Pharmaceutical Nanotechnology
Liposomes as drug delivery systems
Biophysical and Thermodynamical considerations

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What is nanotechnology? Milestones

- Nanotechnology is defined as the application of the technology in the gray area between the classical mechanism and quantum mechanics.
- The first report of nanotechnology was from Richard Feyman.
- Prof. Nario Tanaguchi was the first to introduce the term nanotechnology from the University of Sciences in Tokyo, in 1974.
- In 1995, FDA approved Doxil (liposomal doxorubicin) to cure cancer.
- In 2012 the first report on bio-mimetic drug delivery nano systems was published.
- In 2015 ThermoDox (thermosensitive liposomal doxorubicin) successfully evaluated in clinical trials (Phase III).
- In 2015 FDA approves Onivyde (liposomal irinotecan) for advanced pancreatic cancer.

Nanotechnology is the art for producing little devices and machines at the mesoscopic and molecular scale.

Pharmaceutical Nanotechnology

is considered to be an attractive area of colloidal drug delivery nanocarriers.

Nanotechnological platforms can

- alter the pharmacokinetic properties and tissue distribution
- ↑ therapeutic efficiency
- ↓ adverse effects

According to National Nanotechnology Initiative (U.S.A)

Nanotechnology is the understanding and control of matter at dimensions between 1 to 100 nanometers

According to E.M.A :
Nanotechnology is defined as the production and application of structures, devices and systems by controlling the shape and size.....
Biomimetic materials with self-assembly abilities are used for producing nano-systems.

These nano-systems can be used as drug delivery platform, in imaging and in diagnosis.

- Liposomes
- Lipidic nanostructures
- Nanotubes
- Nanoemulsions
- Polymeric micelles
- Polyelectrolytes
- Dendrimers
- Chimeric nanostructures
- Nanocapsules and nanospheres (10-1000nm)
- Nanoshells
- Nanocrystals Qd
- Magnetic nanoparticles
- etc.
Nanocarriers belong to Nanocolloidal dispersed systems. Their physicochemical characteristics are important in order to correlate their behavior with that of the living cells.
What is a liposome?

- Pseudo-Spherical vesicles with a phospholipid bilayer
Lipidic self-assembled nano-systems

LIPOSOMES

Liposomes are colloidal dispersed systems that are able to incorporate bioactive molecules (drugs) and deliver them to the tissues increasing their effectiveness.

Phospholipidic bilayer

Aqueous cavity

Liposomal technology

Drug Delivery

Cosmetics

Food Technology

They are composed of

ARTIFICIAL PHOSPHOLIPIDIC MEMBRANES FORMING BILAYER CAN MIMIC THE FUNCTIONALITY OF BIOMEMBRANES AND MIMIC THEIR BEHAVIOUR.

The Differences in fatty acid acyl chain of phospholipids explain differences in fluidity of lipid bilayers while the tans–gauche conformational transitions are affect biophysical properties and their thermodynamics.
CONVENTIONAL LIPOSOMAL DRUG DELIVERY nanoSYSTEMS

- Simple liposomes
- Anionic liposomes
- Cationic liposomes
- PEGylated (stealth) liposomes
Abelcet® ABLC

Ribbon-like particles
Carrier lipids: DMPC, DMPG
Particle size (μm): 1.6-11

Amphotec® ABCD

Disk-like particles
Carrier lipids: Cholesteryl sulfate
Particle size (μm): 0.12-0.14

Ambisome® L-AMB

Unilaminar liposome
Carrier lipids: HSPC, DSPG, cholesterol
Particle size (μm): 0.08
VIDEO

LIPOSOMAL AMPHOTERICIN B

Ambisome® L-AMB

Water

Unilaminar liposome
Carrier lipids: HSPC, DSPG, cholesterol
Particle size (µm): 0.08
DOXORUBICIN HCL

Simple liposomes

PEGylated (stealth) Liposomal formulation

Myocet™ (liposomal doxorubicin)

Caelyx 2 mg/ml
concentrate for solution for infusion

PEGylated liposomal doxorubicin hydrochloride
### Liposomal medicines in market

<table>
<thead>
<tr>
<th>Encapsulated drug</th>
<th>Trade Name</th>
<th>Company</th>
<th>Indication</th>
<th>Approval</th>
<th>Innovator Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Abelcet</td>
<td>Sigma-Tau PharmaSource, Inc, Indianapolis, IN</td>
<td>Sever fungal infections</td>
<td>1995</td>
<td>The Liposome Company</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Ambisome</td>
<td>Gilead Sciences, Inc, San Dimas, CA</td>
<td>Sever fungal infections</td>
<td>1997</td>
<td>Vestar</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>DepoCyte</td>
<td>Enzon/Skye Pharma</td>
<td>Lymphomatous meningitis (intrathecal administration)</td>
<td>1999</td>
<td>Chiron Corporation and SkyePharma</td>
</tr>
<tr>
<td>Daunorubicine</td>
<td>DaunoXome</td>
<td>Gilead Sciences, Inc</td>
<td>Kaposi sarcoma</td>
<td>1996</td>
<td>Gilead</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>LipoDox (generic of Doxil)</td>
<td>TTY Biopharm Company Ltd, Taipei Taiwan</td>
<td>Kaposi’s sarcoma, ovarian/breast cancer</td>
<td>2013 (FDA approved; USA)</td>
<td>Sun Pharma</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Doxil (USA), Caelyx (Europe)</td>
<td>Essex (Europe) Ortho Biotech (USA)</td>
<td>Breast and ovarian cancer, Kaposi sarcoma</td>
<td>1995 (conditional)</td>
<td>Sequus, Inc.</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Myocet</td>
<td>Novartis Pharma AG, Basel, Switzerland</td>
<td>Breast cancer</td>
<td>2000 (EU)</td>
<td>The Liposome Company</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Onivyde</td>
<td>Merrimack Pharmaceutical Inc. of Cambridge, Massachussetts</td>
<td>Advanced pancreatic cancer</td>
<td>2015 (FDA approved; USA)</td>
<td>Merrimark Pharmaceuticals</td>
</tr>
<tr>
<td>Verteporfin</td>
<td>Visudyne</td>
<td>Novartis Pharma AG, Basel, Switzerland</td>
<td>Age-related molecular degeneration, pathologic myopia, ocular histoplasmosis</td>
<td>2000</td>
<td>QLT</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Marquibo</td>
<td>Spectrum Pharmaceuticals Inc.</td>
<td>Philadelphia chromosome–negative (Ph−) acute lymphoblastic leukemia (ALL)</td>
<td>2012 (FDA approved; USA)</td>
<td>Inex and Enzon</td>
</tr>
</tbody>
</table>
Advanced Liposomal Drug Delivery nanoSystems

- STIMULI - RESPONSIVE LIPOSOMAL nanoSYSTEMS

- CHIMERIC LIPOSOMAL nanoSYSTEMS
Thermodox® is a nanoengineered drug delivery system in Phase III and is a temperature sensitive liposomal formulation incorporating doxorubicin (anthracycline) for the treatment of metastatic malignant melanoma and liver cancer.

ThermoDox is the first heat-activated liposomal formulation, which is consisted of three synthetic low phase transition temperature phospholipids and release the anticancer agent at 39.5°C.
The presence of the polymeric component plays a key role in the thermal behavior of mixed lipid based nanovectors due to the structural rearrangement of liposomal membrane.

Temperature-dependent release of IND was observed from chimeric liposomes, due to the well known thermotropic conformational transition of the grafted PNIPAM chains.
Polymers in Pharmaceutics:

1) Polymers are materials that are widely used in the pharmaceutical industry in various technological formulations and in bioactive molecule delivery system coating.

2) Polymer behavior is directly related to their chemical structure. Also, their abilities depend on the way that monomers are connected to each other.

3) Polymers can have linear or branched chains that may cross each other. Copolymers are composed of more than one monomer and develop new polymers with completely new properties.
Chimeric liposomal systems are composed of phospholipids and polymers.

Polymer grafted liposomes

PEO-b-PCL–DPPC chimeric nanocarriers: self-assembly aspects in aqueous and biological media and drug incorporation†

Natassa Pippa,⁎⁎ Eleni Kaditi,⁎ Stergios Pispas⁎⁎ and Costas Demetzos


RESEARCH PAPER

DPPC/poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) chimeric nanostructures as potential drug nanocarriers

Natassa Pippa · Eleni Kaditi · Stergios Pispas · Costas Demetzos
Carbon nanotubes in drug delivery:

Functionalyzed CNTs

- Diagnosis and Imaging
  - Cancer therapy
  - Gene therapy
  - Tissue engineering

- Therapeutics
  - As antioxidants
  - Biosensors
  - Enantioseparation of chiral drugs
  - Extraction of chemicals

Delivery of drugs:

- Reduced toxicity
- Increased circulation period
- Active targeting
- Reduced side effects
- Controlled toxicity
Synthesis

Functionalized CNTs

MWCNTs

(4:1 v/v) \( \text{H}_2\text{SO}_4, \text{H}_2\text{O}_2 \) →

\( \text{NaHCO}_3 \) → CN1

(i) \( \text{SO}_3 \)
(ii) (1:1 v/v) \( \text{SO}_3, \text{HNO}_3 \) →

\( \text{NaHCO}_3 \) → CN2
Advanced drug delivery nano systems:

- Co-delivery of si-RNA and cytotoxic drugs
- Efficient cell uptake
- High drug loading
- Biomimetic molecular transport systems
- Lab-on-chip applications

- Carbon nanotubes – liposome conjugate
- Carbon nanotube – liposome supramolecular nanotrails
- Cationic liposome-multiwalled carbon nanotube hybrids

*Miyako et al., 2012
*Karchemchi et al., 2012
*Pereira et al., 2015
What we need ...

We need specific tools that can meet the requirements for characterizing nanoparticulate medicines and to control the manufacturing process for their development.

**Determination of the physicochemical functionality of nanomedicines is related to their surface characteristics.**

- size, size distribution, ζ-potential, shape, surface properties, etc

- The nature and stability of surface properties/coatings in the final nanomedicine product can be very important in determining safety and efficacy.

- Thus, the need for sensitive and accurate **analytical methods** to identify and quantify the nanomedicine is considered as essential for its quality in part and as final product.
Methods:

Thermodynamic and Biophysical Characterization
- Differential Scanning Calorimetry

Physicochemical Characterization
- Dynamic and Electrophoretic Light Scattering

Morphological Characterization
- Static Light Scattering, cryo-TEM, Atomic Force Microscopy

Biological evaluation
- In vitro screening, in vivo toxicity
BASIC SCIENTIFIC TOOLS FOR STUDYING LIPOSOMAL PHOSPHOLIPIDIC MEMBRANES

The **Biophysics** and **Thermodynamics** are considered as the basic scientific elements for studying artificial cell membranes and provide projection of the behavior of nano systems as artificial cell models.

The synergy regarding the biophysical behavior of artificial biomembranes and of cell biology has promoted nanoparticulate systems as drug delivery nano-platforms, while their thermotropic behavior can be correlated with cell functionality.

Mini-Review

Biophysics and Thermodynamics: The Scientific Building Blocks of Bio-inspired Drug Delivery Nano Systems

Costas Demetzos¹,²

Received 9 March 2015; accepted 7 April 2015; published online 22 April 2015

Abstract. Biophysics and thermodynamics are considered as the scientific milestones for investigating the properties of materials. The relationship between the changes of temperature with the biophysical variables of biomaterials is important in the process of the development of drug delivery systems. Biophysics is a challenge sector of physics and should be used complementary with the biochemistry in order to discover new and promising technological platforms (i.e., drug delivery systems) and to disclose the ‘silence functionality’ of bio-inspired biological and artificial membranes. Thermal analysis and biophysical approaches in pharmaceuticals present reliable and versatile tools for their characterization and for the successful development of pharmaceutical products. The metastable phases of self-assembled nanostructures such as liposomes should be taken into consideration because they represent the thermal events can affect the functionality of advanced drug delivery nano systems. In conclusion, biophysics and thermodynamics are characterized as the building blocks for design and development of bio-inspired drug delivery systems.

KEY WORDS: biophysics; drug delivery nano systems; pharmaceuticals; thermal analysis; thermodynamics.
Thermodynamics is the fundamental scientific element that could efficiently be used for studying and analyzing the behavior of artificial biological membranes that could be correlated with biological networks and create scientific platforms for the system therapeutics concept.

Liquid crystalline phases of phospholipids

The regulatory issues concerning the drug development process have incorporated DSC as a valuable technique for the analysis of the physical and energetic properties of drugs and of excipients, and its use is essential under standard procedures, as is described in the *United States Pharmacopeia*.

**Differential Scanning Calorimetry (DSC): A Tool to Study the Thermal Behavior of Lipid Bilayers and Liposomal Stability**

**COSTAS DEMETZOS**
Department of Pharmaceutical Technology, School of Pharmacy, University of Athens, Greece

DSC is a useful tool for designing lipidic drug delivery systems by determining the cooperativeness of the mixed substances.
VINBLASTINE is an anticancer drug.
It is toxic to the normal tissues and appropriate DDSs are needed in order to reduce its toxicity and improve its pharmacokinetic parameters.
Studies concerning the thermotropic properties of Lipid Bilayers composed of DPPC phospholipids incorporating Vinblastine and changes of the thermodynamic parameters can be used to predict the Physical Stability of Liposomal Formulation composed by DPPC Phospholipids.

Ref: Maswadeh, Demetzos, Mavromoustakos et al., BBA, 2002
Large Unilamellar liposomal Vesicles and Multi Lamellar liposomal Vesicles have been prepared.

The changes of the parameters $T_m$ and $\Delta H$ were measured and it was found that they were affected by the liposomal composition (pure DPPC or DPPC incorporated Vinblastine or DPPC incorporated cholesterol or cholesterol and Vinblastine).

<table>
<thead>
<tr>
<th>Lipid Composition</th>
<th>LUV $T_m$ ($^\circ$C)</th>
<th>LUV $\Delta H$ (Kcal/mol)</th>
<th>MLV $T_m$ ($^\circ$C)</th>
<th>MLV $\Delta H$ (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC</td>
<td>41.2</td>
<td>5.3</td>
<td>42.3</td>
<td>7.5</td>
</tr>
<tr>
<td>DPPC/VIN (100:17)</td>
<td>41.7</td>
<td>7.7</td>
<td>40.5</td>
<td>10.4</td>
</tr>
<tr>
<td>DPPC/CHL (10:1)</td>
<td>41.5</td>
<td>3.6</td>
<td>40.6</td>
<td>4.2</td>
</tr>
<tr>
<td>DPPC/CHL/VIN (100:10:17)</td>
<td>41.0</td>
<td>3.8</td>
<td>40.0</td>
<td>5.8</td>
</tr>
<tr>
<td>DPPC/CHL (10:3)</td>
<td>40.4</td>
<td>0.5</td>
<td>42.8</td>
<td>1.9</td>
</tr>
<tr>
<td>DPPC/CHL/VIN (100:30:17)</td>
<td>40.2</td>
<td>0.4</td>
<td>43.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Quantitative thermal data for DPPC LUVs and MLVs liposomal vesicles.
The results from the DSC experiments based on calculated thermodynamic parameters could be used to select appropriate biomaterials for designing an effective and stable liposomal DDnS incorporating the anticancer drug vinblastine.
An innovative medicine consists of the bioactive substance (drug) and of the excipient that are mentioned as innovative excipient.

An Innovative excipient is an excipient that has self-assembled behavior and can create functionality and new surface properties.

INNOVATIVE PHARMACOLOGICALLY INACTIVE EXCIPIENTS-BIOMATERIALS THAT ARE ABLE TO CREATE SELF-ASSEMBLED NANOSTRUCTURES

- Globules
- Micelles
- Polymersomes
- Liposomes
It has been therefore a great personal pleasure to write the Preface of the present book by Professor Costas Demetzos. His monograph, ‘Pharmaceutical Nanotechnology’, is a unique publication.
Laboratory of Pharmaceutical Nanotechnology University of Athens, Greece

Conclusions:

➢ Basic sciences such as Physics (biophysics and thermodynamics) and Mathematics are needed to assist the research and development process of nanomedicines.

➢ A regulatory approach that promotes self-assembled nanoparticulate systems as innovative excipients complementary to the already existing regulatory framework for the classical and functional excipients, could produce new guidelines for the regulatory authorities to create a framework process for the approval of nanomedicines and consequently of nanosimilars.

➢ An analytical concept on fractal analysis based on non-Euclidian approach of the geometry of nanoparticles (complementary to the QbD approach) has been proposed from our lab. to EMA offering an added value to the ‘mapping process’ for developing nanomedical products.

➢ The regulatory authorities should take into consideration that efficient analytical evaluation of nanomedicines should be developed and new analytical tools should be considered as part of the dossier to be submitted to the regulatory body of experts.

The interplay between the rate of release from polymer grafted liposomes and their fractal morphology

Natassa Pippa\textsuperscript{a,b}, Aristides Dokoumetzidis\textsuperscript{a}, Stergios Pispas\textsuperscript{b}, Costas Demetzos\textsuperscript{a,}\textsuperscript{\ast}

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\textsuperscript{b} Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, Athens, Greece

DPPC/poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) chimeric nanostructures as potential drug nanocarriers

Natassa Pippa \cdot Eleni Kaditi \cdot Stergios Pispas \cdot Costas Demetzos

Fractal Analysis of Liposomal Aggregation.

Natassa Pippa, Costas Demetzos\textsuperscript{\ast}

Faculty of Pharmacy, Department of Pharmaceutical Technology, University of Athens, University Campus Zografou, 15784 Athens, Greece
Our contribution......

Our research group has published several research articles on the fractal morphology of nanoparticles. A comment was submitted to the EMA regarding the ‘concept paper on the revision of the guideline on immunogenicity assessment of biotech therapeutic proteins –EMA/CHMP/BMWP/42832/2005). This comment included the adaption of fractal analysis in the evaluation process of biotech biosimilar and nanomedicinal products.
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