"ωφελέειν, εἰ μη βλάπτειν"

'benefit and do not harm'

National & Kapodistrian University of Athens

Pharmaceutical Nanotechnology

SELF-ASSEMBLED *CHIMERIC* LIPOSOMAL SYSTEMS
Biopysical and Thermodynamical considerations

Costas Demetzos

Professor in Pharmaceutical Nanotechnology

Director in the Laboratory of Pharmaceutical Technology
Faculty of Pharmacy,
National and Kapodistrian University of Athens

President of Hellenic Pharmaceutical Society

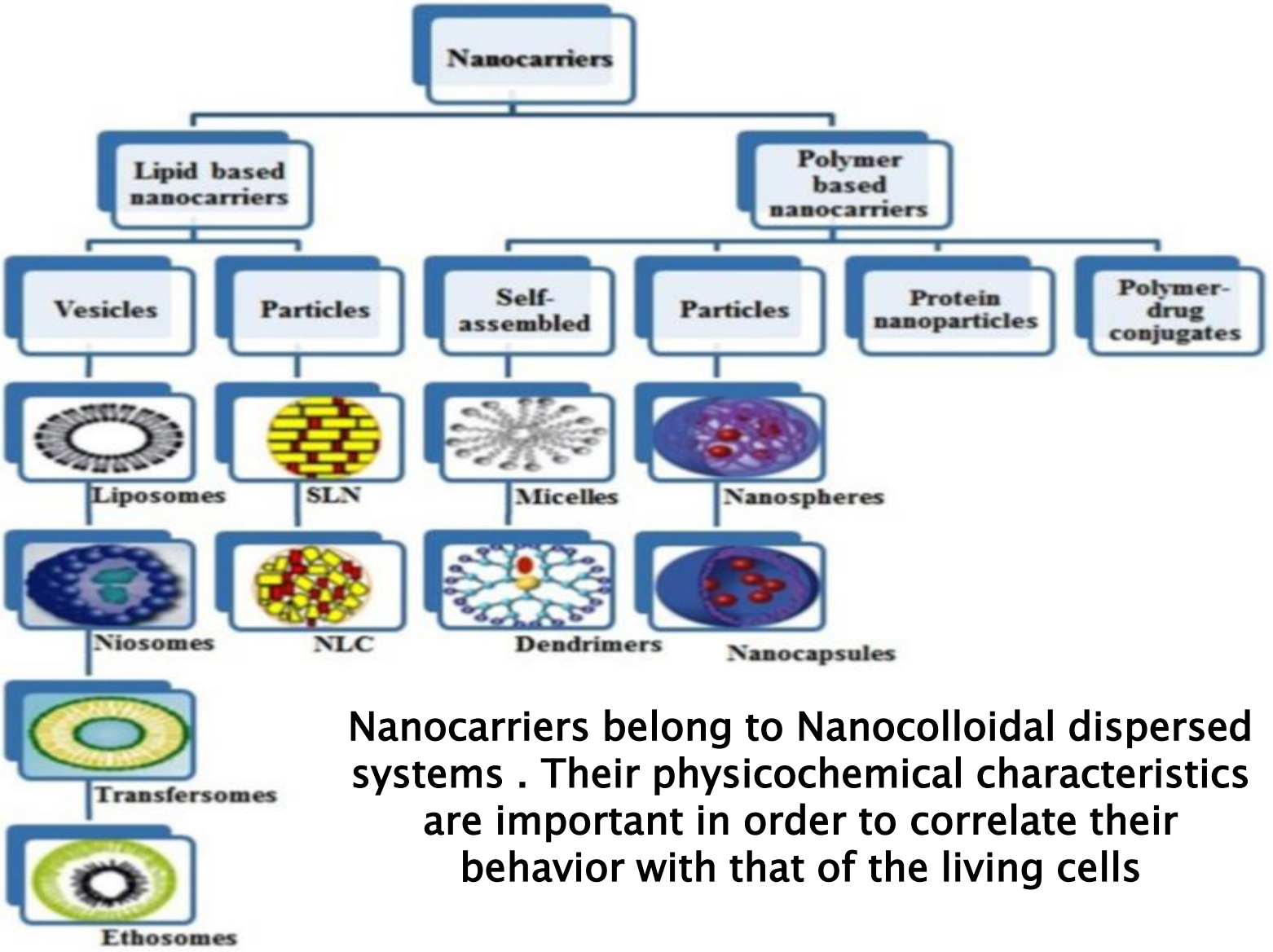
Member of the Executive Committee of European Federation of Pharmaceutical Sciences (EUFEPS) (2014-2016)

What is nanotechnology ? Milestones

- Nanotechnology is multidisciplinary scientific field that deals with the development and use of materials with a dimension equal to one billionth of a meter (1nm = 10-9m).
- 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 (Phse III).
- In 2015 FDA approves Onivyde (liposomal irinotecan) for advanced pancreatic cancer

Ref. Saladin Nanotechnology for the development word. *Chaos Solition Fractals* 30 (4): 769-773, 2006

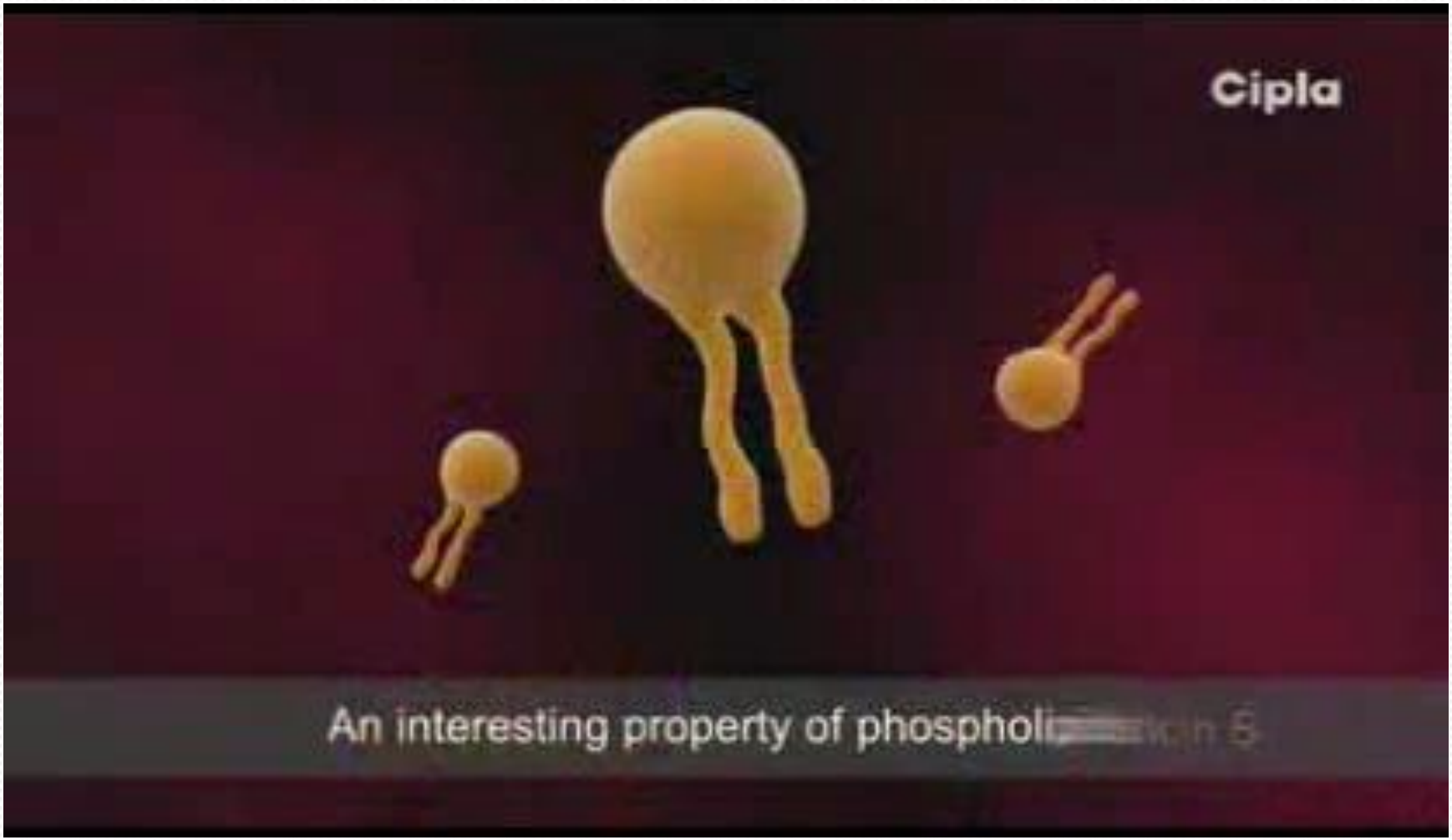
Ref. C. Demetzos '*Pharmaceutical Nanotechnology. Fundamentals and practical applications*' 2016, Springer



Nanocarriers belong to Nanocolloidal dispersed systems . Their physicochemical characteristics are important in order to correlate their behavior with that of the living cells

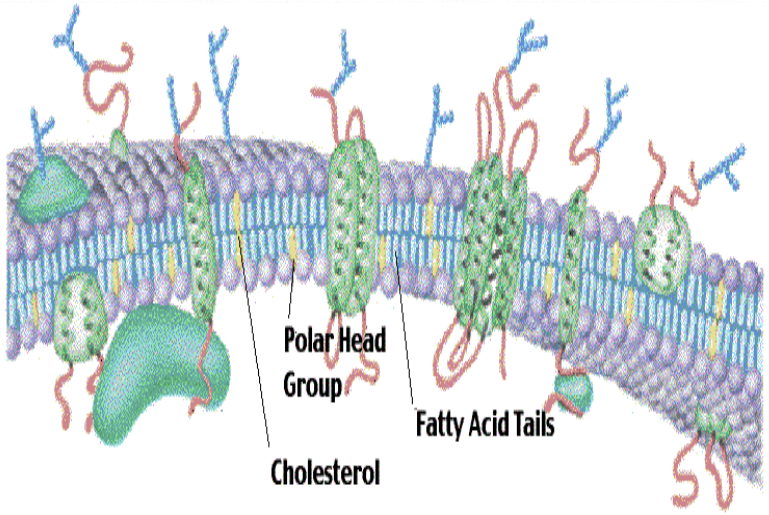
M. Estanqueiro et al. / Colloids and Surfaces B: Biointerfaces 126 (2015)631–648

SELF ASSEMBLY PROCESS OF PHOSPHOLIPIDS FOR PRODUCING PHOSPHOLIPID BILAYERS.

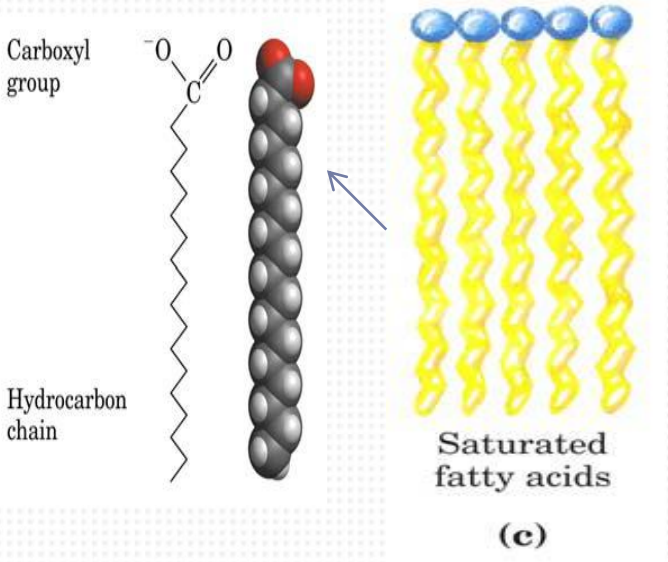


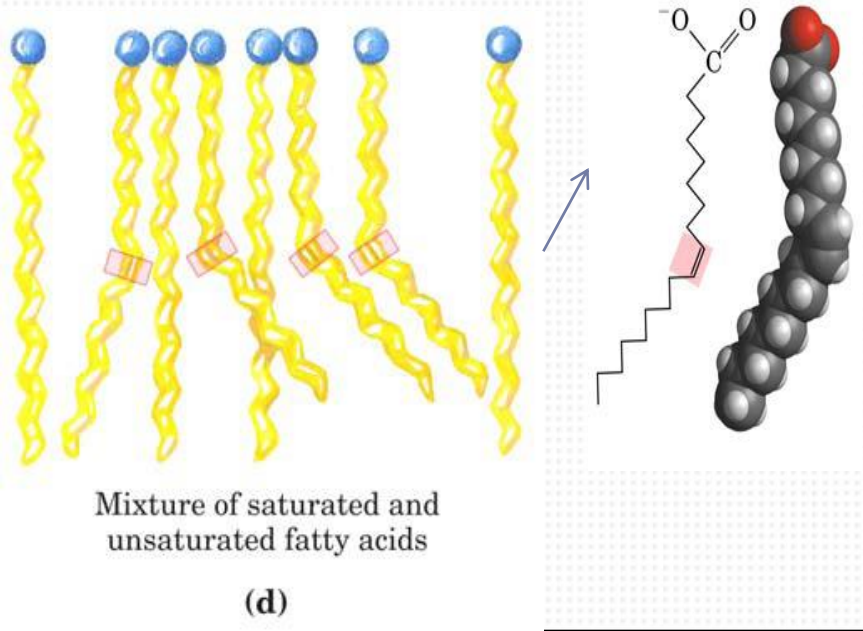
THE SELF ASSEMBLY PROCESS IS BASED ON THE PHYSICOCHEMICAL CHARACTERISTICS OF THE INITIAL BIOMATERIALS

artificial Phospholipidic Membranes FORMING Bilayer CAN MIMIC THE FUNCTIONALITY OF Biomembranes AND MIMIC THEIR BEHAVIOUR.

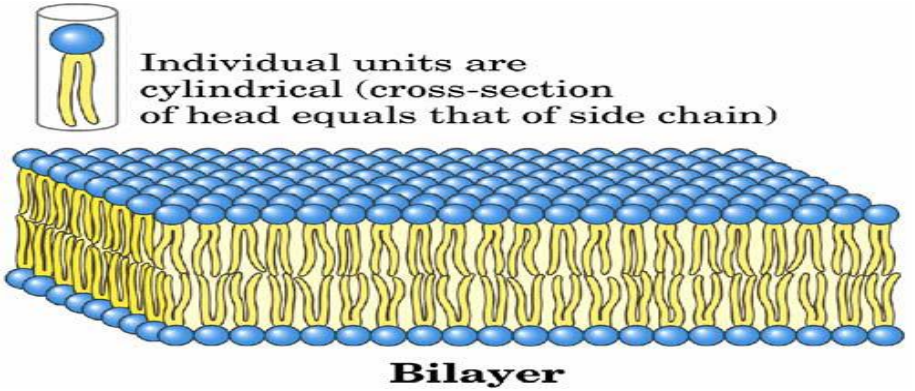


The Differences in fatty acid acyl chain of phospholipids explain differences in fluidity of lipid bilayers while the *tans–gauche* conformational transitions are affect biophysical properties and their thermodynamics

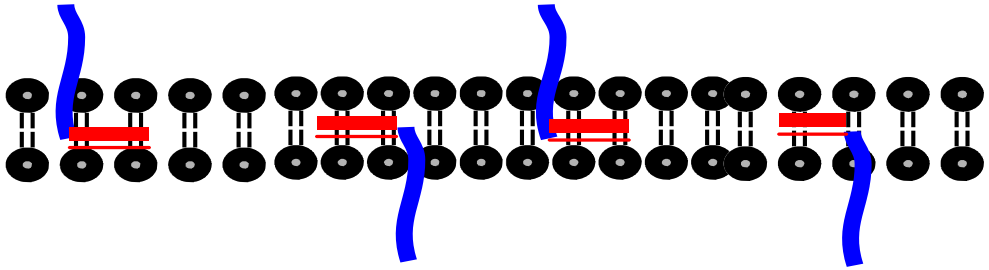




- Artificial **Lipidic Bilayers** are structural components of Liposomal Drug Delivery nano Systems (DDnSs)

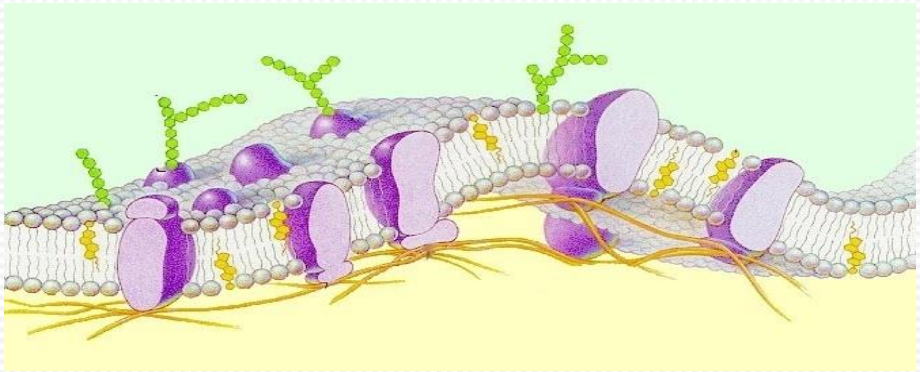


- They are used to study the interactions between lipidic bilayers with the incorporated bioactive molecule



- The design and development of liposomal DDnSs is mainly based on findings from their theromotropic and from their biophysical behavior.

CAN WE MIMIC CELLS' MECHANISM TO OBTAIN ASSEMBLIES WITH CUSTOMIZED ***SHAPE*** AND SIZE ??



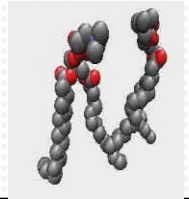
CAN WE MIMIC CELLS' SURFACES BEHAVIOUR TO OBTAIN CUSTOMIZED ***FUNCTIONALITY*** ??

THE CONCEPT IN INNOVATIVE DRUG DELIVERY nanoSYSTEMS IS TO PRODUCE DEVICES THAT CAN BE ABLE TO MIMIC THE FUNCTIONALITY OF CELLS

COOPERATIVITY

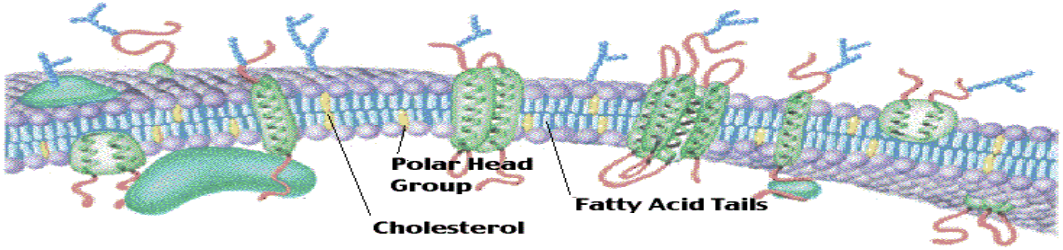
COMPOSITION

METASTABLE PHSES (rafts) ON THE SURFACES' LAYERS OF THE nanoSYSTEM ARE PROVIDING THE '*SMARTNESS*' EFFECT AND IT BECOMES MORE EFFECTIVE

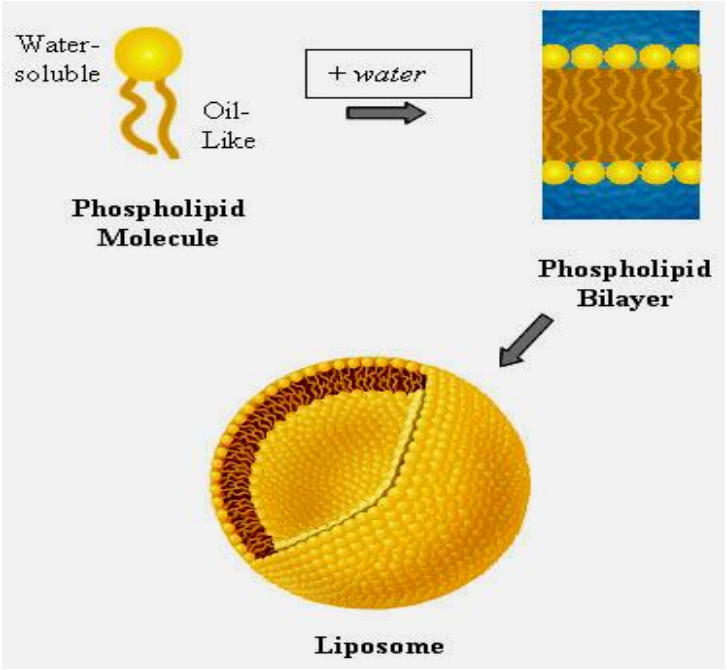


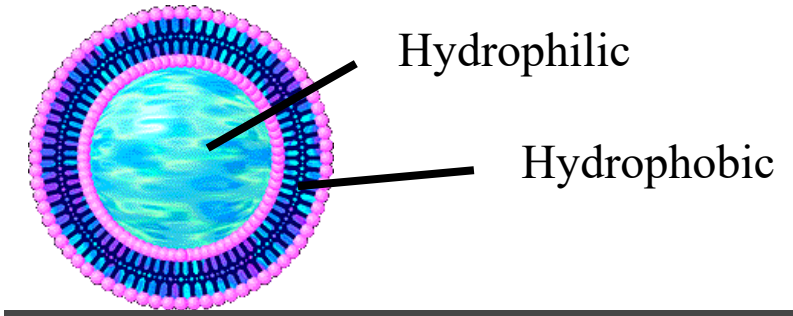
LIPIDIC NANOCARRIERS IN PHARMACEUTICS

What are liposomes ?



- Pseudo-Spherical vesicles with a phospholipid bilayer

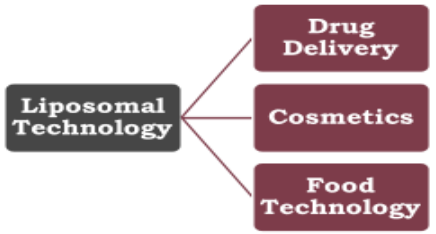




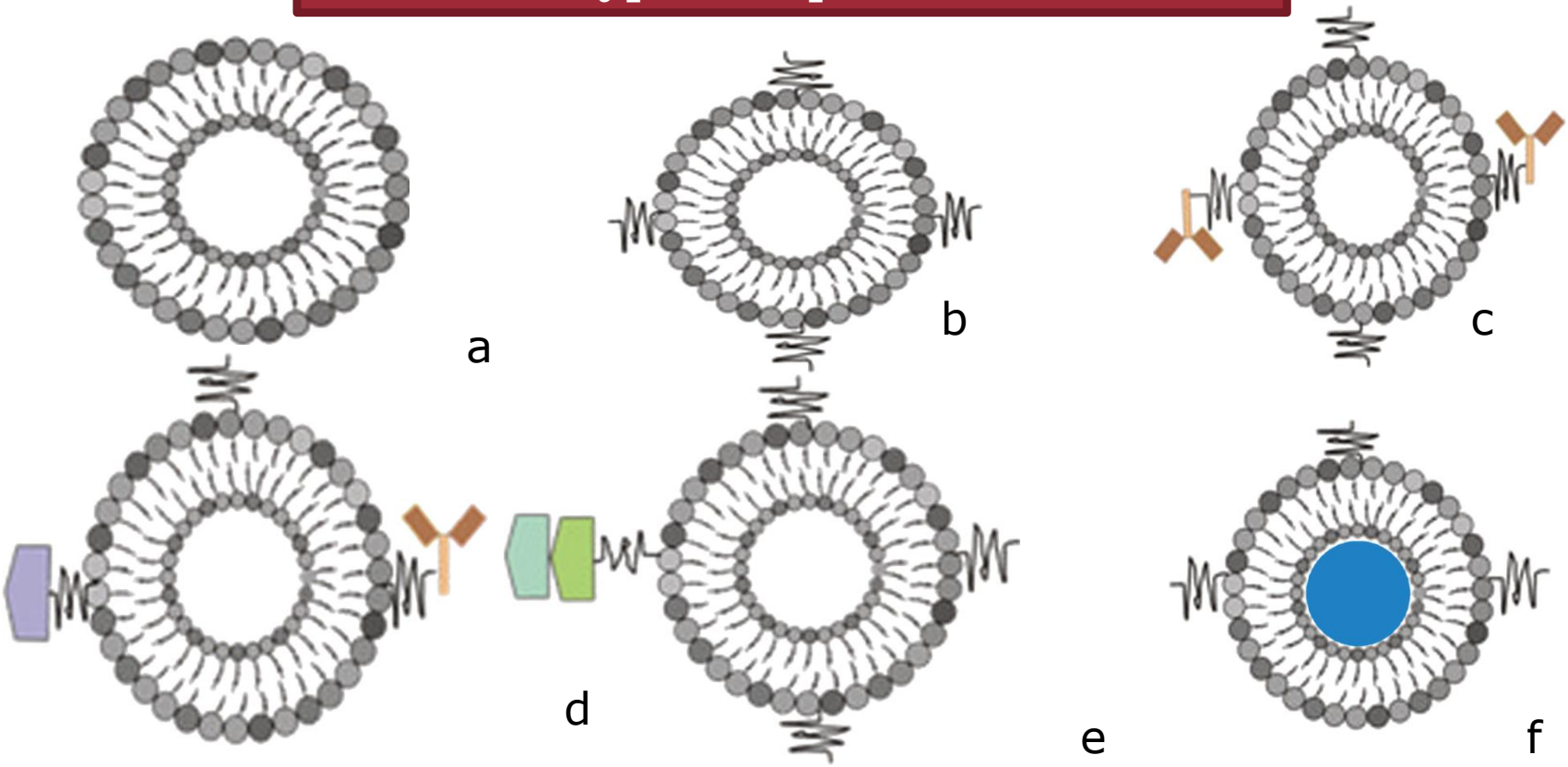
Liposomes in brief.....

- Liposomes were first discovered by A.D. Bangham in 1960 in an attempt to create lipid – water systems that behave as biomembranes.

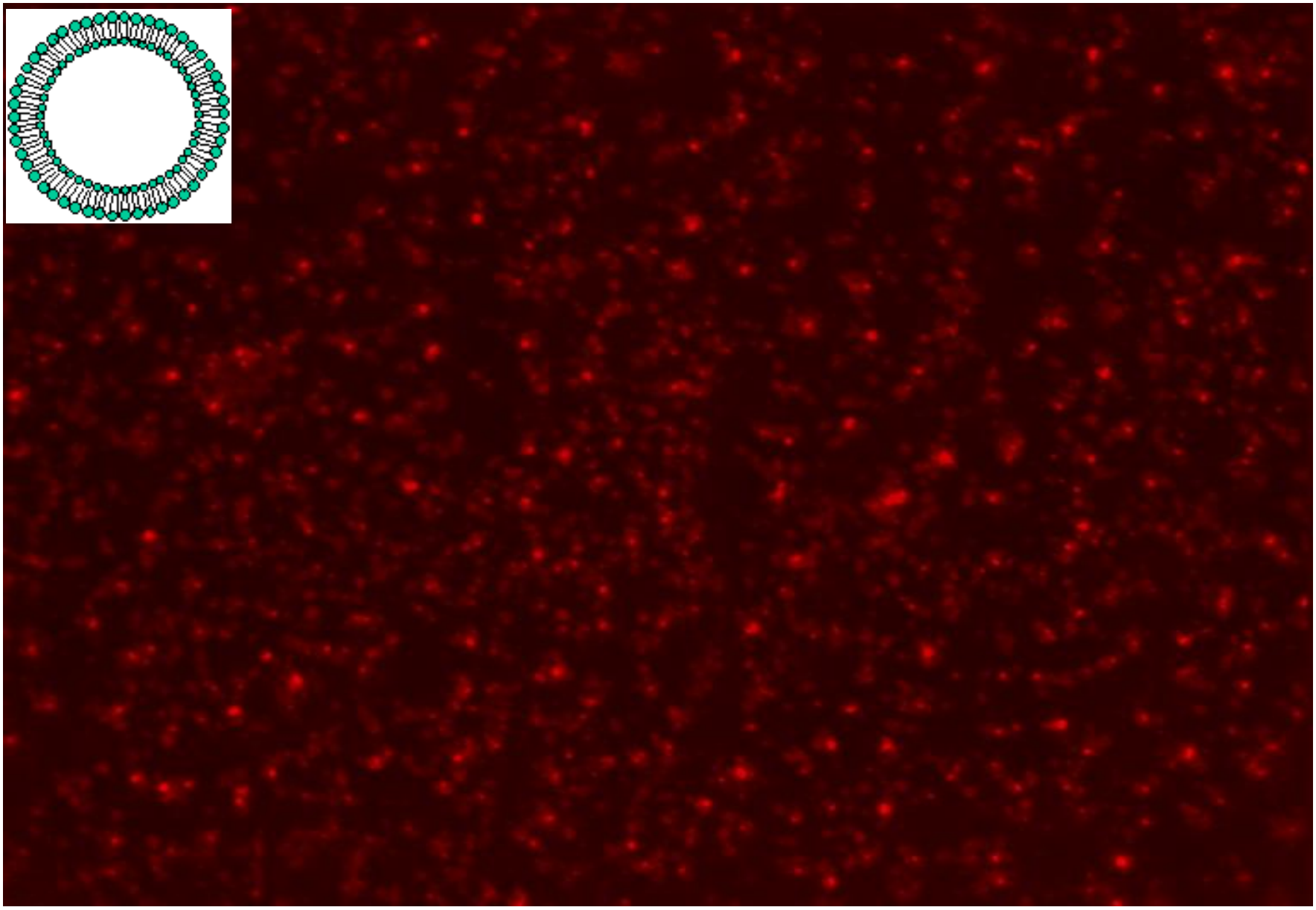
- Liposomes belong to the class of bio-colloids and could affect the physical properties (release, solubility, pharmacokinetics) or ADME profile of the encapsulated drug.



Schematic representation of different types of liposomes



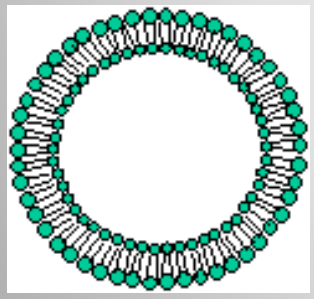
a) Conventional liposomes.
b) Stealth liposome coated with a polymeric conjugate such as PEG.
c) Stealth liposome coupled with a functionalized ligand.
d) Liposome with a single ligand and antibody.
e) Duplicated ligand with repeated peptide sequence.
f) Liposome loaded with per fluorocarbon gas (Adapted from Mufanadi et al., 2011)



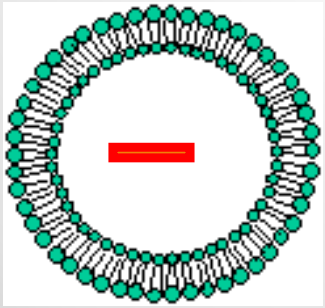
Liposomes colored with Rhodamine B. Image from Confocal Microscope

CONVENTIONAL LIPOSOMAL DRUG DELIVERY nanoSYSTEMS

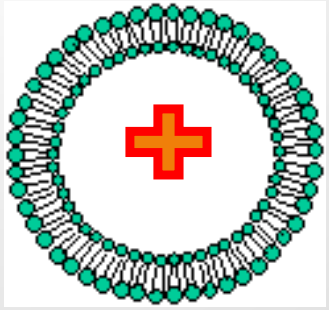
Simple liposomes



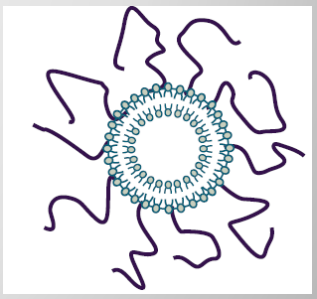
Anionic liposomes



Cationic liposomes



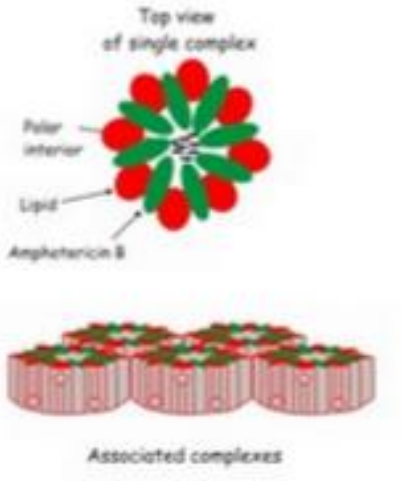
PEGylated (stealth) liposomes



Lipidic and Liposomal formulations of Amphotericin B

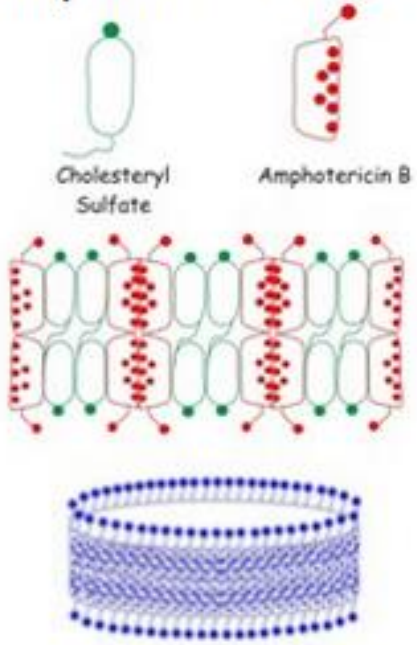
Amphotericin **B** **L**ipid **C**omplex

Abelcet ® ABLC

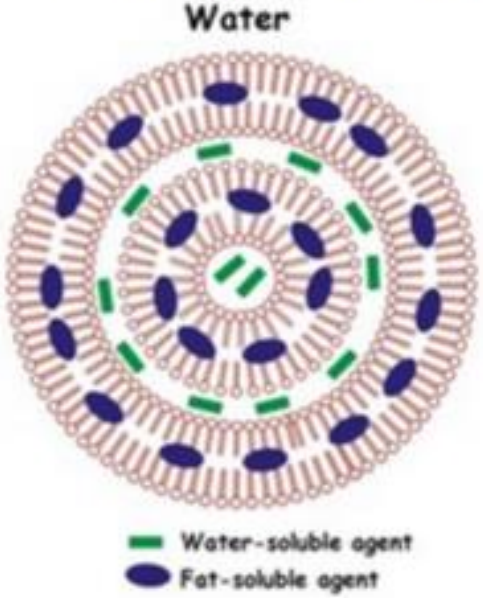


Amphotericin **B** **C**olloidal **D**ispersion

Amphotec ® ABCD



Ambisome ® L-AMB



Ribbon-like particles

Carrier lipids: DMPC, DMPG

Particle size (μm): 1.6-11

Disk-like particles

Carrier lipids: Cholesteryl sulfate

Particle size (μm): 0.12-0.14

Unilaminar liposome

Carrier lipids: HSPC, DSPG, cholesterol

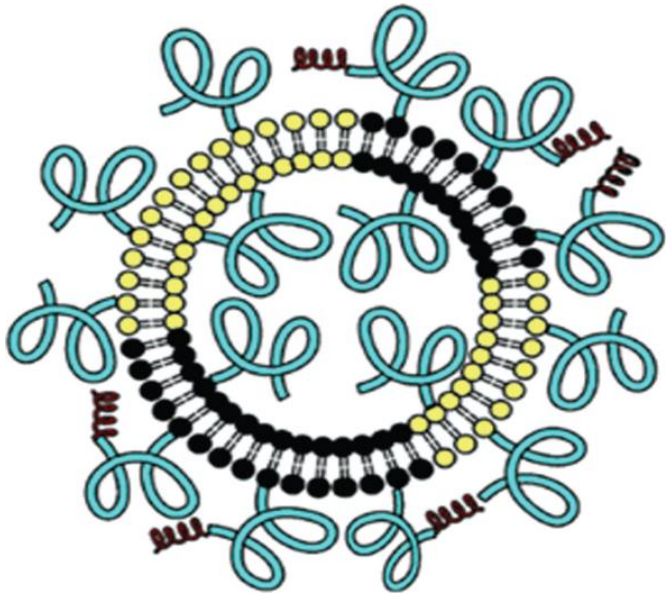
Particle size (μm) : 0.08

AMBISOME (LIPOSOMAL AMPHOTERICIN B)

Stealth liposomal formulations

Stealth liposome technology is one of the most often used liposomal formulations for delivery of active molecules.

This strategy was developed to overcome most of the challenges encountered by conventional liposome technology such as the inability to evade interception by the immune system, toxicity due to charged liposomes, low blood circulation half-life, and steric stability.

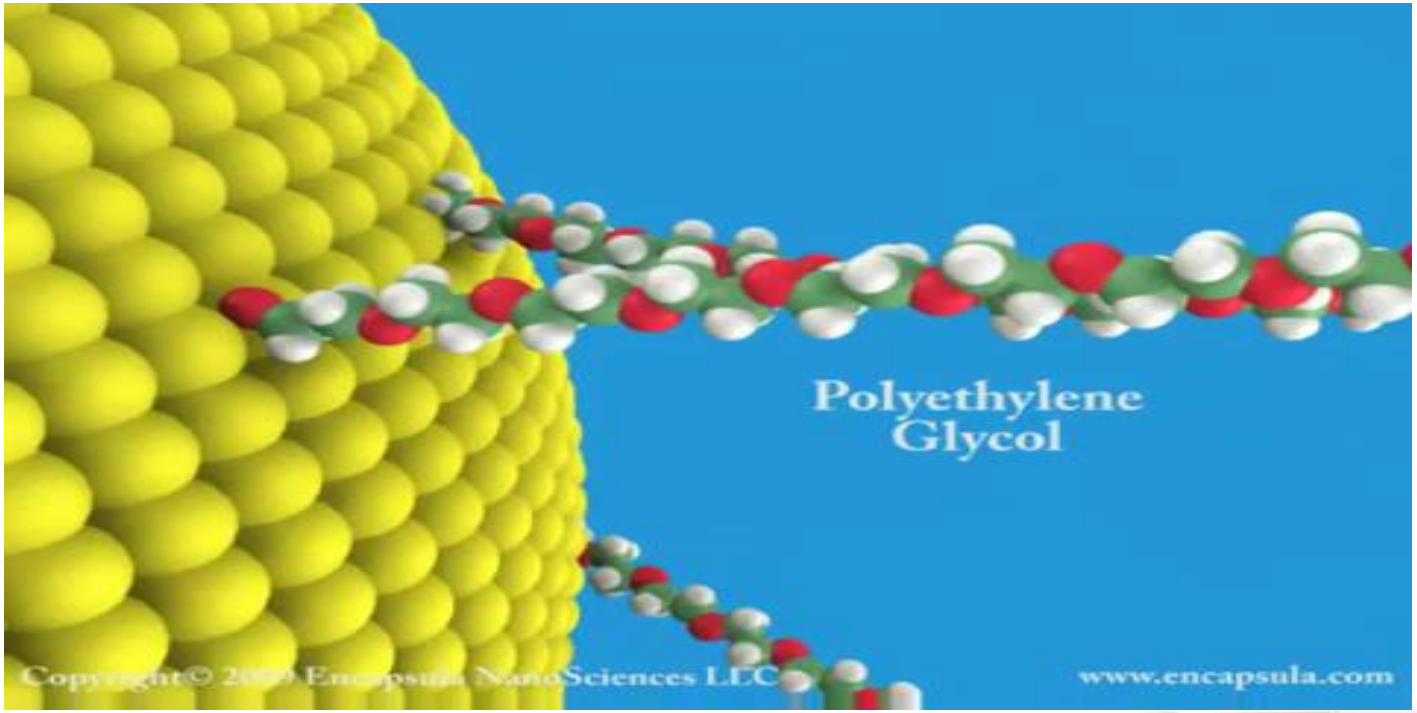


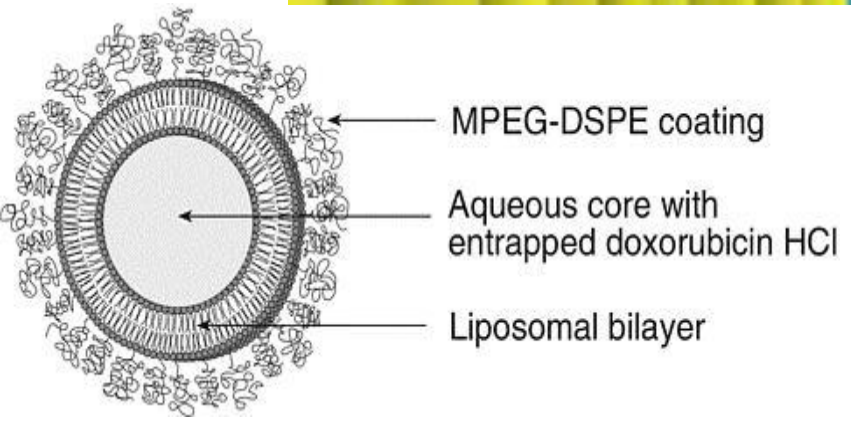
Schematic depicting of a stealth PEGylated liposome.

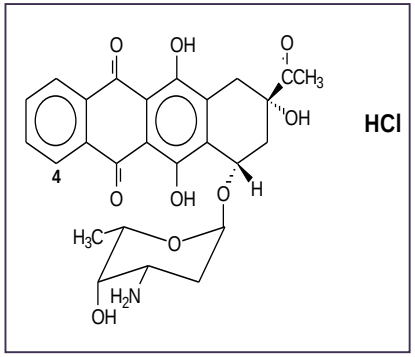
Sterically stabilized liposomes (SSL): **The steric repulsion of the stealth liposome appears to not only stabilize liposome suspensions against aggregation but also inhibit the absorption of various opsonins onto the liposomal surface and degrade biological interactions.**

The short polymer chains can provide a physical barrier or force buffer around the bilayer to prevent contact between liposomes. (Liu et al., 2003)

STEALTH LIPOSOMAL FORMULATION







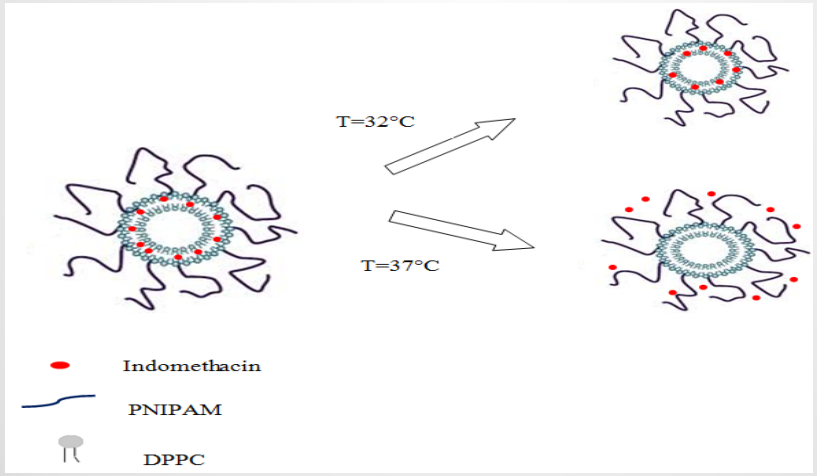
Σημειώσεις

Liposomal medicines in market

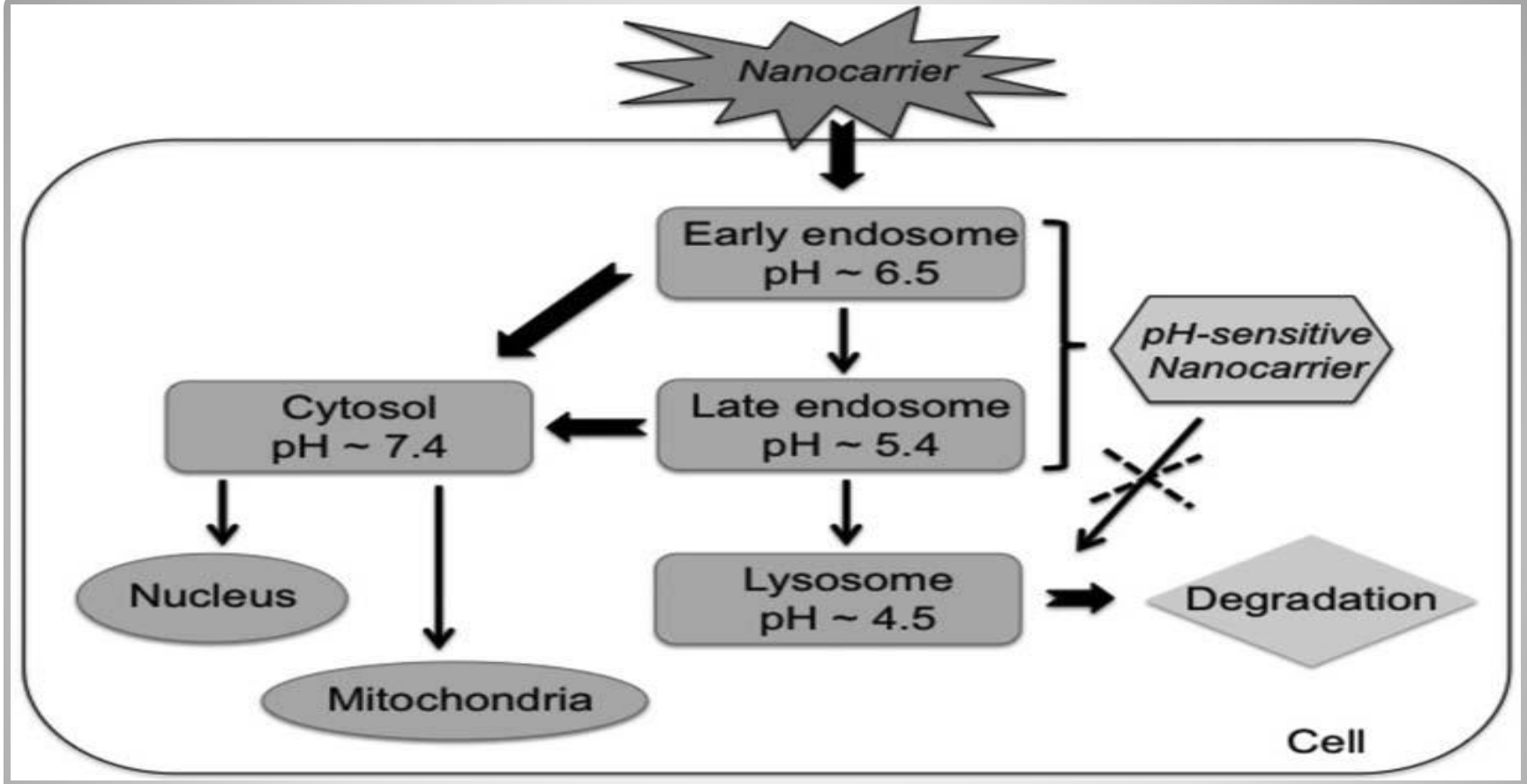
Encapsulated drug	Trade Name	Company	Indication	Approval	Innovator Company
Amphotericin B	Abelcet	Sigma-Tau PharmaSource, Inc, Indianapolis, IN	Sever fungal infections	1995	The Liposome Company
Amphotericin B	Ambisome	Gilead Sciences, Inc, San Dimas, CA	Sever fungal infections	1997	Vestar
Amphotericin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

Advanced Liposomal Drug Delivery nanoSystems

- **STIMULI - RESPONSIVE LIPOSOMAL nanoSYSTEMS**



- ***CHIMERIC*** **LIPOSOMAL nanoSYSTEMS (Chi-aDDnSs)**

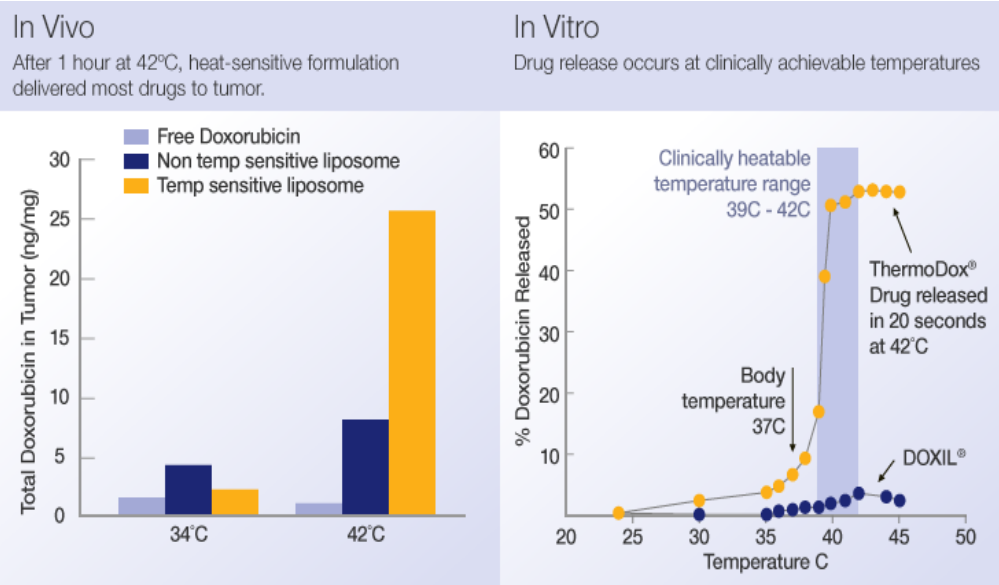


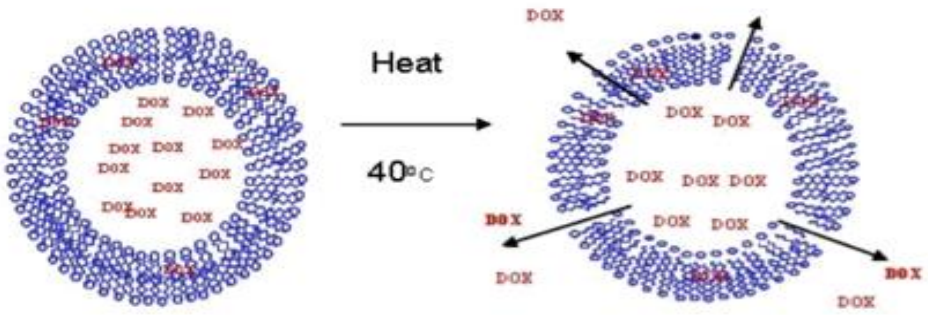
Scheme of the intracellular trafficking of a nanocarrier after cell uptake. The nanocarrier with pH-sensitive properties can undergo several processes that result in selective targeting to the cytosol, nucleus or other subcellular organelles

Ref. Nanotechnology Approaches to Target Endosomal pH: A Promising Strategy for an Efficient Intracellular Drug, Gene and Protein Delivery by Daniele Rubert Nogueira, Montserrat Mitjans and M. Pilar Vinardell*in Drug Delivery Letters 2014

Stimuli-responsive liposomes
Thermosensitive liposomes
as drug delivery systems

ThermoDox Chemotherap





▸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 consists of three synthetic low phase transition temperature phospholipids and release the anticancer agent at **39.5ºC**

International Journal of Pharmaceutics 485 (2015) 374–382

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journal homepage: www.elsevier.com/locate/ijpharm

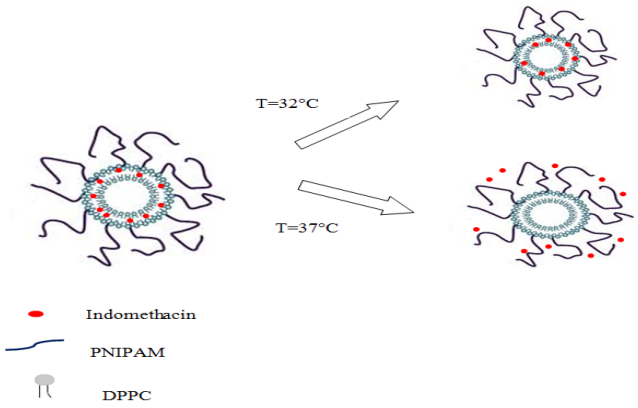
Pharmaceutical nanotechnology

Temperature-dependent drug release from DPPC:$C_{12}H_{25}$-PNIPAM-COOH liposomes: Control of the drug loading/release by modulation of the nanocarriers' components

Natassa Pippa [a,b], Anastasia Meristoudi [b], Stergios Pispas [b], Costas Demetzos [a,*]

[a] Department of Pharmaceutical Technology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis Zografou 15771, Athens, Greece

[b] Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, Athens 11635, Greece



Stimuli-responsive thermosensitive liposomes as drug delivery (aDDnSs) incorporating Indomethacin

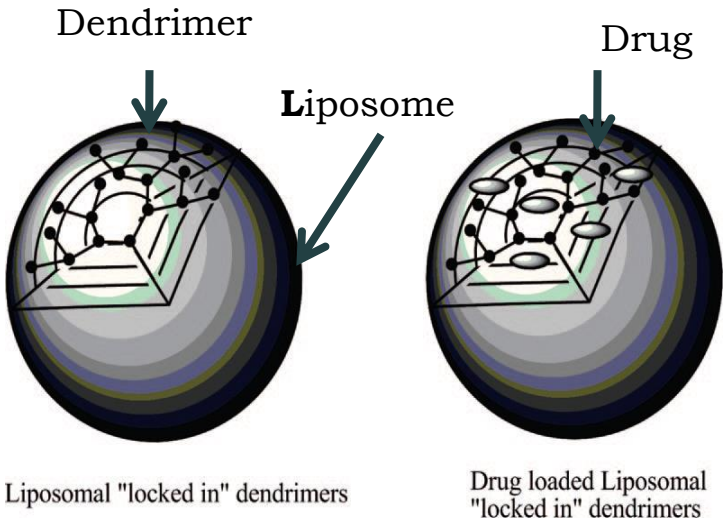
- The presence of the polymeric component plays a key role in the thermal behavior of mixed lipid based nanovectors due to the structural rearrangement of liposomal membrane.
- Temperature-dependent release of IND was observed from chimeric liposomes, due to the well known thermotropic conformational transition of the grafted PNIPAM chains.

Chimeric Chi- aDDnS

Liposomes + Pamam + Doxorubicin

Liposomes + Pamam + Methotraxate

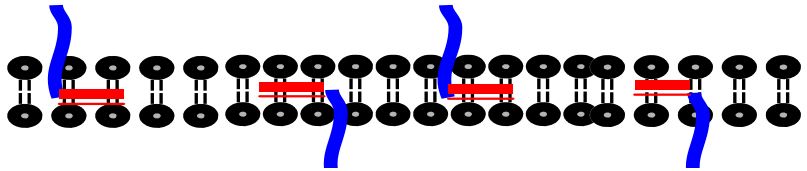
Ref. Klopade et al.,Int. J. Pharm., 2002



Ref. 1. Papagiannaros, Demetzos et al., *Int. J. Pharm.* 2005

2. Papagiannaros, Demetzos, National Patent, 2006

3. Gardikis, Demetzos et al.,, J.Pharm.Sc., 2010



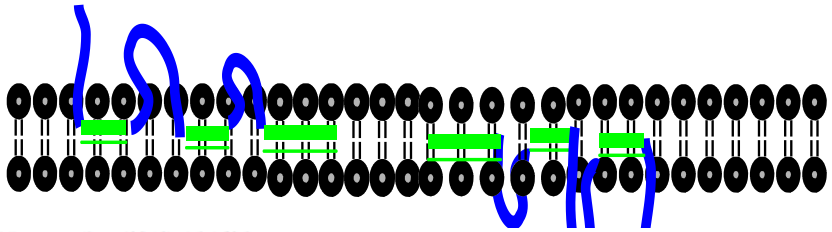
Soft Matter RSCPublishing

PAPER

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

Cite this: *Soft Matter*, 2013, 9, 4073

Natassa Pippa,[ab] Eleni Kaditi,[a] Stergios Pispas*[a] and Costas Demetzos[b]



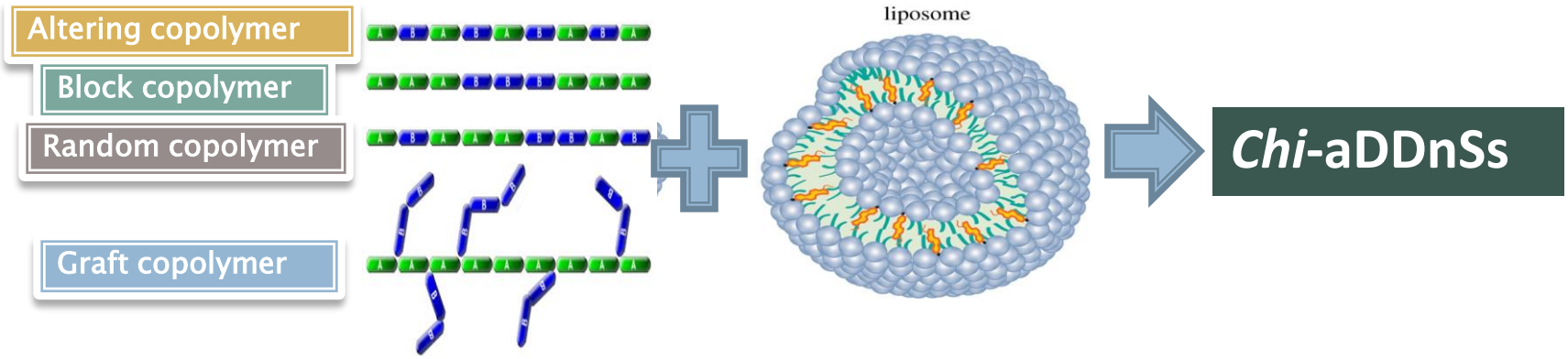
J Nanopart Res (2013) 15:1685
DOI 10.1007/s11051-013-1685-3

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

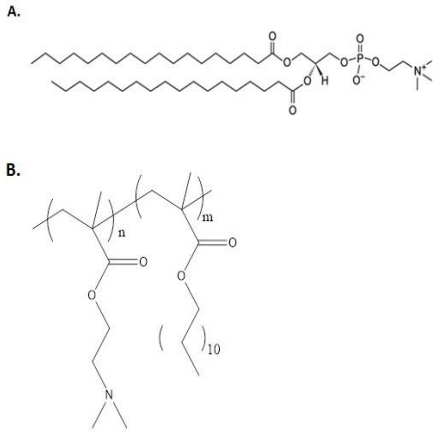
CHIMERIC (polymer and Liposome) NANOCARRIERS IN PHARMACEUTICS



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.

Morphological Diversity of Block Copolymer/Lipid Chimeric Nanostructures

N. Naziris, N. Pippa, V. Chrysostomou, S. Pispas*, C. Demetzos*,M. Libera, B Trzebicka*



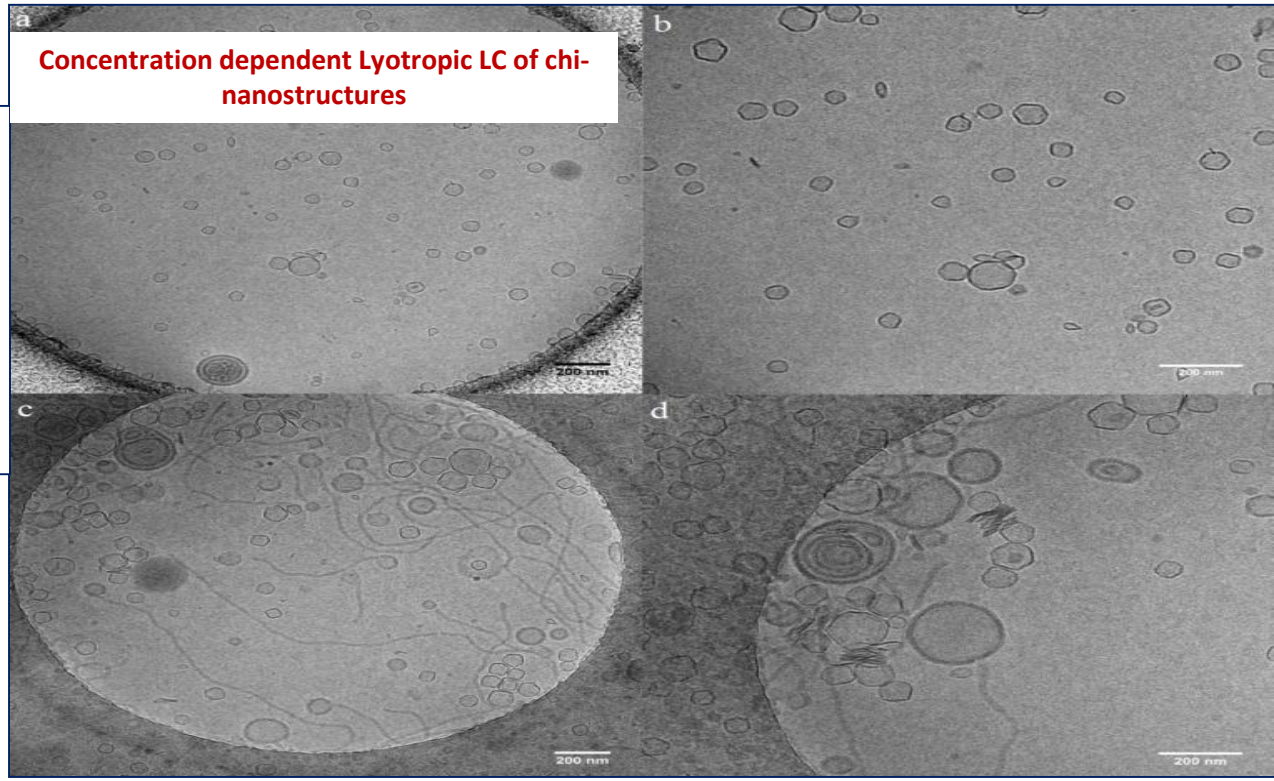
Chemical structures of A) HSPC phospholipid and B) PDMAEMA-b-PLMA block copolymer

These data have been presented in the International Congress of FIP, in Sweden , 2017

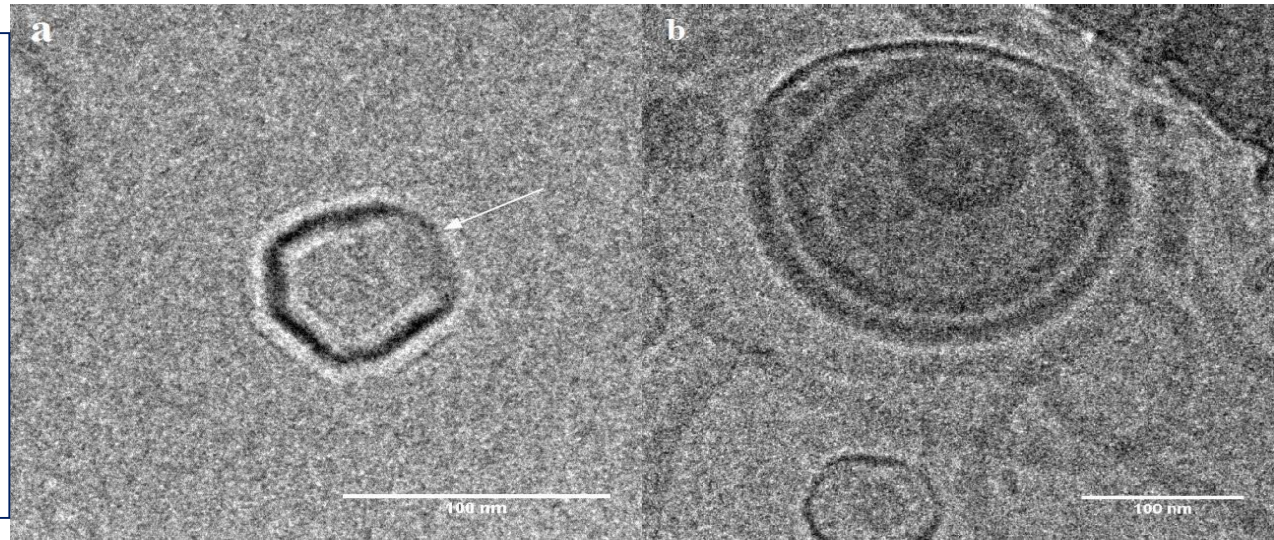
The creation of different in structure self assemblies is a concentration dependent phenomenon and Cryo-TEM and light scattering techniques are employed, in order to extract information on the vesicle **physicochemical characteristics and morphology The elucidation of the details in the morphology of chimeric nanostrutures helps in understanding the biophysical mechanism of their formation and will assist future studies to clarify their interactions with biological Substrates i.e proteins.**

Concentration dependent Lyotropic LC of chi-nanostructures

Cryo-TEM images of aggregates of HSPC:PDMAEMA-b-PLMA 2 chimeric systems, in molar ratios 9:0.1 (a, b) and 9:0.5 (c, d).



Cryo-TEM images of heterogeneous membrane liposome (a) and heterogeneous membrane polymersome (b) of HSPC:PDMAEMA-b-PLMA 2 chimeric system, in molar ratio 9:0.5.



Conclusions I
Biophysical remarks

It is important to figure out that the self-assembly process and the organization of nanoparticulate systems such as liposomes as well as the physical stability of *chimeric*-liposomal systems based on the *lyotropic effect* and on the *metastable phases* of the phospholipid bilayers, is of great importance and could be used as an artificial substrate for evaluating human diseases

SELF-ASSEMBLED SUPRAMOLECULAR ARCHITECTURES

LYOTROPIC LIQUID CRYSTALS

Edited by
Nissim Garti
Ponisseril Somasundaran
Raffaele Mezzenga

A JOHN WILEY & SONS, INC., PUBLICATION

Ref. '*Domains and Rafts in lipid membranes*' published by W. H. Binder, V. Barragan, and F. M. Menger in the Journal of *Angew. Chem. Ind. Ed*, 2003, 42, 5802-5827.

What we need …

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

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

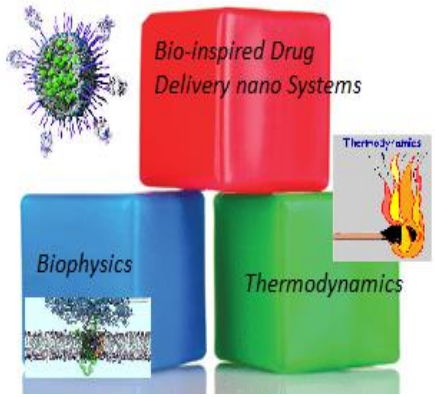
size, size distribution, ζ-potential, shape, surface properties, etc

- 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

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



Ref. Demetzos C (2015) *Biophysics and Thermodynamics: the scientific blocks of bio-inspired drug delivery nano systems.* AAPS PharmSciTEch 16(3):491-495.

AAPS PharmSciTech, Vol. 16, No. 3, June 2015 (© 2015)
DOI: 10.1208/s12249-015-0321-1

Mini-Review

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

Costas Demetzos[1,2]

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; pharmaceutics; thermal analysis; thermodynamics.

J Therm Anal Calorim (2014) 116:1–3
DOI 10.1007/s10973-013-3616-z

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Chemistry and Physics of Lipids 144 (2006) 85–95

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CHEMISTRY AND PHYSICS OF LIPIDS
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Special Chapter on Current Advancement of Thermal Analysis with Special Focus on Bio- and Pharmaceutica Researches from the Mediterranians

The role of the anticancer drug vinorelbine in lipid bilayers using differential scanning calorimetry and molecular modeling

C. Koukoulitsa[a], I. Kyrikou[a], C. Demetzos[b], T. Mavromoustakos[a,*]

Thermodynamics of bioactive molecules, New Molecular Entities, drug delivery systems and excipients is considered as important tool for the development of drugs. Thermodynamics contributes to the laboratory research and to the scale up process in the Pharmaceutical Industry.

Guest Editor

Prof. Costas Demetzos
In Pharmaceutical Technology and Nano-technology
Director of the Laboratory of Pharmaceutical Technology
Faculty of Pharmacy, University of Athens, Greece
E-mail: demetzos@pharm.uoa.gr

Colloids and Surfaces B: Biointerfaces 82 (2011) 8–12

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Interaction of cationic phosphorus dendrimers (CPD) with charged and neutral lipid membranes

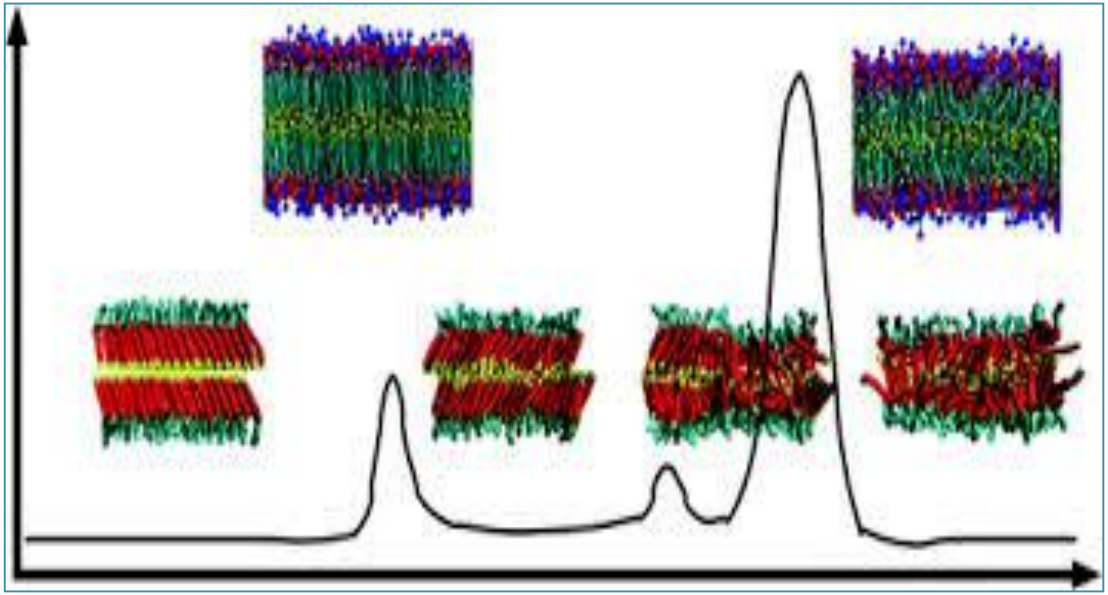
Maksim Ionov[a,*], Konstantinos Gardikis[b], Dominika Wróbel[a], Sophia Hatziantoniou[b], Helena Mourelatou[b], Jean-Pierre Majoral[c], Barbara Klajnert[a], Maria Bryszewska[a], Costas Demetzos[b]

The modulation of thermal properties of vinblastine by cholesterol in membrane bilayers

Ioanna Kyrikou[a], Ioanna Daliani[a], Thomas Mavromoustakos[a,*], Hamzah Maswadeh[b], Costas Demetzos[b], Sophia Hatziantoniou[b], Sarantis Giatrellis[c], George Nounesis[c]

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



Ref. Koynova R., Caffrey M., Phases and phase transition of the phosphatidylocholines, Biochim. Biophys. Acta 1376, 91-145, 1998

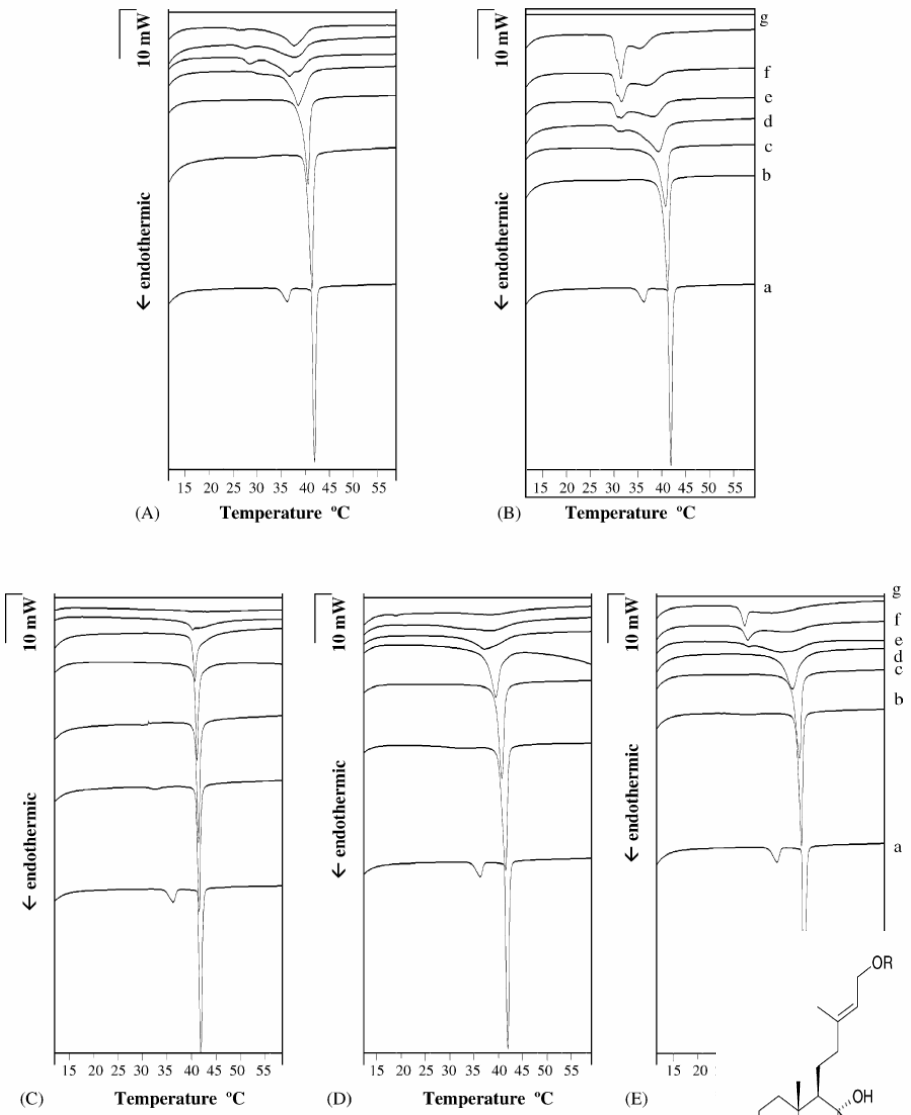
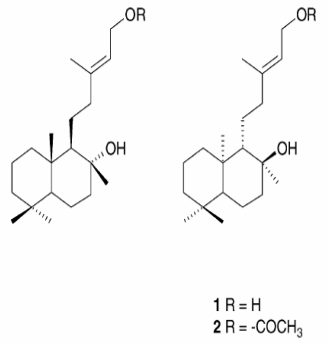


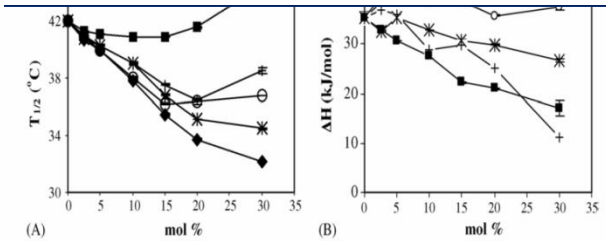
Fig. 2. DSC thermograms of fully hydrated bilayers of DPPC with varying amounts (a, 0 mol%; b, 2.5 mol%; c, 20 mol%; g, 30 mol%) of compound **1** (A), compound **2** (B), cholesterol (C), equimolar mixture of cholesterol a mixture of cholesterol and compound **2** (E).

Table 1
Calorimetric parameters

Sample (x = mol%)	T_{onset} (°C)	S.D.	T_m (°C)	S.D.	$T_{1/2}$ (°C)	S.D.	ΔH (kJ/mol)	S.D.
DPPC	41.28	0.16	41.67	0.15	41.96	0.17	35.48	1.14
DPPC/cholesterol (x=2.5)	40.72	0.01	41.31	0.02	41.24	0.01	32.79	0.08
DPPC/cholesterol (x=5)	40.57	0.01	41.15	0.01	41.09	0.01	30.87	0.02
DPPC/cholesterol (x=10)	40.35	0.01	40.84	0.00	40.86	0.01	27.56	0.13
DPPC/cholesterol (x=15)	39.87	0.00	40.49	0.00	40.90	0.00	22.24	0.24
DPPC/cholesterol (x=20)	38.64	0.03	40.18	0.03	41.55	0.02	21.10	0.14
DPPC/cholesterol (x=30)	38.83	0.89	41.20	0.02	44.48	0.43	17.21	1.51
DPPC/**1** (x=2.5)	40.02	0.01	41.07	0.02	40.86	0.01	38.64	0.06
DPPC/**1** (x=5)	38.96	0.04	40.39	0.00	39.98	0.01	39.28	0.10
DPPC/**1** (x=10)	35.77	0.12	38.38	0.02	38.05	0.01	38.56	0.17
DPPC/**1** (x=15)	32.45	0.18	36.52	0.09	36.20	0.05	38.74	0.20
DPPC/**1** (x=20)	31.03	0.31	37.69	0.11	36.37	0.19	35.83	0.09
DPPC/**1** (x=30)	33.61	0.04	37.50	0.06	36.76	0.02	37.52	0.66
DPPC/Chol/**1** (x=1.25+1.25)	39.91	0.02	41.21	0.04	40.95	0.01	37.04	0.10
DPPC/Chol/**1** (x=2.5+2.5)	39.22	0.02	40.47	0.01	40.27	0.01	35.98	0.11
DPPC/Chol/**1** (x=5+5)	37.31	0.03	39.27	0.03	39.06	0.02	28.88	0.23
DPPC/Chol/**1** (x=7.5+7.5)	32.84	0.10	37.07	0.12	37.52	0.04	29.84	0.12
DPPC/Chol/**1** (x=10+10)	28.75	0.22	38.54	0.14	36.49	0.03	25.21	0.15
DPPC/Chol/**1** (x=15+15)	31.71	0.49	38.58	0.09	38.52	0.18	11.06	0.33
DPPC/**2** (x=2.5)	39.76	0.01	40.99	0.02	40.71	0.00	39.81	0.17
DPPC/**2** (x=5)	38.64	0.08	40.60	0.05	39.99	0.05	38.88	0.21
DPPC/**2** (x=10)	34.33	0.24	39.19	0.08	37.76	0.06	39.14	0.29
DPPC/**2** (x=15)	29.68	0.26	31.39	0.06	35.45	0.09	40.19	0.22
DPPC/**2** (x=20)	29.55	0.01	31.45	0.07	33.65	0.11	40.57	0.09
DPPC/**2** (x=30)	29.83	0.23	31.31	0.02	32.17	0.02	39.82	0.04
DPPC/Chol/**2** (x=1.25+1.25)	40.04	0.01	40.96	0.00	40.79	0.00	32.71	0.01
DPPC/Chol/**2** (x=2.5+2.5)	39.08	0.03	40.63	0.03	40.29	0.02	35,41	0.24
DPPC/Chol/**2** (x=5+5)	36.63	0.05	39.25	0.05	38.98	0.02	32.91	0.10
DPPC/Chol/**2** (x=7.5+7.5)	30.21	0.31	36.85	0.20	36.82	0.04	30.80	0.22
DPPC/Chol/**2** (x=10+10)	28.63	0.07	30.14	0.04	35.11	0.03	29.96	0.12
DPPC/Chol/**2** (x=15+15)	28.27	0.10	29.56	0.04	34.48	0.04	26.72	0.29

T_{onset}, temperature at which the thermal effect starts; T_m, temperature at which heat capacity (ΔC_p) at constant pressure, is maximum; $T_{1/2}$, ich the transition is half completed; ΔH, transition enthalpy normalised per mole of DPPC; **1**, labd-13(E)-ene-8α,15-diol; **2**, E)-ene-8α-ol-15-yl-acetate.



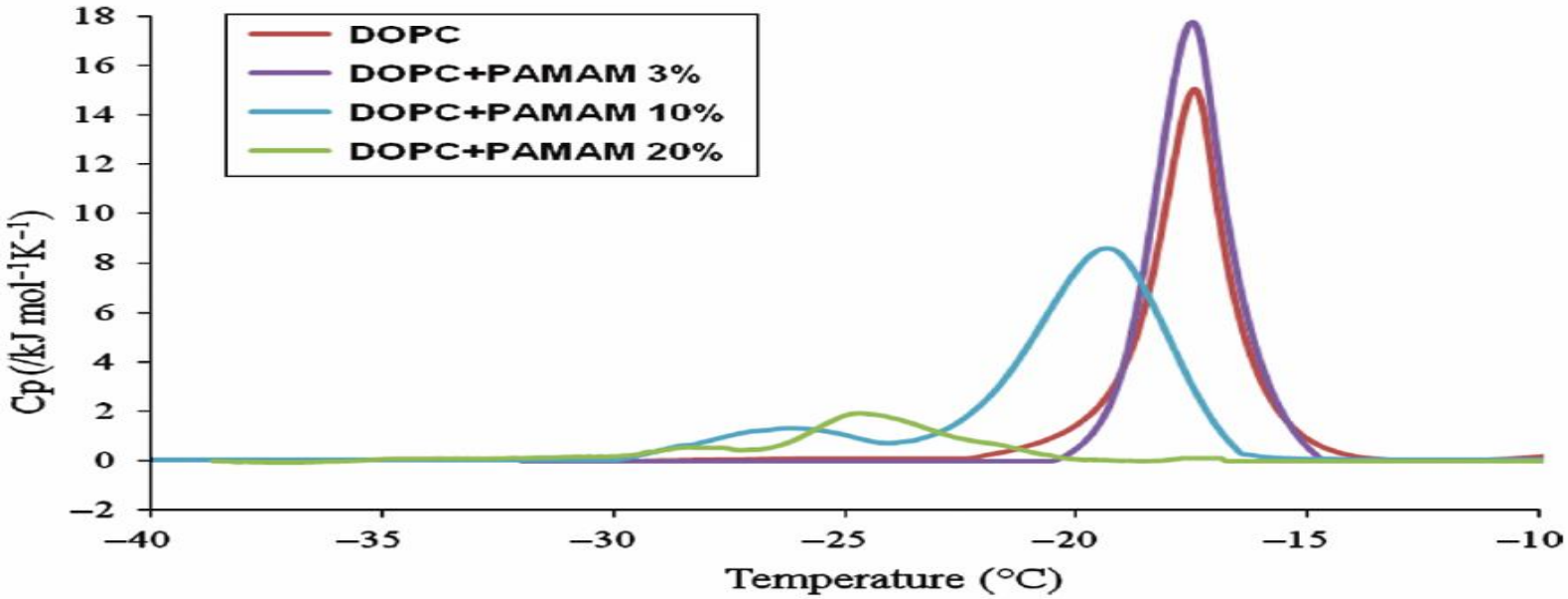


and ΔH (J/mol DPPC) values of DPPC bilayers vs. concentrations of compound **1** (○), **2** (♦), cholesterol (■), equimolar mixture (+) and equimolar mixture of cholesterol/**2** (★).

DSC thermograms of fully hydrated bilayers of DPPC with varying amounts (a: 0 mol%, b: 2.5 mol%, c: 5 mol%, d: 10 mol%, e: 15 mol%, f: 20 mol%, g: 30 mol%) of compound 1 (A), compound 2 (B), cholesterol (C), equimolar mixture of cholesterol and compound 1 (D) and equimolar mixture of cholesterol and compound 2 (E). (Adapted with permission from Elsevier, from *Chem Phys Lipids,* 2005 138, 1-11. Labdane-type diterpenes: thermal effects on phospholipid bilayers, incorporation into liposomes and biological activity. Matsingou, C; Hatziantoniou, S; Georgopoulos, A; Dimas, K; Terzis, A; Demetzos, C.)

Applications of DSC on Lipid Bilayers and on Liposomes

Lipidic bilayers (structural components of liposomes) Incorporating PAMAM Dendrimer



DSC thermograms of DOPC lipid bilayers in the presence of increasing concentrations of PAMAM dendrimer. (**Adapted from *J NanosciNanotechnol,* 2011 11, 3764-3772. A New Chimeric Drug Delivery Nano System (chi-aDDnS) Composed of PAMAM G 3.5 Dendrimer and Liposomes as Doxorubicin's Carrier. Gardikis, K; Fessas, D; Signorelli, M; Dimas, K; Tsimplouli, C; Ionov, M; Demetzos, C.**)

Thermal Transitions Metastable Phases

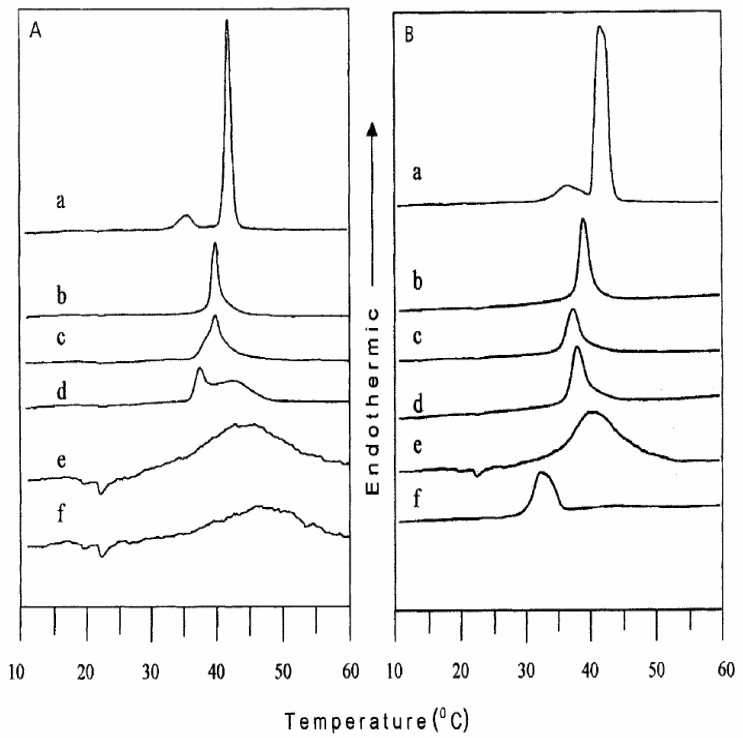
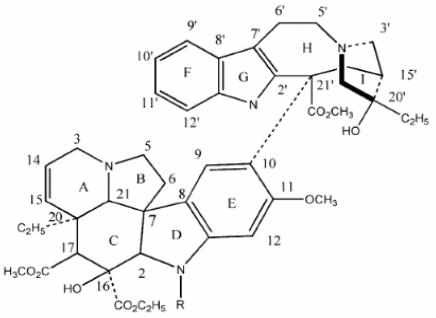


Figure 4 A. DSC calorimetry scan of: DPPC (a); DPPC–cholesterol (x = 0.10) (b); DPPC+cholesterol (x = 0.10)+vinblastine (x = 0.045) (c); DPPC+cholesterol (x = 0.10)+vinblastine (x = 0.17) (d); DPPC–cholesterol (x = 0.30) (e); and DPPC–cholesterol (x = 0.30) +vinblastine (x = 0.17) (f). B. DSC calorimetry scan of: DPPG (a); DPPG–cholesterol (x = 0.10) (b); DPPG+cholesterol (x = 0.10)+vinblastine (x = 0.045) (c); DPPG+cholesterol (x = 0.10)+vinblastine (x = 0.17) (d); DPPG–cholesterol (x = 0.30) (e); and DPPG–cholesterol (x = 0.30)+vinblastine (x = 0.17) (f). x represents molar ratio.



VINBLASTINE

Table 1 Values of pretransition temperature ($T_{pretrans}$), half-width temperature ($T_{m\frac{1}{2}}$), peak temperature ($T_{m\frac{1}{2}}$), peak temperature (T_m) and enthalpy change (ΔH) of phospholipid bilayers without and with vinblastine and phospholipid-cholesterol without or with vinblastine.

Samples	$T_{pretrans}$	T_m (°C)	$T_{m\frac{1}{2}}$ (°C)	ΔH (cal g^{-1})
DPPC	34.8	41.2	2.8	1.11±0.04
			1.0	9.96±0.08
DPPC+cholesterol (x = 0.10)		39.5	1.1	6.65±0.44
DPPC+cholesterol+vinblastine (x = 0.045)		38.9	3.0	8.11±0.56
DPPC+cholesterol+vinblastine (x = 0.17)		36.2	7.5	8.31±0.32
DPPC+cholesterol (x = 0.3)		41.7	16	3.02±0.15
DPPC+cholesterol+vinblastine (x = 0.17)		44.9	17.5	1.69±0.26
DPPG	36.3	40.7	5.0	1.08±0.02
			2.25	10.74±0.04
DPPG+cholesterol (x = 0.10)		38.0	1.9	7.97±0.50
DPPG+cholesterol+vinblastine (x = 0.045)		35.9	2.5	5.54±0.24
DPPG+cholesterol+vinblastine (x = 0.17)		36.7	2.5	7.48±0.27
DPPG+cholesterol (x = 0.30)		39.2	8.5	4.53±0.13
DPPG+cholesterol+vinblastine (x = 0.17)		31.8	4.0	4.88±0.04

Marqibo , liposomal vincristine 2012 approved FDA, lymphoblastic leukemia (Orphan Drug approval process)

C. Demetzos, D. Angelopoulou, A. Kolocouris, I. Daliani, and T. Mavromoustakos (2001) Structure Elucidation, Conformational Analysis and Thermal Effects on Membrane Bilayers of an Antimicrobial Myricetin Ether Derivative ; H. Maswadeh, C. Demetzos, K. Dimas, Y. L. Loukas, A. Georgopoulos, T. Mavromoustakos and G. Th. Papaioannou (2002) In-vitro cytotoxic/cytostatic activity of anionic liposomes containing vinblastine against leukaemic human cell lines

Metastable phases play an important role in the behavior of lipid membranes. Topics touched upon include the experimental detection of domains, their composition, domain induction, properties of rafts (a special form of domain), and **the relationship of rafts to human diseases**.

The results from the DSC experiments based on calculated thermodynamic parameters could be used not only to select appropriate biomaterials for designing an effective and stable liposomal DDnS
BUT
to study the metastable phases in artificial phospholipid membrane bilayers

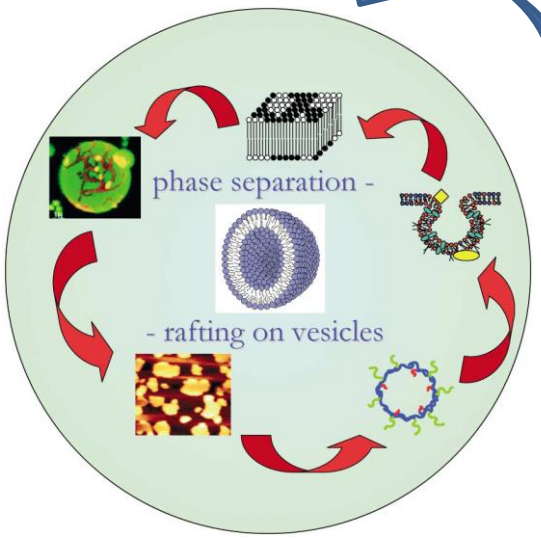
These metastable phases, can cause biophysical and thermodynamic abnormalities that could be defined as ' ***biophysical disease factors***'

'Pharmaceutical Nanotechnology. Funtamentals and practical applications',

by

Costas Demetzos

Springer, 2016



Domains and Rafts in Lipid Membranes
by
Wolfgang H. Binder,*
Veronique Barragan,
and Fredric M. Menger

Angewandte Chemie

Keywords:
block copolymers · domains · lipid membranes · rafts · vesicles

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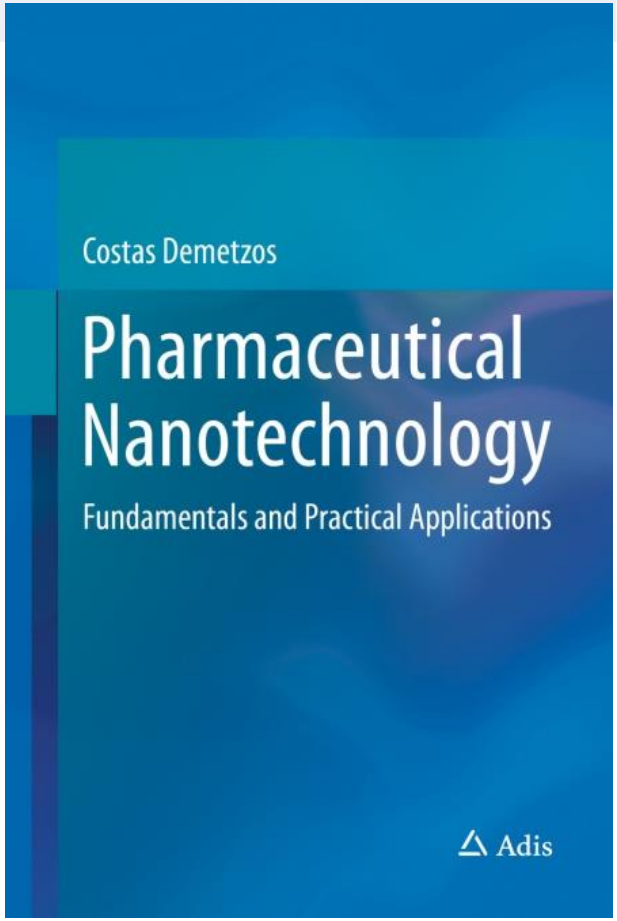


Biophysical Disease Factor

Προλογίζει

Gregory Gregoriadis, PhD, DSc

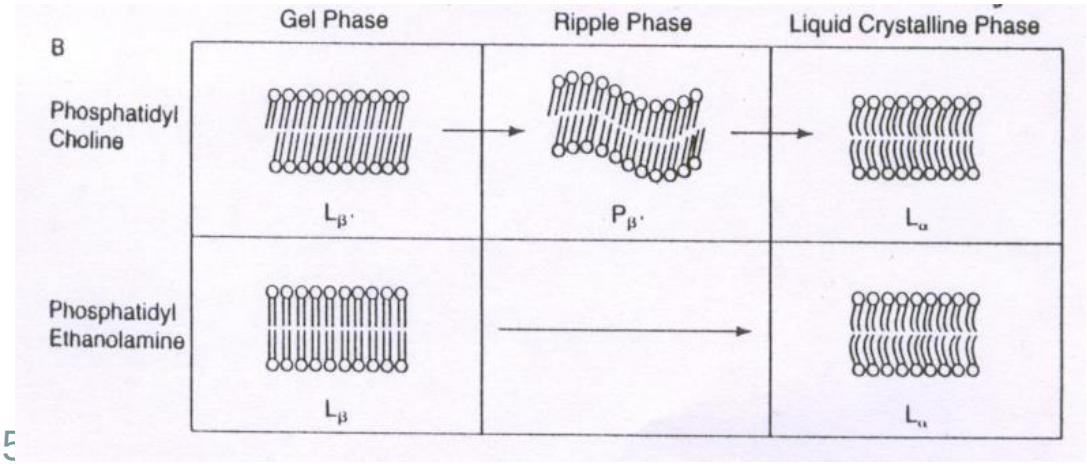
Professor Emeritus,
UCL School of Pharmacy London

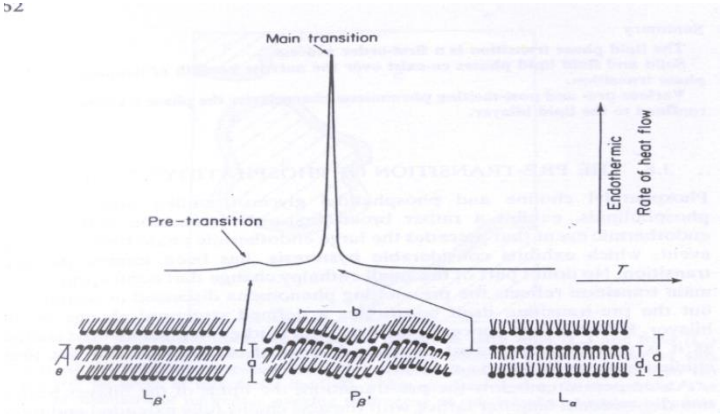
. . . . . It has been therefore a great personal pleasure to write the Preface of the present book by Professor Costas Demetzos.
His monograph, 'Pharmaceutical Nanotechnology', is a unique publication . . . . . .

Going a little further…..

Nano-Thermodynamics

Nanosystems such as liposomal systems are mostly nonequilibrium systems, with **dynamic structural characteristics** and their interactions with the environment being dominated by thermal fluctuations. Pressure in a nanosystem cannot be considered isotropic **Properties considered in classical thermodynamics as intensive at equilibrium, like internal energy, specific enthalpy, specific entropy, specific free Gibbs energy, are not intensive any more.** These are related to the fact that chemical potential (**μ**) for **nanophases depends on the size** (number of atoms) and for the microenviromental factors. **For example …a polymeric guest or drugs affect the microenviroment of lipidic bilayer and create phase separation and metastable phases.**





Thermal Transitions Metastable Phases and Tsallis' entropic index

Thermal transitions well known as METASTABLE PHASES of liposomal membranes and of the living cells, are considered as an emerged field that should be studied based on nano-thermodynamics concept and on the **Tsallis' theory and Hills'** thermodynamics of small systems. The Tsallis' theory introduced the term '***entropic index q***'. ***q:*** describes the degree of non-extensivity of the system. A new kind of entropy, called non- extensive entropy, has been proposed in the literature

- **Inetrfacial energy , must be taken into consideration**
- **Fluctuation of thermodynamic variables should also be taken into consideration**
- **In nanosystems properties such as *T* and *P* are not stable**
- **Quantum effects for small systems may also become important**

$$S_q(p_i) = \frac{k}{q-1}\left(1 - \sum_i p_i^q\right),$$

k is a positive constant, *pi* is the probability of the system at the quantum state *i* , ***q*** is the *'entropic index''*.

This equation can be applied in nano-non extensive systems while preserving the fundamental property of entropy in the Second Law of Thermodynamics.

Entropy is considered to be an extensive property, i.e., that its value depends on the amount of material present. Constantino Tsallis has proposed a nonextensive entropy *(Tsallis entropy) is an extension of the traditional Boltzmann–Gibbs entropy*.
The rationale behind the theory is that Gibbs-Boltzmann entropy leads to systems that have a strong dependence on initial conditions. In reality most materials behave quite independently of initial conditions.
Nonextensive entropy leads to nonextensive statistical mechanics, whose typical functions are power laws, instead of the traditional exponentials.

The concept was introduced in 1988 by Constantino Tsallis as a basis for generalizing the standard statistical mechanics, within Information Theory.

proposed by Claude E. Shannon in 1948 to find fundamental limits on signal processing and communication operations

- **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*.**

Journal of Liposome Research, 18:159–173, 2008

ISSN: 0898-2104 print / 1532-2394 online
DOI: 10.1080/08982100802310261

informa
healthcare

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

Conclusions II
Metastable phases of lipidic membranes

The behavior of bio-membranes as highly complex bio-systems, leads to the formation of **metastable phases**.

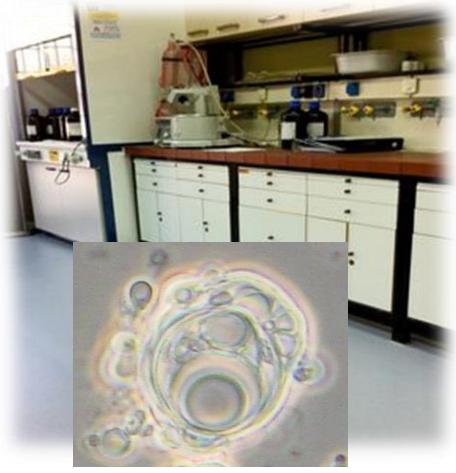
It can be **assumed that new strategies for fighting viral infections, lipid storage disease, cancer, and other diseases** will **arise through the understanding of membrane metastable phases.**

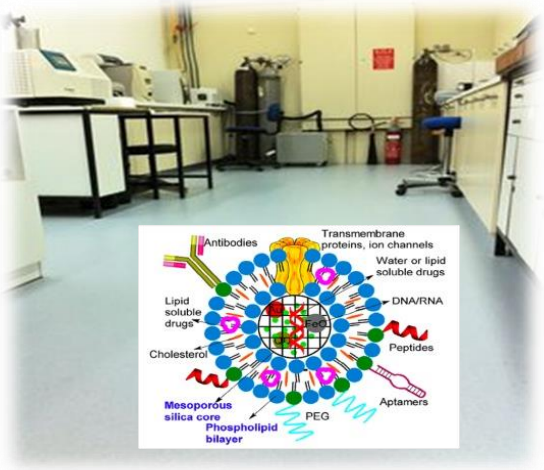
However, lipidic nanoparticulate systems such as liposomes could be efficient technological platforms not only to deliver bioactive molecules but to act as substrates for studying human diseases.

Ref. Costas Demetzos *'Pharmaceutical Nanotechnology. Funtamendals and practical Applicatio*n', 2016, Springer
W. H. Binder, V. Barragan, and F. M. Menger in the Journal of *Angew. Chem. Ind. Ed*, 2003, 42, 5802-5827

Laboratory of Pharmaceutical Nanotechnology University of Athens, Greece

http://nanopharmlab.gr/index.php/en/





National and Kapodistrian University
of Athens
School of Health Sciences
Department of Pharmacy
Lab of Pharmaceutical
Nanotechnology

"ωφελέειν, εἰ μὴ βλάπτειν"

'benefit and do not harm'

Nanotechnology Based Immunotherapy for Glioblastoma

EURONANOMED 2

Partner 5

Prof. Costas Demetzos

Lab of Pharmaceutical Nanotechnology

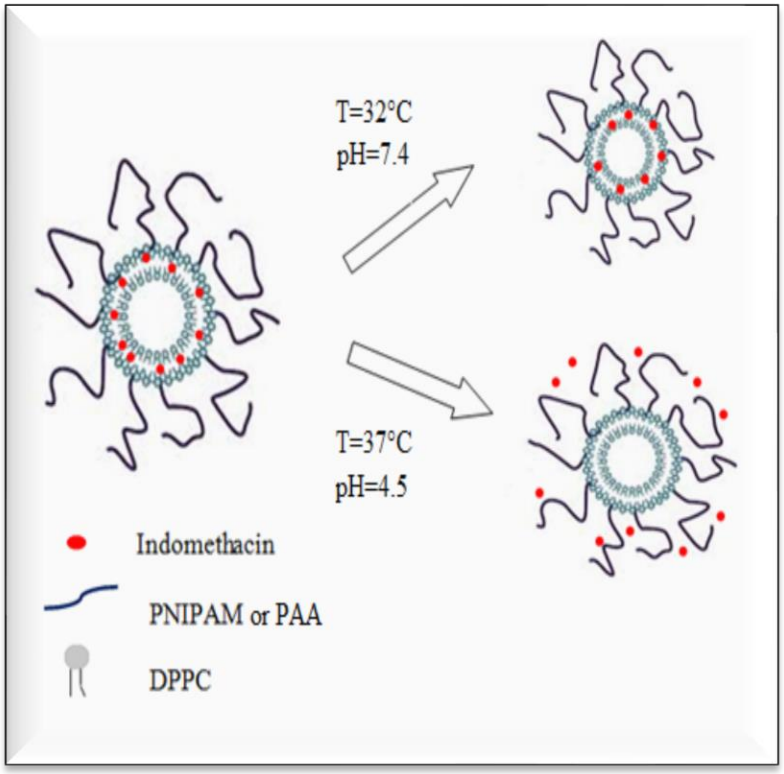
National and Kapodistrian University of Athens (UoA)

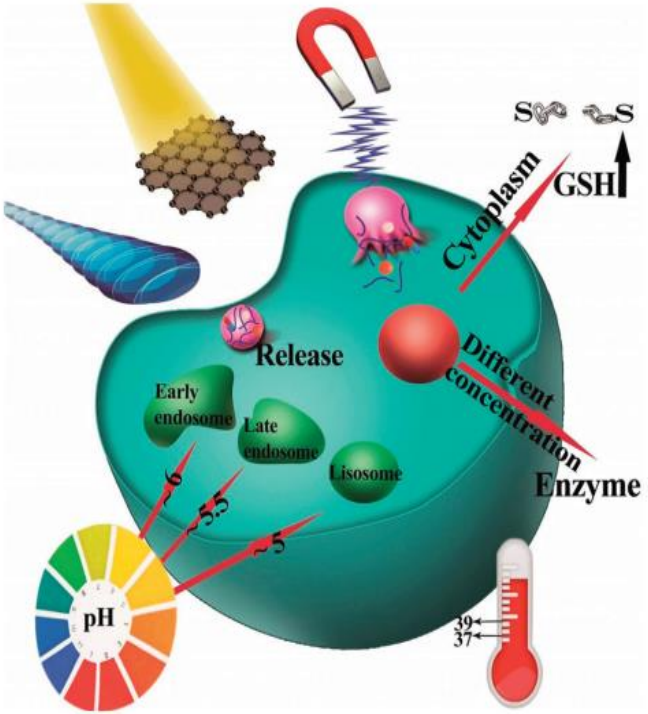
EURONANOMED II
Joint Transnational Call for Proposals (2016)
for
"EUROPEAN INNOVATIVE RESEARCH &
TECHNOLOGICAL
DEVELOPMENT PROJECTS IN

7 Partners

- Santoni (Italy, Clinical) (Immunomodulation)
- Kaminska (Poland, Academia) (Anticancer)
- Peng (France, Academia) (DDSs)
- Videira (Portugal, Academia) (DDSs)
- Demetzos (Greece, Academia) (DDSs)
- Pricl (Italy, Academia) (Computer Modeling)
- March (France, Academia) (Toxicity)

Chimeric Stimuli-Responsive Liposomes





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Dr.N. Tagmatarchis, NHRF,

Institute of Theoretical and Physical Chemistry

- Dr. **Stergios Pispas**

Prof. **Nissim Garti**
Casali Institute of Applied Chemistry, The Institute of Chemistry, The Hebrew University of Jerusalem, Israel

Dr. ***Zoe Cournia***, BRFAA

Prof. **Dimitrios Fessas,**
Department of Food Science, Technology and Microbiology, University of Milan, Milan, Italy

Prof. D.Tomalia Prof. **M. Makropoulou**, NTUA,

Thank you for your kind attention

Vincent van Gogh
Branch of almond – tree in flowers, 1890