SOLID LIPID NANOPARTICLES AS COLLOIDAL DRUG CARRIER SYSTEMS

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MICROPARTICLES AND NANOPARTICLES: THE BASIC CONCEPTS
“The recent tremendous advances in health science technology and inexorable progress in related scientific innovations, coupled with changes in population demographics and the colouring of political agendas with the economics of disease, are all acting in concert to take the pharmaceutical industry into exciting and challenging new dimensions”.

G. Gregoriadis (2002)
DIFFERENT APPROACHES

PHYSICAL APPROACH
• Macroscopic systems activated by physical processes (capsules, granules, etc)

CHEMICAL APPROACH
• Chemical modification of drugs (derivative preparation, salts, pro-drugs, association to polymers, etc.)

BIOCHEMICAL / BIOTECHNOLOGICAL APPROACH
• Naturally occurring macromolecules
• Cells
• Colloidal polymeric or lipid systems

GENERAL CONCEPTS IN MODIFIED DRUG RELEASE

in vitro

in vivo

Drug concentration in serum

Time (hour)
GENERAL CONCEPTS IN MODIFIED DRUG RELEASE

Convencional Release  
Modified Release  
Delayed Release  
Prolonged Release  
Controlled Release  
Drug Targeting

"NEW DRUG DELIVERY SYSTEMS"

SKF  
Spansule

ALZA  
Progestasert  
Oros  
Ocusert
### FORMULATION OF NEW DRUG MOLECULES

#### PROTEIN DRUGS

<table>
<thead>
<tr>
<th>Physicochemical problems</th>
<th>Biological problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight and size</td>
<td>Biodegradation by digestive enzymes</td>
</tr>
<tr>
<td>Complex structure</td>
<td>Short in vivo half-life</td>
</tr>
<tr>
<td>Conformational stability</td>
<td>Immunogenicity</td>
</tr>
<tr>
<td>Solubility</td>
<td>Complex metabolism</td>
</tr>
<tr>
<td>Sensitivity (light, temperature, pH)</td>
<td>Difficulty in crossing mucosal barriers</td>
</tr>
<tr>
<td>Crystallization</td>
<td>No access to some compartments</td>
</tr>
<tr>
<td>Molecularar interactions</td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td></td>
</tr>
<tr>
<td>Aggregation</td>
<td></td>
</tr>
<tr>
<td>Presence of protein impurities</td>
<td></td>
</tr>
</tbody>
</table>

![Pharmacokinetic Problems](image)

![Analytical Problems](image)

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#### PROTEIN DRUGS

- Investigation of alternative administration routes (nasal, rectal, pulmonary, etc.);
- Investigation of alternative prolonged or pulsed release drug delivery systems;
- Investigation of new dosage forms that can provide protection against premature degradation;
- Investigation of new site specific drug delivery systems.
The term refers to a state of subdivision, implying that the molecules or polymolecular particles dispersed in a medium have at least in one direction a dimension roughly between 1 nm and 1 µm, or that in a system discontinuities are found at distances of that order.
### Microparticles
Solid particles made of polymeric or solid lipid materials
Podem conter o fármaco disperso, encapsulado ou adsorvido à superfície.

**Microspheres (≥1 μm)**
- Microcapsules (reservoir systems)
- Microparticles (matrix systems)

**Nanospheres (<1 μm)**
- Nanocapsules (reservoir systems)
- Nanoparticles (matrix systems)

### Polymeric Micro- and Nanoparticulate Systems

**Main Polymers**

Biocompatibility versus Biodegradation

Biodegradation mechanisms: Type I, II e III

- poly(lactide-co-glycolide) (PLGA)
- poly-ε-caprolactone (PCL)
- poly(hydroxibutiric acid) (PHB)
- polyorthoesters
- polyanhydrides
- polyphosfazes
- polyalkylcianoacrylates (PIBCA ou PHCA)
- proteins (albumine, gelatine, gliadines)
- starch
- chitosan
- alginate
POLYMERIC MICRO- AND NANOPARTICULATE SYSTEMS

Biodegradation Mechanisms

Type I

Type II

A B

A C

Type III


POLYMERIC MICRO- AND NANOPARTICULATE SYSTEMS

BIODEGRADABLE IMPLANTS

Poly(lactide-co-glycolide) (PLGA)

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Drug release by polymer enzymatic degradation.
Drug release rate is controlled by the rate of the enzymatic reaction.
Predominant use in controlled release from biodegradable polymers often used in particulate carriers (e.g. albumine, gelatine, polyalklycianoacrylates).

Main Technologic and Therapeutic Uses

- Diagnostic systems
- Controlled drug delivery systems
  - Implants
  - Aerosols
  - Solid dosage forms
  - Vaccines
  - Site-specific drug delivery
### MAIN MICRO / NANOENCAPSULATION TECHNIQUES

<table>
<thead>
<tr>
<th>Technique</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spray coating; Pan-coating, Spray-drying</td>
<td></td>
</tr>
<tr>
<td>2. Droplet extrusion</td>
<td></td>
</tr>
<tr>
<td>3. Coacervation / Phase separation</td>
<td></td>
</tr>
</tbody>
</table>
| 4. Emulsion solidification | 4.1. Solvent evaporation (simple or multiple emulsion)  
4.2. Solvent extraction (simple or multiple emulsion)  
4.3. Melting and solidification (simple or multiple emulsion) |
| 5. Polymerization methods | 5.1. Interfacial polymerization  
5.2. Emulsion polymerization |

### Colloidal Systems

**Aqueous phase** + **surfactant** → **Polymer in organic solvent** → **Drug suspended in polymer solution** → **Drug** → **Evaporation or Extraction of organic solvent** → **Particle isolation by filtration or centrifugation**

### MAIN MICRO / NANOENCAPSULATION TECHNIQUES

#### Solvent Evaporation/Extraction

<table>
<thead>
<tr>
<th>Simple Emulsion</th>
<th>Multiple Emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td>Drug</td>
<td>Drug + water + emulsifying agent</td>
</tr>
<tr>
<td>Aqueous phase + surfactant</td>
<td>Aqueous phase + surfactant</td>
</tr>
<tr>
<td>Polymer in organic solvent</td>
<td>w/o emulsion</td>
</tr>
<tr>
<td>Drug suspended in polymer solution</td>
<td><strong>Evaporation or Extraction of organic solvent</strong></td>
</tr>
</tbody>
</table>

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MAIN MICRO / NANOENCAPSULATION TECHNIQUES

Melting and Solidification (1)

Simple Emulsion

1. Melted lipid
2. Drug
3. Aqueous phase heated at same temperature
4. Drug suspended in melted lipid
5. Simple [A] or multiple [B] emulsion
6. Cooling to solidify disperse phase
7. Particle isolation by filtration or centrifugation

Multiple Emulsion

1. Melted lipid
2. Drug + Water + Emulsifying agent
3. Aqueous phase heated at same temperature
4. W/O emulsion
5. Particle isolation by filtration or centrifugation

MAIN MICRO / NANOENCAPSULATION TECHNIQUES

Melting and Solidification (2)

1. Protein Solution (albumin or gelatine)
2. Drug
3. Emulsion 1
4. Oil
5. Oil at 180°C + surfactant
6. Emulsion 2
7. Cooling
8. Particle isolation by filtration or centrifugation

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**MAIN MICRO / NANOENCAPSULATION TECHNIQUES**

**Emulsion Polymerization**

- A single liquid (or dissolved) reacting monomer
- Drug dissolved or suspended
- Polymerisation started by chemical agent or radiation
- Polymer MW and particle size dependent on:
  - Monomer concentration
  - Initiator concentration
  - Temperature
- Emulsion stabilizer needed
- Particles >20 nm

Ex.: PIBCA; polyacrylamide

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**Droplet Extrusion**

- Gelatine + alginate + drug
- Washing
- Fluid-bed drying

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Ferreira Almeida and Almeida (2004). J Control Release
### MICRO- AND NANOPARTICULATE SYSTEMS

#### CONTROL OF FORMULATIONS

- Particle size
  - Electron microscopy
  - Electric sensing zone
  - Laser diffraction
  - Photon correlation spectroscopy (PCS)
  - Optical sensing zone
- Encapsulation efficiency
- Drug loading
- Surface charge (zeta potential)
- Hydrofobicity
  - Contact angle
  - Partition coefficient
  - Hydrophobic interaction chromatography
- Release profile

![Sterilization](image)

![Stability](image)

### MICRO- AND NANOPARTICULATE SYSTEMS

#### DRUG RELEASE

Dependent on:
- Drug location inside the particle
- Type and amount of polymer
- Particle size and density
- Cross-linking
- Physicochemical properties of drug and polymer
- Drug MW and concentration
- Presence of excipients
- Release medium

![General drug release kinetics](image)
PARTICULATE CARRIERS IN THE PHARMACEUTICAL MARKET

LIPOSOMES
✓ physical and chemical stability problems
✓ no “cheap” liposome available
✓ marketed products but behind expectations

POLYMERIC NANOPARTICLES
✓ input: 30 years of research
✓ no output: no products on the market

SITE-SPECIFIC DRUG DELIVERY
The *magic bullet* concept

The term used to describe a specific cure for syphilis, which would attack the syphilis spirochaete while having no effect whatsoever on human tissue.

Paul Ehrlich (1854-1915)

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**RATIONALE FOR SITE-SPECIFIC DRUG DELIVERY**

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Translocation across biological barriers</th>
<th>Protection against inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target site</td>
<td>Carrier</td>
<td>Drug</td>
</tr>
<tr>
<td>Other sites</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Puisieux and Roblot-Treupel (1989) STP Pharma
**RATIONALE FOR SITE-SPECIFIC DRUG DELIVERY**

- Exclusive delivery to specific compartments (and/or diseases)
- Access to previously inaccessible sites (e.g. intracellular infections)
- Protection of drug and body from unwanted deposition, which could lead to unwanted reactions and metabolism, etc.
- Controlled rate and modality of delivery to pharmacological receptor
- Reduction in the amount of drug employed

↑ Drug safety  
↑ Drug efficacy  
↑ Patient compliance

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**SITE-SPECIFIC DRUG DELIVERY: DRUG TARGETING**

### PASSIVE TARGETING

- Uptake by MPS and tropism for the lysosomes  
  - macrophages  
  - Kupffer cells
- Translocation to the tissues  
  - hepatocytes  
  - bone marrow  
  - splenocytes  
  - tumors (?)
- Capillary embolism  
  - lung targeting (iv)  
  - specific regions (ia)

### ACTIVE TARGETING

- Magnetic fields  
- Lymphotropic adjuvants  
- MPS blocking  
- Receptor mediated processes  
- Immunotherapy

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DRUG TARGETING

- Soluble carriers (monoclonal antibodies, dextran, soluble synthetic polymers)
- Particulate carriers (liposomes, microspheres, nanoparticles, etc.)
- Target-specific recognition moieties (monoclonal antibodies, carbohydrates)
- Antibody-directed enzyme/prodrug therapy
- Virus-directed enzyme/prodrug therapy

Utilização de lipossomas como transportadores de fármacos

MAIN DRUG TARGETING SYSTEMS

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Crommelin et al. (http://www.drugdeliverypartnerships.com/lip.html)

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## SOLID LIPID NANOPARTICLES

- Lipophilic colloidal delivery system (R.H. Müller, Berlin; M.R. Gasco, Turin);
- Efficient and non-toxic drug carrier specially for lipophilic drug molecules;
- Composed of physiological / well tolerated excipients e.g. GRAS (= similar to emulsions and liposomes);
- Possess solid matrix (= similar to polymeric nanoparticles);
  - Protective properties
  - Controlled release properties
- Colloidal dimensions and controlled release behaviour enable drug protection and administration by parenteral and non-parenteral routes.
## LIPID NANOPARTICLES

- Versatility
  - Parenteral administration (Fundarò et al., 2000)
  - Brain delivery (Fundarò et al., 2000)
  - Ocular delivery (Cavalli et al., 2002)
  - Rectal delivery (Sznitowska et al., 2001)
  - Oral delivery (García-Fuentes et al., 2002)
  - Topical delivery (Souto et al., 2004)
- Potential vaccine delivery systems (Almeida et al., 1997)
- Large scale production possible using lines available in pharmaceutical plants (i.e. lines for parenteral emulsions (Müller et al., 2001)

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## SLN PREPARATION

- **High Pressure Homogenization** (R.H. Müller et al.)
  - SkyePharma PLC, Berlin, Germany
  - PharmaSol GmbH, Berlin, Germany

- **Microemulsion Technology** (M.R. Gasco)
  - Vectorpharma/Eurand Spa, Turin, Italy

- **Solvent Evaporation**

- **Phase Inversion Type**
SLN PREPARATION BY HIGH PRESSURE HOMOGENIZATION (HPH)

HOT HOMOGENIZATION PROCESS

A
Melted Lipid
Drug
Aqueous phase at same temperature
Drug solution in melted lipid
Emulsification using Ultra-turrax single [A] or double [B]
High-Pressure Homogenisation
Cooling for solidification and particle hardening
SLN suspension

B
Melted Lipid
Emulsion w/o
Aqueous phase at same temperature
Drug + Water + surfactant

APV Gaulin LAB40 discontinuous (40 g batch)

HIGH PRESSURE HOMOGENIZATION (HPH)

Dept. Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin
SLN PREPARATION BY HPH

HOT HOMOGENIZATION PROCESS

Basic principles:

✓ equipment can be qualified and validated
✓ accepted by regulatory authorities in production lines used for parenterals
✓ existing industrial production lines for i.v. parenteral emulsions can be used
✓ all production lines developed for SLN are usable

 Dept. Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin
**SLN PREPARATION BY HPH**

**COLD HOMOGENIZATION PROCESS**

- Melted Lipid
  - Drug
  - Drug suspension in melted lipid
  - Liquid nitrogen
  - Micronization
  - Suspension in water using Ultra-turrax
    - High-Pressure Homogenisation
    - SLN suspension

**DRUG ENTRAPMENT INTO SLN**

- **A**
  - m.p. drug ≈ m.p. lipid → homogeneous matrix

- **B**
  - m.p. drug < m.p. lipid → lipid-enriched core

- **C**
  - m.p. drug > m.p. lipid → drug-enriched core
DRUG ENTRAPMENT INTO SLN

PROBLEMS ASSOCIATED WITH SLN
Formation of “perfect” crystalline structure during storage ($\beta$ modification) $\Rightarrow$ drug expulsion

SLN PROCESSING DISADVANTAGES

- Physical stability of aqueous solution
- Gel formation
- Particle aggregation
- High water content of dispersions (70 - 95%)
- Need to remove too much water in tablet / pellet production
- Dosing problems (e.g. dispersion for soft gelatin capsules, lipid particle content max. 30%)
# SLN PROCESSING DISADVANTAGES

<table>
<thead>
<tr>
<th>SLN – Solid Lipid Nanoparticles</th>
<th>![Solid lipid particle]</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Produced from solid lipids</td>
<td></td>
</tr>
<tr>
<td>✓ Tend to form perfect crystals</td>
<td>⇒ drug expulsion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NLC – Nanostructured Lipid Carriers</th>
<th>![Nanostructured lipid carriers]</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Produced from blend of solid and liquid lipids</td>
<td></td>
</tr>
<tr>
<td>✓ Particles are in solid state at body temperature</td>
<td></td>
</tr>
<tr>
<td>✓ Inhibit crystallization process by mixing “spatially” very different molecules ⇒ imperfections in lattice</td>
<td></td>
</tr>
</tbody>
</table>

- not “just mixing” solid lipids but:
  - controlled “nanostructuring” of lipid matrix
    - to accommodate drug
    - to control release
    - to trigger release

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# LIPID NANOPARTICLES

<table>
<thead>
<tr>
<th>NLC – a “multiple” carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN: one phase (= solid lipid)</td>
</tr>
<tr>
<td>NLC: multiple O/F particle (oil nanodroplets in solid fat nanoparticles)</td>
</tr>
</tbody>
</table>

**Advantages:**

- higher drug load, firm incorporation (e.g. 1% Retinol in SLN, 6% in NLC)
LN AS A POTENTIAL PROTEIN / ANTIGEN DELIVERY SYSTEM

- **Lipid composition**
  - Hard fats (Softisan 142® and Witepsol® E85)
  - Cetyl alcohol
  - Propylene glycol palmitostearate (Monosteol®)
  - PEG 300 mono-, di-stearate (Superpolystate®)

- **Surfactant**
  - Poloxamer 188
  - Tween 80

PROTEIN NANOENCAPSULATION IN SLN

- Lipid + poloxamer 188 melting
- Solidification in liquid nitrogen
- Micronisation in a powder-mill
- Pre-mix using a high-speed homogeniser
- HPH at room temperature

STABILITY OF LYSOZYME THROUGHOUT FORMULATION

Influence of temperature (50°C)

Influence of HPH treatment (N° cycles/Pressure)

MW Water     S/CA  W/CA    Mono  Sup

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NANOENCAPSULATION OF LYSOZYME IN LN

Lysozyme activity (Units/g of lipid)

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NANOENCAPSULATION OF LYSOZYME IN LN

#### Lipid matrix

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Lys Content (%)</th>
<th>Particle Size Laser diffraction (µm ±sd)</th>
<th>Particle Size PCS (nm ±sd)</th>
<th>Zeta Potential (mV ±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S: 142/CA</td>
<td>0.22</td>
<td>D50 0.60±0.00</td>
<td>644±12.9</td>
<td>-11.0±0.2</td>
</tr>
<tr>
<td>W: E85/CA</td>
<td>0.30</td>
<td>D50 0.58±0.00</td>
<td>549±7.0</td>
<td>-9.8±0.7</td>
</tr>
</tbody>
</table>

#### DEVELOPMENT OF OVA-CONTAINING LN

- **Lipid composition**
  - gliceryl behenate (Compritol® 888 ATO)
  - gliceryl palmitostearate (Precirol® ATO 5)
  - cetyl Alcohol
- **Surfactant**
  - Tween 80
- **Speed rate**
  - 8 000 - 10 000 rpm

Particle size (PCS)
- 100-400 nm
- PI - 0.250

Videira and Almeida (1998), Proceed III SPLC-CRS Conf
NANOPARTICLE CHARACTERISATION: PHYSICAL STABILITY

<table>
<thead>
<tr>
<th>Lipid (%)</th>
<th>SLN (nm)</th>
<th>OVA –SLN (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d_{m,3d}$</td>
<td>$d_{m,30d}$</td>
</tr>
<tr>
<td>6 Pr</td>
<td>103</td>
<td>115</td>
</tr>
<tr>
<td>6 Cp</td>
<td>171</td>
<td>168</td>
</tr>
<tr>
<td>W/AC</td>
<td>284</td>
<td>290</td>
</tr>
<tr>
<td>8 Pr</td>
<td>196</td>
<td>205</td>
</tr>
<tr>
<td>8 Cp</td>
<td>240</td>
<td>253</td>
</tr>
<tr>
<td>W/AC</td>
<td>252</td>
<td>265</td>
</tr>
<tr>
<td>10 Pr</td>
<td>230</td>
<td>200</td>
</tr>
<tr>
<td>10 Cp</td>
<td>373</td>
<td>378</td>
</tr>
<tr>
<td>W/AC</td>
<td>307</td>
<td>289</td>
</tr>
</tbody>
</table>

ENTRAPMENT EFFICIENCY

OVA-6SLN 92% EE with 2% surfactant
FORMULATION STUDIES OF OVA OF LYSOZYME IN LN

![Graph showing protein integrity over time]

**Protein Integrity**

<table>
<thead>
<tr>
<th>MW</th>
<th>LNsup</th>
<th>1W</th>
<th>2W</th>
<th>3W</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.0 KDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66.2 KDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.0 KDa</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>31.0 KDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.5 KDa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.4 KDa</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Time (day)**

0 5 10 15 20 25 30 35

**FORMULATION STUDIES OF OVA OF LYSOZYME IN LN**

Videira et al. (2002). Proceed V SPLC-CRS Conf

ADSORPTION STUDIES

<table>
<thead>
<tr>
<th>OVA/lipid (%)</th>
<th>4</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>90</td>
<td>80</td>
<td>82</td>
</tr>
<tr>
<td>15°C</td>
<td>80</td>
<td>70</td>
<td>79</td>
</tr>
</tbody>
</table>

![Graph showing desorption profiles]

**Desorption profiles**

- 25°C
- 15°C

**Time (min)**

0 250 500 750 1000 1250 1500

**Adsorption Studies**

Videira et al. (2002). Proceed V SPLC-CRS Conf
LN AS A POTENTIAL PROTEIN / ANTIGEN DELIVERY SYSTEM

- LN are potential protein carriers with high protein binding
- Formulations are stable
- Partial adsorption of protein may not be precluded
- Production conditions seem not to damage protein integrity
- Following a burst effect, a sustained release profile is obtained
- Loading capacity is suitable to the usual antigen doses

PULMONARY DELIVERY OF SLN
PULMONARY ADMINISTRATION

The Lung

Unique features that can facilitate systemic delivery via pulmonary administration of drugs:

- Large surface area (~75 m²).
- Good vascularisation.
- Large capacity for solute exchange.
- Ultra-thinness of the alveolar epithelium (0.1-0.5 mm).
- First-pass metabolism is avoided.

A.J. Almeida, 2007

PULMONARY DELIVERY USING PARTICULATE CARRIERS

Advantages

- An alternative non-invasive means for both local and systemic drug delivery using particulate carriers.
- Avoids instability in blood stream and uptake by the MPS.
- Allows high concentrations of drug in the lungs minimizing side toxic effects.
- A potential route for drug therapy and immunisation.

A.J. Almeida, 2007

LYMPHATIC TARGETING AND LUNG CANCER THERAPY

• A leading cause of death around the world, accounting for ≈13% of newly diagnosed cancers and ≈30% of all cancer deaths.

• Very low survival rate (≤14% in 5 years; mean survival time 14 months).

• Metastatic disease starts at very early stages, particularly in SCLC, the majority of patients with lung cancer having metastatic disease at the time of diagnosis.

• Metastasis spread mainly via the lymphatics to the lymph nodes, liver, brain, bones and adrenal glands.

• Early diagnosis is crucial to increase survival rate and staging is critically dependent upon the involvement of the lymph nodes that drain the region containing the tumour.

• Chemotherapy plays an important role alongside surgery and radiation therapy, but lung cancer is usually diagnosed at an incurable stage.

IMAGING USING PARTICULATE CARRIERS

• Lung perfusion imaging is based on the trapping of i.v. injected radiolabelled albumin microspheres (10-50 μm) in the capillary bed of the lung: Technetium [99m Tc] Microspheres Injection (Ph Eur).

• I.v. injected radiolabelled PLGA microspheres have also been proposed for lung imaging (Delgado et al. 2000).

• Several particulate carriers have been used for lymphoscintigraphy, such as liposomes, sulfur colloid, PMMA and PHCA nanoparticles.
PULMONARY ADMINISTRATION OF LIPID NANOPARTICLES

Lipid Nanoparticles
- Administration have been studied by parenteral and non-parenteral routes.

- To investigate LN as a potential drug carrier to the lungs and, through alveolar airways, to the lymphatic system, thus optimising concentration at the tumour site or at distant metastasis sites.

- To evaluate LN in vivo fate after pulmonary absorption upon nebulisation and delivery to laboratory animals.

NANOPARTICLE PRODUCTION

Lipid
- Lipid composition
  - gliceryl behenate (Compritol® 888 ATO)
  - gliceryl palmitostearate (Precirol® ATO 5)
  - cetyl Alcohol
- Surfactant
  - Tween 80
- Speed rate
  - 8 000 - 10 000 rpm

Aq. phase
- Melting
- Heating
- Emulsification
- High-speed homogenization
  - 8 000 - 10 000 rpm
- Solidification

Videira et al (2002), J Drug Targeting
LABELLING AND ADMINISTRATION

\[
\begin{align*}
\text{D,L-hexamethypropyleneamine oxime (HMPAO)} & \rightarrow \text{Lipid Nanoparticles} \rightarrow \text{Radiolabelled LN} \\
\gamma - \text{Scintigraphy} & \rightarrow \text{Labelling efficiency} \\
\text{Ultrasonic nebulization} & \rightarrow \text{Particle size} \\
\text{anaesthetised male Wistar rats} &
\end{align*}
\]

CHARACTERISATION OF 99mTc-HMPAO-LN

<table>
<thead>
<tr>
<th>ASPECT</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>PARTICLE SIZE</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Before aerosolisation</th>
<th>After aerosolisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_{50}) (nm)</td>
<td>PI</td>
</tr>
<tr>
<td>(D_{50}) (nm)</td>
<td>PI</td>
</tr>
<tr>
<td>197.5</td>
<td>0.231</td>
</tr>
<tr>
<td>218.3</td>
<td>0.378</td>
</tr>
<tr>
<td>194.8</td>
<td>0.291</td>
</tr>
<tr>
<td>220.3</td>
<td>0.379</td>
</tr>
<tr>
<td>196.1</td>
<td>0.261</td>
</tr>
<tr>
<td>219.3</td>
<td>0.379</td>
</tr>
</tbody>
</table>
CHARACTERISATION OF $^{99m}$Tc-HMPAO-LN

Radiochemical Purity (TLC)

Labelling efficiency: $97\pm 2\%$

$^{99m}$Tc-HMPAO

| 10' | 130' |
|-----|-----|-----|
| Sys I | Sys II | Sys I | Sys II |

butanone 0.9% aq. NaCl

$^{99m}$Tc-HMPAO-LN

| 10' | 130' |
|-----|-----|-----|
| Sys I | Sys II | Sys I | Sys II |

butanone 0.9% aq. NaCl

Other hydrophilic species

$^{99m}$Tc-HMPAO

Reduced-hydrolysed $^{99m}$Tc

Na $^{99m}$TcO$_4$

Videira et al (2002), J Drug Targeting

BIODISTRIBUTION

A.J. Almeida, 2007
**BIODISTRIBUTION**

- $^{99m}$Tc-HMPAO
- $^{99m}$Tc-HMPAO-LN

**LYMPHATIC DISTRIBUTION OF $^{99m}$Tc-HMPAO-LN**
LYMPHATIC DISTRIBUTION

A.J. Almeida, 2007


BIODISTRIBUTION IN RATS 4h AFTER INHALATION

A.J. Almeida, 2007

BIODISTRIBUTION OF $^{99m}$Tc-HMPAO IN RATS AFTER i.v. INJECTION

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Dose in Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min</td>
</tr>
<tr>
<td>Brain</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Blood</td>
<td>11.0±0.4</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>Kidneys</td>
<td>6.5±1.1</td>
</tr>
<tr>
<td>Intestines</td>
<td>15.4±1.7</td>
</tr>
<tr>
<td>Liver</td>
<td>13.9±1.9</td>
</tr>
<tr>
<td>Urine</td>
<td>0.4±0.4</td>
</tr>
</tbody>
</table>

Radioactivity in Rat Lungs after Endotracheal Administration

(n=4; mean±sd)
LYMPHATIC DISTRIBUTION IN RATS AFTER ENDOTRACHEAL ADMINISTRATION

99mTc-HMPAO-LN

(n=4; mean±sd)

LYMPHATIC DISTRIBUTION IN RATS AFTER ENDOTRACHEAL ADMINISTRATION

99mTc-HMPAO

(n=4; mean±sd)
ALVEOLAR CLEARANCE

Uptake of 1.1 μm fluorescent polystyrene particles administered i.n. to mice
Eyles et al (2001). Vaccine

Alveolar clearance of 400 nm fluorescent polystyrene particles administered i.n. to mice

PARTICLE UPTAKE AT THE LUNGS

Possible Mechanisms
- Dissolution
- Macrophage phagocytosis
- Direct passage
- Uptake by vascular system
- Movement into the lymphatic system
- Axonal translocation to CNS

Elimination
- Mucociliary escalator
- Exhaled air

Valentine and Kennedy (2001) In: Principles and Methods of Toxicology
TREATMENT OF BREAST CANCER LUNG METASTASIS

PRELIMINARY STUDY

% mice metastised per group

- non-treated
- LN-paclitaxel aero (last 15 days)
- Taxol® i.v. (last 15 days)
- LN aero control
- LN-paclitaxel aero (first 15 days)
- Taxol® i.v. (first 15 days)
- LN iv control

LIPID NANOPARTICLES AS TOPICAL DELIVERY SYSTEMS
LIPID NANOPARTICLES AS TOPICAL DELIVERY SYSTEMS

BACKGROUND

- Topical application of LN have been used with promising results either for therapeutic or cosmetic purposes (Jenning et al. 2000; Müller et al. 2002).
- SLN have shown some protective activity on skin surface, such as a UV-blocking potential (Wissing et al. 2003).
- SLN may be formulated in creams, gels, sprays.
- SLN allow modulated drug release.

MECHANISMS OF UV PROTECTION

Background:

- Topical application of SLN have been used for therapeutic or cosmetic purposes.
- SLN act as an efficient particulate UV blocker (scattering effect) SLN and molecular sunscreens have synergistic effect.
- Reduced release of sunscreens from SLN when compared to emulsions.
- Reduced side effects.
- SLN may be formulated in creams, gels, sprays, allowing modulated drug release.

UV Particulate sunscreen

UV

Molecular sunscreen

heat, light

Is it so?
NANOPARTICLE PRODUCTION

- **Lipid composition**
  - Gliceryl behenate (Compritol® 888 ATO)
  - Gliceryl palmitosterarate (Precirol® ATO 5)

- **Surfactant**
  - Tween 80

- **Speed rate**
  - 8,000 - 10,000 rpm

- **Model Drugs**
  - Octylmethoxycynamate (OMC)

NANOPARTICLE CHARACTERISATION: PHYSICAL STABILITY

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Time (months)</th>
<th>Particle size (nm)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (empty)</td>
<td>1</td>
<td>147,0 174,3 206,6</td>
<td>0.516</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>80,4 320,4 320,4</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>102,2 201,8 201,8</td>
<td>0.529</td>
</tr>
<tr>
<td>C-OMC</td>
<td>1</td>
<td>16,5 83,6 105,3</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>33,9 41,4 41,4</td>
<td>0.443</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>14,9 49,5 49,5</td>
<td>0.418</td>
</tr>
<tr>
<td>P (empty)</td>
<td>1</td>
<td>221,6 247,3 247,3</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>269,4 269,4 269,4</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>289,1 289,1 289,1</td>
<td>0.614</td>
</tr>
<tr>
<td>P-OMC</td>
<td>1</td>
<td>144,7 203,3 243,0</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59,3 139,8 186</td>
<td>0.589</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>87,2 202,4 268</td>
<td>0.586</td>
</tr>
</tbody>
</table>
**OMC RELEASE STUDIES**

Membrane: Silicone  
Receiving medium: 2% albumin in phosphate buffer (pH=7.4)

**IN VITRO PERCUTANEOUS ABSORPTION OF OMC**

Membrane: Human skin  
Receiving medium: 2% albumin in phosphate buffer (pH=7.4)
IN VIVO STUDIES

- 16 female volunteers (24 to 54 years old; phototypes II and III)
- 10-day application trial on the antero-external surface of both legs
- 3 different areas studied corresponding:
  - LN-OMC formulation
  - LN blank formulation
  - non-treated area (1 negative control per leg).
- Experimental challenge: controlled exposure of volunteers to a UV radiation source (Sunny HB-406 Solarium, Phillips)
- Parameter measured: skin colorimetry (erythema and melanisation indexes), TEWL and elasticity.

IN VIVO RESULTS

Erythema

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>LN</th>
<th>Neg. Cont.</th>
<th>LN-OMC</th>
<th>Neg. Cont.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>5</td>
<td></td>
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<tr>
<td>7</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
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</table>

Melanisation

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>LN</th>
<th>Neg. Cont.</th>
<th>LN-OMC</th>
<th>Neg. Cont.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
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<tr>
<td>10</td>
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MARKETED PRODUCTS

Nanobase®
Yamanouchi/Poland

Cutanova®
Dr. Rimpler GmbH/Germany

PRODUCTION OF LIPID MICRO- OR NANOPARTICLES USING SUPERCRITICAL FLUIDS
**AIM**

Production of drug-lipid micro- and nanoparticles by a PGSS (particles from gas saturated solutions) process, as an alternative method.

**MAIN ADVANTAGES**
- Avoid the use of solvents
- Particles are obtained as a dry powders, instead of suspensions
- Mild pressure and temperature conditions

---

**LIPID MICROPARTICLES USING SUPERCritical FLUIDS**

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>Pressure</th>
<th>Model drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripalmitin</td>
<td>120-180 Bar</td>
<td>theophiline</td>
</tr>
<tr>
<td>Temperature</td>
<td>&lt; 60°C</td>
<td></td>
</tr>
</tbody>
</table>

**PGSS System**

- (A) CO₂ supply cylinder
- (B) Gas compressor
- (C) CO₂ storage cylinder
- (D) Mixing cell
- (E) Collecting chamber
**LIPI D MICROPARTICLES USING SUPERCRITICAL FLUIDS**

![Image 1](image1.png)  
![Image 2](image2.png)

**LYSOZYME-CONTAINING LN PREPARED USING SUPERCRITICAL CO₂**

**Lysozyme Biological Activity**

Lysozyme particles produced from solutions with an ethanol mole fraction of C = 0.28 showed no significant loss of activity.

![Image 3](image3.png)
**CONCLUSIONS (I)**

- LN show suitable properties to become one of the most versatile nanoparticulate carriers for drug and protein delivery.

- Several drug incorporation techniques are applicable, including protein adsorption.

- Inhalation may be an effective route to deliver LN, representing an alternative to the intraperitoneal route for targeting colloidal carriers to the lymphatics.

- LN present lymphatic tropism, thus providing the possibility of using radiolabelled LN as a lymphoscintigraphic agent, and allowing the direct delivery of cytotoxic drugs to lung cancer in limited or extensive stage.

- The limited amount of particle retention in lungs may allow the use of LN as a sustained release formulation in chronic lung therapy.

- LN show an effective control release of lipophilic drugs, which is suitable for percutaneous absorption, with a minimum permeation thus making LN a safe vehicle for sunscreen agents.

---

**CONCLUSIONS (II)**

- *In vivo* results do not confirm early reports, i.e. LN on their own no not improve significantly TEWL and elasticity (results not shown).

- Melanisation index was the only parameter that showed a trend, demonstrating that LN present some protective capacity against UV radiation *in vivo*.

- SCF techniques can be successfully applied to the formulation of solid lipid micro- and nanoparticles with controlled-release characteristics.

- Using a modified SCF technique, it is possible to produce LN containing intact and biologically active protein molecules.
<table>
<thead>
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<th>ACKNOWLEDGEMENTS</th>
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<tr>
<td>Faculdade de Farmácia, Lisboa</td>
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<tr>
<td>Ana Cerdeira</td>
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<td>Ana Filipa Azevedo</td>
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<td>Helena Florindo</td>
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<tr>
<td>Jorge Galhardas</td>
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<td>Jorge Mota</td>
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<td>José A. G. Morais</td>
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<td>Luís F. Gouveia</td>
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<td>Luís M. Rodrigues</td>
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<td>Mafalda Videira</td>
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<tr>
<td>Paulo Ferreira Almeida</td>
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<tr>
<td>Freie Universität, Berlin</td>
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<td>Rainer H. Müller</td>
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<tr>
<td>Stephan Runge</td>
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<td>Ana Cristina Santos</td>
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<td>J.J. Pedroso de Lima</td>
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<td>Institut de Recerca Oncològica, Barcelona</td>
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A.J. Almeida, 2007
MODERN LISBON

Thank you for your attention!

A.J. Almeida, 2007