of animal weight from controls were observed. Indicating minimal or no systemic toxicity effects.
In-Vivo Experiment Overview: Tumor Volume Response

**In vivo release profile of EHDA microparticles**

14% drug loaded EHDA microparticles

Weight loss observed in control group over first seven days.
No weight loss observed for EHDA group.
Control group showed Paclitaxel cleared from Plasma after 10 days.
Sustained delivery observed for Experimental group for up to 28 days.
The research efforts of this project are to develop various biomedical devices for applications in controlled release of bioactive materials using electrohydrodynamic atomization techniques. Through investigation of the processing parameters during the EHDA process, controllable size and morphology of particles, controllable diameters of fibers and controllable thicknesses of films were successfully achieved.

Electrohydrodynamic atomization (EHDA) is a process, also called electrospray, where a liquid jet breaks up into fine droplets under the influence of electrical forces. With increasing electric forces, different spray modes can be obtained from dripping mode, single cone-jet mode to multiple-cone mode. After the solvent evaporates, polymeric particles can be obtained.

Electrospinning of liquids involves the introduction of electrostatic charges to a stream of polymeric fluid in the presence of strong electric field. After the fluid evaporates, fibers can be formed.

Representative optical images of C6 glioma cells after being treated by different concentrations of paclitaxel-loaded PLGA microparticles.

Tumor volume variation with time.
Nanoparticle fabrication of biodegradable polymers using supercritical antisolvent: Effects of mixing and thermodynamic properties
Nanoparticle fabrication

**Methods of Fabrication**

- Emulsion methods (O/W; W₁/O/W₂)
- Electrohydrodynamic atomization (EHDA) (Micro and nanoparticles)
- Dialysis (nanoparticles)
- Spray drying (Micro particles)
- Supercritical fluid techniques (Micro and nanoparticles)

**Advantages of CO₂**

- “green” solvent, environmentally benign
- Readily available and inexpensive
- Low critical pressure and temperatures

**References**

- **RESS** Rapid Expansion of Supercritical Solutions
  (Debenedetti et al. (1993) Fluid Phase Equilibria 82, 311-321)
- **ASES** Aerosol Solvent Extraction System
  (Bleich et al. (1993) Int. J. Pharma., 97, 111-117)
- **SEDS** Solution Enhanced Dispersion by Supercritical Fluids
- **SAS** Supercritical AntiSolvent
- **SASEM** Supercritical Antisolvent with Enhanced Mass Transfer
Supercritical antisolvent

- Most organic solvents are soluble in supercritical \( \text{CO}_2 \)
- Low critical temperature (31.1 deg C)
  - Suitable for processing thermally labile pharmaceuticals
- Removal of organic solvent from product

- **Capillary nozzle**
  - Small orifice
- **Coaxial nozzles**
  - Assisted jet breakup
  - Enhanced mixing
- **Ultrasonic nozzles**
  - Uniform atomization
  - Enhanced mass transfer in vessel
Hydrodynamics and thermodynamics

- Both hydrodynamics and thermodynamics play a role in influencing the Supercritical antisolvent process for polymer particle formation.
- Diego and coworkers (2005) identified the two regimes of particle formation for precipitation of polymers in the PCA process:
  - Below mixture critical pressure, the solution droplets were obtained and mass transfer takes place between the solution droplets and CO$_2$.
    - Initial size of droplets influences the particle size.
  - Above mixture critical pressure, solution enters the supercritical CO$_2$ as a gaseous plume.
    - Turbulent mixing of solution and CO$_2$.
    - Mass transfer and mixing influence the particle size.
    - Fibers or discrete particle may be obtained.

Diego et al. (2005) Operating regimes and mechanism of particle formation during the precipitation of polymers using the PCA process. *J. Supercritical fluids*, **35**, 147 - 156
Hydrodynamics

Carretier and coworkers (2003) investigated the hydrodynamics of the SAS process for precipitation of PLA particles from methylene chloride (DCM) solution.

Jet formation and breakup at supercritical pressures
- Varying liquid flow rates (0.25 – 3 ml/min)
- Jet breakup length dependent on the spray Reynolds number
- Fibers or microparticles were obtained depending on liquid flow rates

Chattopadhyay and Gupta (2001) developed the supercritical antisolvent with enhanced mass transfer (SASEM) for production of uniform sized nanoparticles.
- Ultrasonic assisted atomization of jet
- Enhanced mass transfer between organic solution and supercritical CO₂ phases

P. Chattopadhyay and R. B. Gupta, Production of griseofulvin nanoparticles using supercritical CO₂ antisolvent with enhanced mass transfer. *Int. J. Pharma*. 228 (2001) 19 – 31
Objectives

Fabrication of nanoparticles of biodegradable polymers
- Controlled release purposes
- Model drug: Paclitaxel (hydrophobic)
- Polymer studied: Poly L lactide (PLA)
- Organic solvent used: Methylene chloride (DCM)

1. Effect of thermodynamic conditions on particle properties
   - Constant operating temperature
     - 35 deg C
   - Varying operating pressure
     - 73.8 bar – 95 bar

2. Effect of hydrodynamics
   - Jet formation in supercritical fluid
     - Jet flow rate

3. Effect of mixing on particle formation
   - Supercritical antisolvent (SAS) process
   - Ultrasonication for mixing with the high pressure vessel
SAS setup
Modified SASEM setup

U1: Ultrasonic system; Branson sonifier and converter, Sonics and Materials probe (3/8” probe tip diameter)
Particle fabrication

1. **Pressurization (CO\textsubscript{2})**
   - High pressure pump was used to deliver liquefied CO\textsubscript{2} to high pressure vessel
   - Temperature in high pressure vessel was maintained using circulating water bath

2. **Spraying (Organic solution jet)**
   - High pressure liquid pump was used to deliver organic solution (Solvent + pharmaceutical) into the high pressure vessel via a capillary nozzle
     - Vertical jet (SAS)
     - Horizontal jet (modified SASEM with ultrasonication)
   - Flowrate may be controlled by HPLC pump

3. **Venting**
   - Organic solvent – CO\textsubscript{2} mixture was vented off to a fume cupboard from the bottom of the vessel
   - Filter frit (0.22\textmu m) was placed at bottom of the vessel to collect particles

4. **Purging**
   - Vessel was purged using fresh CO\textsubscript{2} to remove any remaining organic solvent
SAS setup

Flow rate = 4ml/min
Reynolds number = 292

Jet breakup after entering high pressure CO₂

Jet breakup length is dependent on spray Reynolds number

No external mixing in the high pressure vessel during precipitation
Effect of varying pressure

Experimental conditions

- Solution flow rate was kept constant at 4ml/min
- System temperature was maintained at 35 deg C
- 2% polymer loading in DCM
- Pressure varied from 73.8 to 95 bars

73.8 bars

80 bars
Effect of varying pressure

Smoother surface morphology particles obtained
Particle sizes were highly polydispersed
Effect of varying pressure

Results obtained

- Particle sizes of 5 – 10 \( \mu \)m were obtained
- At 73.8 bars, particles obtained were agglomerated and surfaces were very rough
- At 80 bars and above, spherical particles with little agglomeration were achieved
- As supercritical pressure increases, particle morphology improves
  - For particles obtained at 90 and 95 bars, smooth surface morphology were obtained
- This suggests that as pressure increases, better mass transfer between CO\(_2\) and DCM during the spraying process
  - More rapid precipitation
  - Less agglomeration
Effect of varying solution flowrate

- Temperature: 35 deg C
- Pressure: 90 bars
- 2% polymer loading in DCM
- Solution flowrate of 2, 4, 6 ml/min

2 ml/min

4 ml/min

6 ml/min
Effect of varying solution flowrate

4ml/min

Mean Size: 3.03 μm
SD: 2.25 μm

6ml/min

Mean Size: 2.05 μm
SD: 1.21 μm
Effect of varying flowrate

Results obtained

- At 90 bar and 35 deg C, powdery particles with little/no agglomeration were obtained
- Particles obtained have similar surface morphologies
- Polydispersed particles
  - Clusters of small particles (< 2 μm)
  - Larger particles (2-10 μm)
- Higher liquid flowrate generally decreases the particle size for the larger particles
  - Shorter jet breakup length
  - Better mass transfer between the organic solvent and supercritical CO₂
Modified SASEM setup

Jet breakup is not due to liquid film disintegration from ultrasonic vibrating surface.

Enhanced turbulence and mixing of jet in the high pressure cell due to ultrasonic vibration.

Jet breakup

Turbulence and mixing
Effect of ultrasonication

Comparison of SAS (No external mixing) and modified SASEM
10w/w% paclitaxel loaded PLA particles
2% polymer loading in DCM

No ultrasonication
Polydispersed particles obtained
Mean Size: 4.13 μm
SD: 1.98 μm
Effect of ultrasonication

Particles obtained with ultrasonication

30 µm vibration amplitude
10% of maximum vibration

60 µm vibration amplitude
20% of maximum vibration
Effect of ultrasonication

Particles obtained with ultrasonication

- 90 μm vibration amplitude
- 30% of maximum vibration
- 120 μm vibration amplitude
- 40% of maximum vibration
Particle properties

- Particle size and size distribution tends to decrease as vibration amplitude increases.
- Recovery yield was comparable to conventional spray drying methods.
- Differential scanning calorimetry was performed to determine the crystalline state of particles fabricated using the SAS/modified SASEM setup.
- Encapsulation efficiency and *in vitro* release profile of the particles were determined.
  - EE% of as high as 80% were obtained.
Differential scanning calorimetry analysis

Thermogram analysis is an useful tool to determine whether paclitaxel is molecularly dispersed in the polymer matrix or phase separated as paclitaxel crystals

**DSC analysis**
- Temperature range = 20 – 280 deg C
- Temperature ramp speed = 10 deg C/min
- Nitrogen flow rate = 5ml/min

**Literature values**
- Pure paclitaxel
  - Endothermic peak @ 223.0 deg C
- PLLA
  - Melting point @ 172-178 deg C
Thermogram properties

- Temperature (°C)
- Exotherm (mW)

- Raw paclitaxel
- raw PLA (before SAS process)
- Paclitaxel loaded PLA with ultrasonication
- Blank PLA with ultrasonication
**In vitro release profiles**

Particles suspended in phosphate buffered saline (PBS)
- Placed in shaker bath (120 rpm, 37 deg C)
- Removed at predetermined time intervals
  - Solution was centrifuged and buffer solution was removed
  - Fresh PBS was added
- Paclitaxel content in PBS was extracted and analysed using HPLC
We have successfully fabricated micro and nanoparticles of PLA for potential application in controlled release purposes.

The effect of thermodynamic properties and hydrodynamics on particle formation was investigated in SAS process:

- Varying the operating pressure from 73.8 bar (critical pressure) to 95 bar significantly alters the surface morphology of microparticles obtained.
- Increasing liquid flowrate reduces particle size and size distribution.

The effect of ultrasonic vibration amplitude on particle size and properties was investigated:

- Nanoparticles were obtained using the modified SASEM setup.
- Particle size may be altered by applying different ultrasonic vibration amplitude.
A supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. Supercritical fluids offer favourable gas-like transport properties and liquid-like solubility. Supercritical CO₂ is used to fabricate biodegradable polymeric controlled release devices using Poly L lactide (PLA) and Poly DL lactide-co-glycolide (PLGA). CO₂ was chosen as it has accessible critical temperature (31.1 deg C) and pressure (73.8 Bars), tunable properties near the critical region, is inexpensive, non-flammable, and generally environmentally benign.

The supercritical fluid fabrication techniques employed in our research group include fabrication of microparticles using supercritical antisolvent (SAS) process, and the fabrication of micro-porous polymeric foams using supercritical gas foaming technique. In the supercritical antisolvent process, the jet disintegration process at near critical conditions was investigated. In vitro release profiles and other properties of the controlled release devices were also characterized.

In vitro release of paclitaxel from PLA microparticles

The research efforts of our group are to develop controlled-release drug delivery devices using supercritical fluid techniques for chemotherapy to treat brain and liver cancers. Through investigation of the operating parameters of the SAS and Foaming process, controllable particle size with anticancer drug encapsulation and foams with tailored pore size have been achieved respectively. As such, the drug release profile may be modulated.