



NANOLIPOSOMES: THE WHAT, WHY, AND HOW

Jeffrey L. Atkinson, Ph.D.

12 December 2019

1

Introduction (EPR) Effect

Education



UAB THE UNIVERSITY OF
ALABAMA AT BIRMINGHAM

Family



Professional Experience

- 23 years in pharmaceutical development and manufacturing of complex parenteral formulations



- Formulation technologies: polymeric microparticles and nanoparticles, hydrophobic salts, liposomes, micelles, solid lipid nanoparticles

Fun



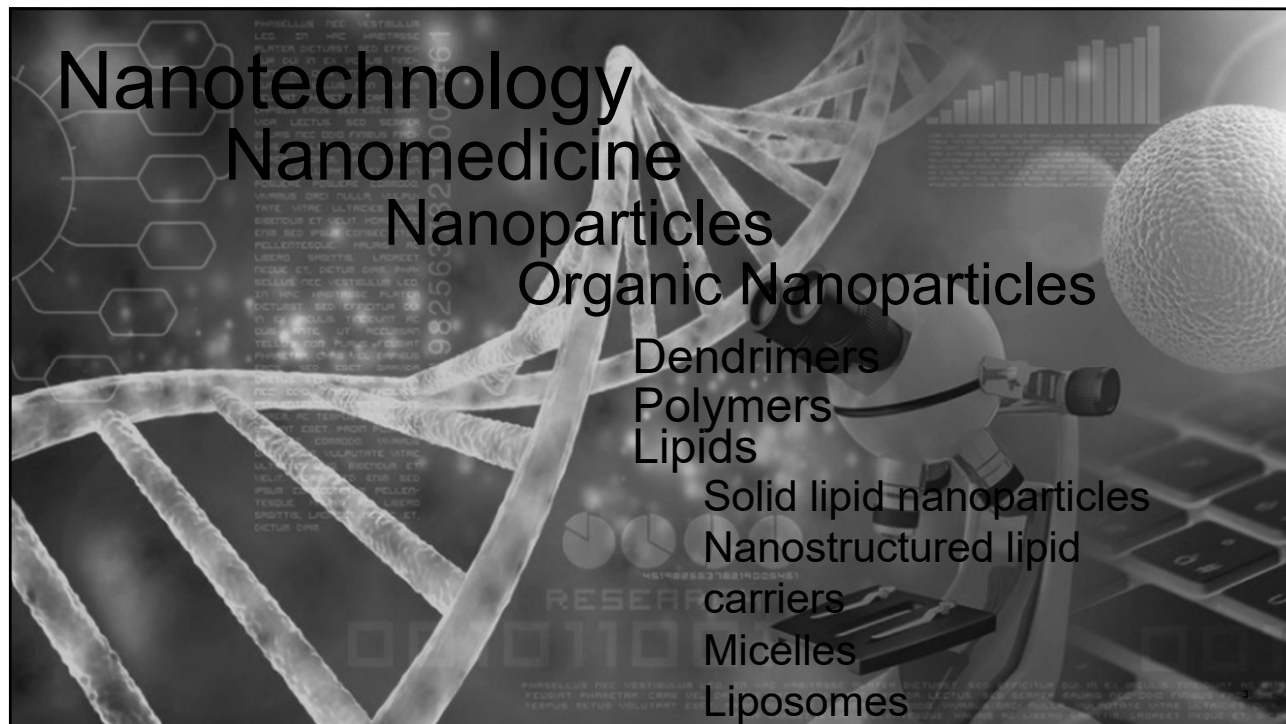
Connecting

Pharmaceutical

Knowledge

ispe.org | 2

2



3

Liposome

Greek *Lipos* “fat” • *Soma* “body”

A structure consisting of one or more concentric spheres consisting of lipid bilayers surrounding an aqueous compartment. Drug molecules are entrapped in the aqueous cavity (hydrophilic drugs) or intercalated into the lipid bilayer (lipophilic drugs). PEG is often incorporated to extend circulation time. Targeting agents may be conjugated to the outside of the bilayer.

Aqueous core
Hydrophobic tail
Hydrophilic head

ISPE
Connecting Pharmaceutical Knowledge
ispe.org | 4

4

| Product | Approval year | Active Agent | Indication |
|-------------|---------------|---|---|
| Epaxal® | 1993 | Inactivated hepatitis A virus (strain RGSB) | Hepatitis A |
| Doxil® | 1995 | Doxorubicin | Ovarian, breast cancer, Kaposi's sarcoma |
| Abelcet® | 1995 | Amphotericin B | Invasive severe fungal infections |
| DaunoXome® | 1996 | Daunorubicin | AIDS-related Kaposi's sarcoma |
| Amphotec® | 1996 | Amphotericin B | Severe fungal infections |
| Ambisome® | 1997 | Amphotericin B | Presumed fungal infections |
| Inflexal V® | 1997 | Inactivated hemagglutinine of Influenza virus strains A and B | Influenza |
| Depocyt® | 1999 | Cytarabine/Ara-C | Neoplastic meningitis |
| Myocet® | 2000 | Doxorubicin | Combination therapy with cyclophosphamide in metastatic breast cancer |
| Visudyne® | 2000 | Verteporfin | Choroidal neovascularization |
| Mepact® | 2004 | Mifamurtide | High-grade, resectable, non-metastatic osteosarcoma |
| DepoDur® | 2004 | Morphine sulfate | Pain management |
| Exparel® | 2011 | Bupivacaine | Pain management |
| Marqibo® | 2012 | Vincristine | Acute lymphoblastic leukemia |
| Onivyde™ | 2015 | Irinotecan | Combination therapy with fluorouracil and leucovorin in metastatic adenocarcinoma of the pancreas |

Approved Liposomal Products



Connecting Pharmaceutical Knowledge

5

ispe.org |

5

Why use
liposomes
for drug
delivery?

- Targeting capabilities
- Improvement of efficacy
- Decreased amount of drug required
- Lower toxicity
- Reduction of side effects
- Protection of drugs (stability and reduced elimination)
- Increase circulation time
- Applicable for both hydrophilic and hydrophobic drugs
- Biocompatible, biodegradable, and non-toxic
- Large body of knowledge publicly available

6

6

The diagram illustrates four types of liposomes and their drug delivery mechanisms:

- (I) Liposome (SUV):** A single unilamellar vesicle. A box indicates: "Drug may be associated with the membrane or aqueous core".
- (II) MLV:** Multiple concentric bilayers.
- (III) Sterically stabilized liposome:** Features a "Stealth layer" of "Long-circulating liposome grafted with a hydrophilic polymer such as PEG".
- (IV) Liposome targeting:** Includes "Targeting ligand (Δ)" which can be "Antibody Y", "Carbohydrate", or "Small molecule". A box states: "Targeting ligand may be: • Directly attached to the liposome surface, or • Attached to the distal tips of the grafted PEG chains".

Source: <https://basicmedicalkey.com/nanotechnologies-for-drug-delivery-and-targeting-opportunities-and-obstacles/>

ISPE Connecting Pharmaceutical Knowledge

Achieving Selective Localization of the Active at Disease Sites

- **Passive Targeting** – Drug carriers that stay in circulation for extended periods of time accumulate in pathological sites with affected and leaky vasculature via the enhanced permeability effect.
- **Active Targeting** – Use of specific ligands attached to the surface of the carrier that recognize and bind to pathological cells.

7

The diagram illustrates the EPR effect across different tissue layers:

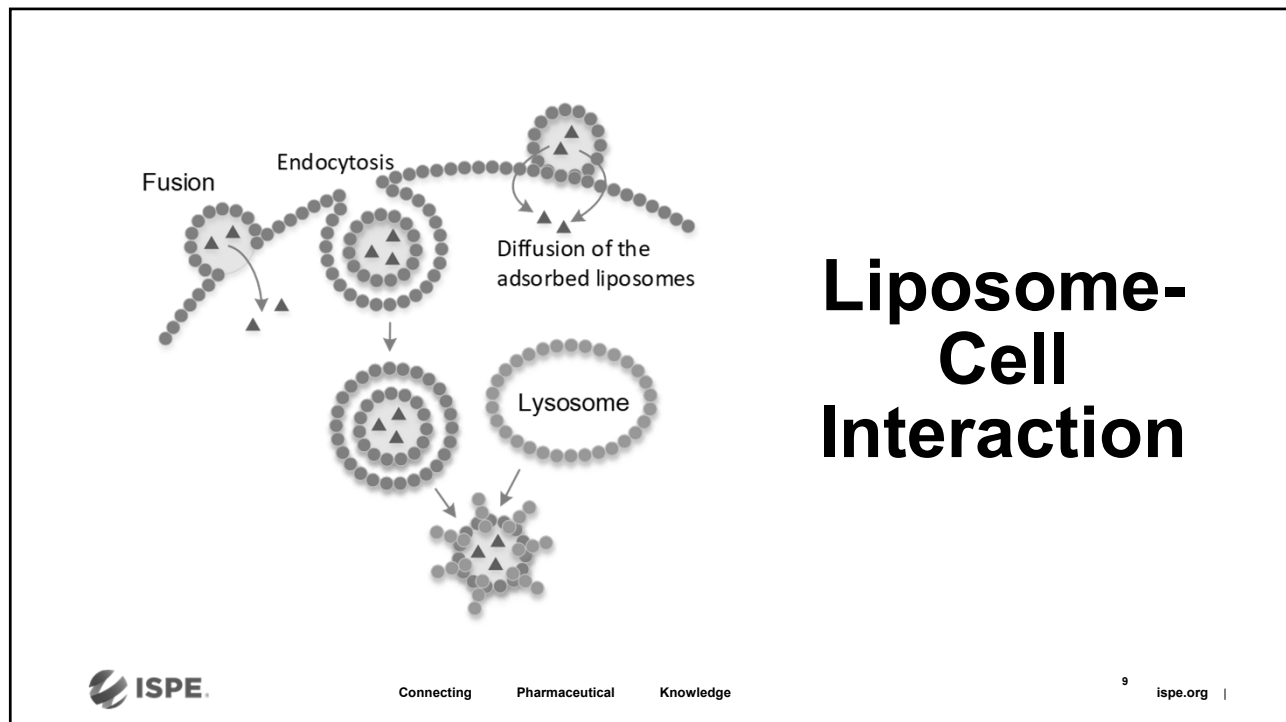
- Healthy tissue:** Shows a tight barrier of cells.
- Lymphatic system:** A vessel for fluid drainage.
- Blood vessel:** A vessel containing nanoparticles.
- Endothelial cells:** The lining of the blood vessel.
- Nanoparticle:** Shown leaking out of the blood vessel into the tumor tissue.
- Tumor tissue:** Shows a leaky vasculature where nanoparticles accumulate.

Credit: C&EN


ISPE Connecting Pharmaceutical Knowledge

Enhanced Permeability and Retention (EPR) Effect

8



9



Methods of Preparation

- Mechanical Agitation
- Solvent Dispersion
- Surfactant Solubilization

Drug Loading

- Passive Loading
- Remote Loading

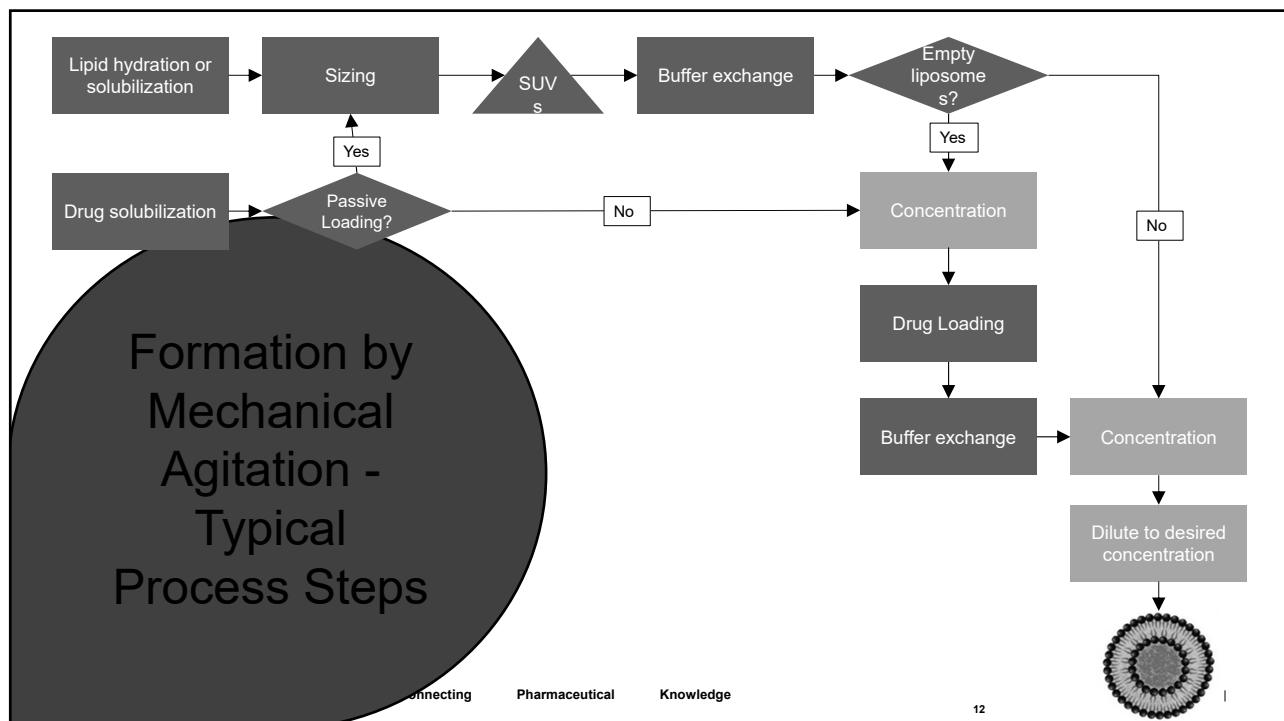
10

10

| Surfactant Solubilization | Solvent Dispersion | |
|--|--|---|
| <ul style="list-style-type: none"> ❑ Formation of mixed micelles using surfactants ❑ Removal of surfactant (dialysis, diafiltration) <p><u>Advantages</u></p> <ul style="list-style-type: none"> ❑ Size control <p><u>Disadvantages</u></p> <ul style="list-style-type: none"> ❑ Low encapsulation of water-soluble molecules ❑ Residual surfactant | <ul style="list-style-type: none"> ❑ Ether Injection <ul style="list-style-type: none"> ▪ Slow addition dissolved lipids into aqueous solution through small orifice ▪ Solvent removal <p><u>Advantages</u></p> <ul style="list-style-type: none"> ❑ High encapsulation ❑ Continuous processing possible <p><u>Disadvantages</u></p> <ul style="list-style-type: none"> ❑ Broad size distribution ❑ Exposure to solvents ❑ Residual solvents ❑ Long production times | <ul style="list-style-type: none"> ❑ Ethanol Injection <ul style="list-style-type: none"> ▪ Rapid addition of dissolved lipids into aqueous solution through small orifice ▪ Solvent removal <p><u>Advantages</u></p> <ul style="list-style-type: none"> ❑ Simple, gentle ❑ High yields <p><u>Disadvantages</u></p> <ul style="list-style-type: none"> ❑ Solubility limitations ❑ Dilute = Large volumes ❑ Low encapsulation ❑ Removal of ethanol |

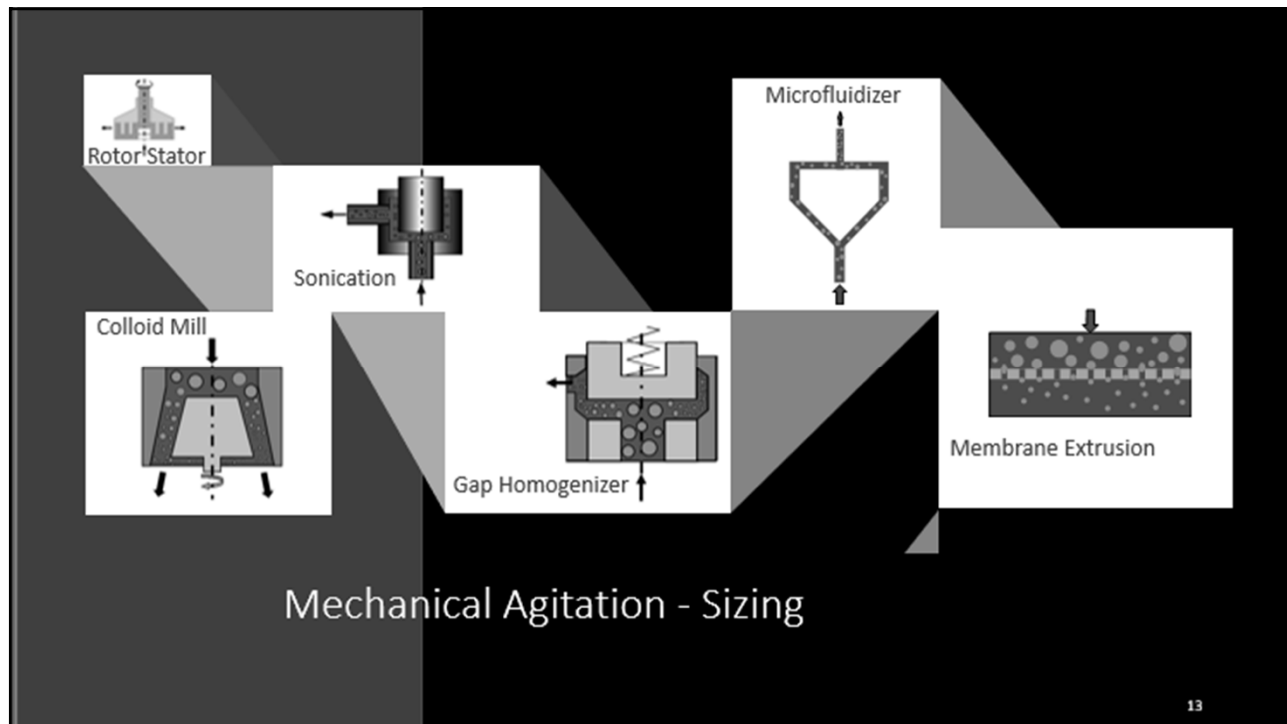
11

11










12

12



13

Disadvantages

-  High production costs
-  Temperature sensitivity (manufacturing and storage)
-  Stability
-  Characterization
-  Control of release rate
-  Sufficient drug loading
-  Biological barriers (e.g., skin, blood-brain barrier)

14

Summary

(A) Conventional liposome

- Hydrophobic drug
- Genetic material i.e. DNA or RNA or siRNA
- Hydrophilic drug
- Phospholipid i.e. anionic or cationic or neutral
- Imaging agent i.e. Gd-DOTA-DSPE for MRI
- Targeting ligands i.e. antibody, etc

(B) PEGylated liposome

- Polyethylene Glycol (PEG)

(C) Ligand targeted liposome

- Aptamer
- Antibody
- Protein
- Peptide
- Small molecule
- Carbohydrate

(D) Multifunctional liposome i.e. theranostic liposome

Figure reference: Riaz, Muhammad & Zhang, Flora & Congcong, Lin & Wong, Ka & Chen, Xiaoyu & Zhang, Ge & Lu, Aiping & Yang, Zhijun. (2018). Surface Functionalization and Targeting Strategies of Liposomes in Solid Tumor Therapy: A Review. International Journal of Molecular Sciences. 19. 195. 10.3390/ijms19010195.

Consider nanoliposomal formulations IF:

- Parenteral route of administration
- Desire/need targeting capabilities
- High value or highly toxic active
- Protection of drugs (stability and reduced elimination)
- Increase circulation time

ISPE Connecting Pharmaceutical Knowledge

15 ispe.org |

15



16