



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

Connecting the Morphology with the Thermodynamic and Biophysical Properties of pH-responsive Chimeric Nanosystems

Nikolaos Naziris, Pharmacist, MSc
PhD Student in Pharmaceutical Nanotechnology



International
Liposome
Society

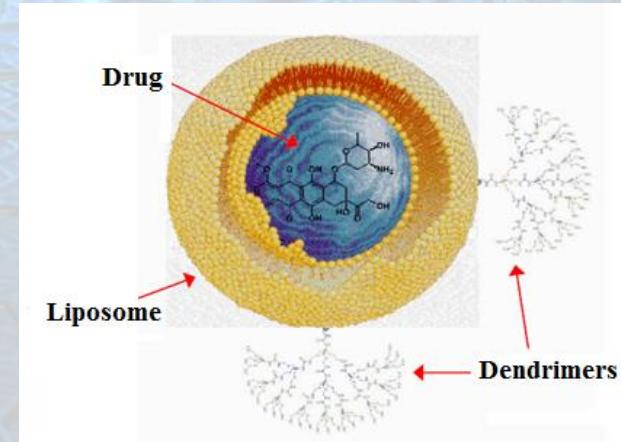
PROGRESS IN LIPOSOMAL
DRUG AND VACCINE DELIVERY



nanolab.pharm.uoa.gr

Chimeric Liposomes

- “Chimera”
- *Mixed or Hybrid*
 - Different Types of Biomaterials
e.g. Lipids + Polymers
or Lipids + Dendrimers
- New Class of Innovative Excipients for Medicine
- New Functionalities



**Drug
Delivery**

<http://informahealthcare.com/drdr>
ISSN: 1071-7544 (print), 1521-0464 (electronic)

Drug Deliv, Early Online: 1-8
© 2013 Informa Healthcare USA, Inc. DOI: 10.3109/10717544.2013.844745

informa
healthcare

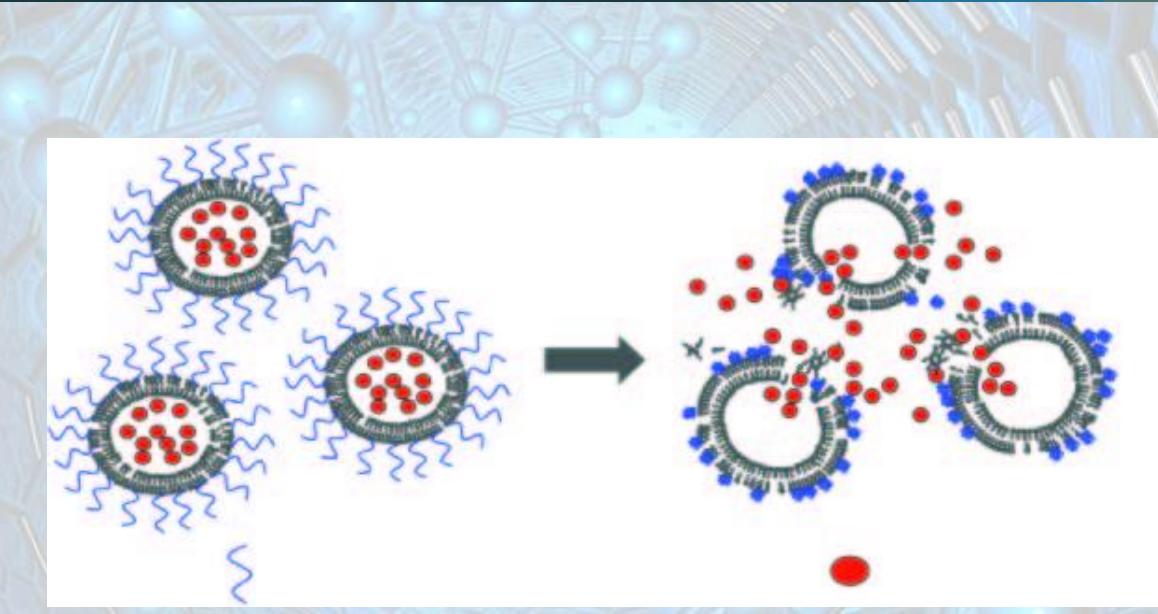
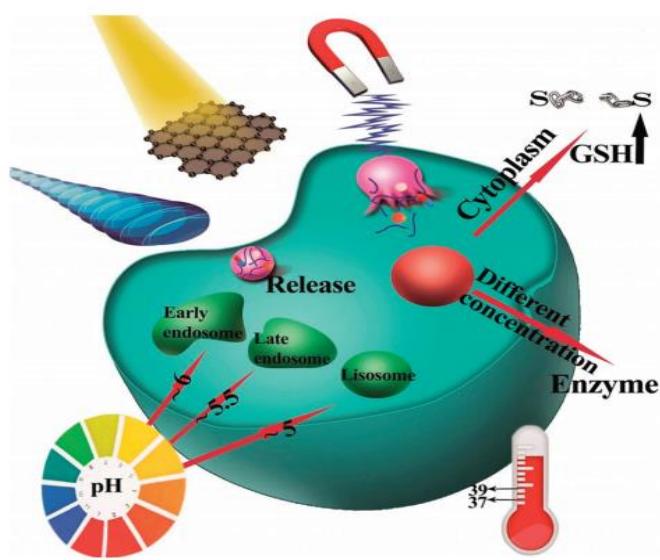
CRITICAL REVIEW

Advanced drug delivery nanosystems (aDDnSs): a mini-review

Costas Demetzos and Natassa Pippa

Department of Pharmaceutical Technology, Faculty of Pharmacy, Panepistimioupolis Zografou, University of Athens, Athens, Greece

Stimuli-Responsive Nanosystems



Current Nanomedicine, 2016, 6, 1-20

1

REVIEW ARTICLE

Stimuli-responsive Drug Delivery Nanosystems: From Bench to Clinic

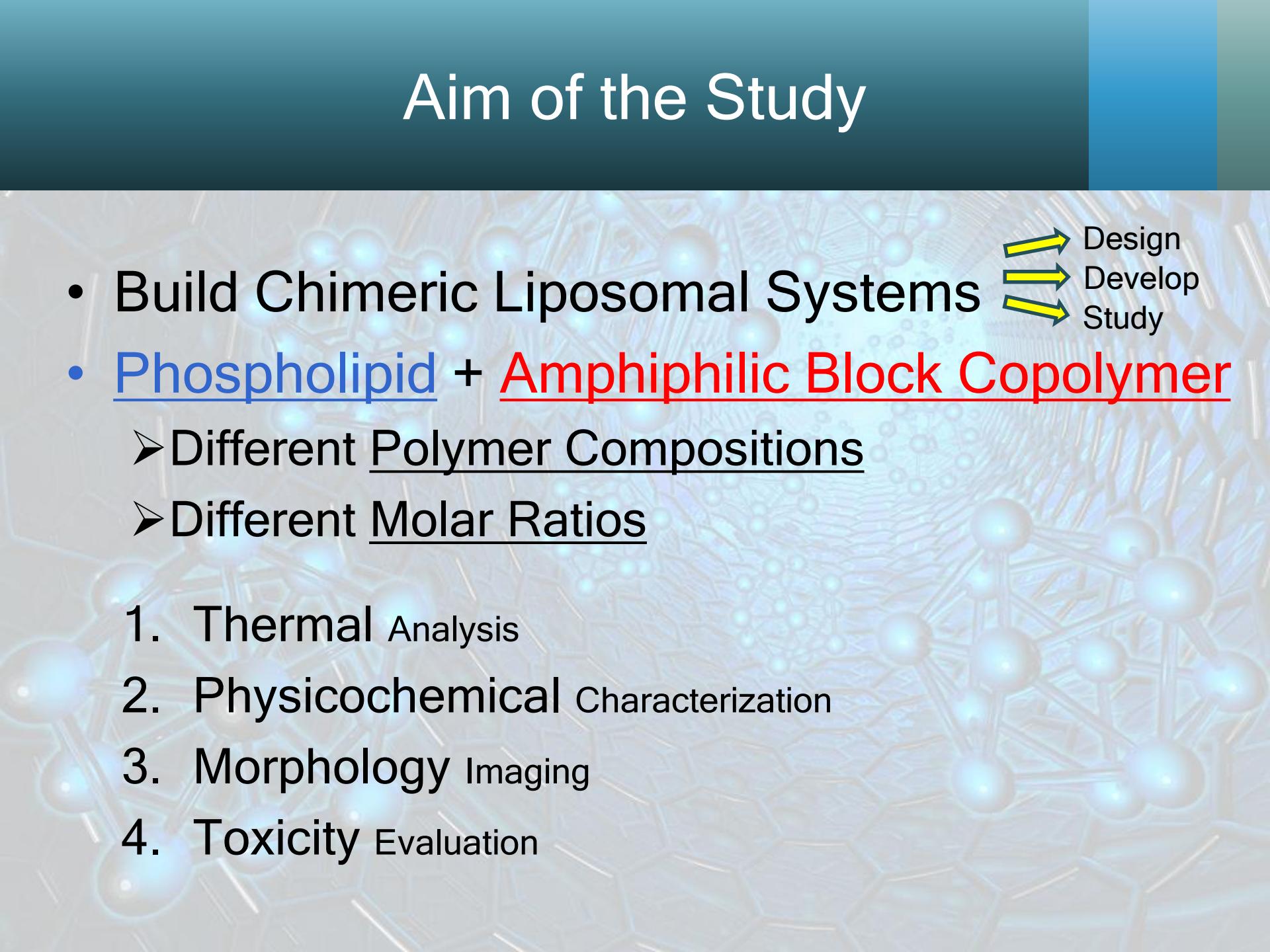
Nikolaos Naziris^a, Natassa Pippa^{a,b}, Stergios Pispas^{b,*} and Costas Demetzos^a

^aDepartment of Pharmaceutical Technology, Faculty of Pharmacy, Panepistimioupolis Zografou 15771, National and Kapodistrian University of Athens, Athens, Greece; ^bTheoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 11635, Athens, Greece

Smart
Functional

Aim of the Study

- Build Chimeric Liposomal Systems
 - Phospholipid + Amphiphilic Block Copolymer
 - Different Polymer Compositions
 - Different Molar Ratios
1. Thermal Analysis
 2. Physicochemical Characterization
 3. Morphology Imaging
 4. Toxicity Evaluation

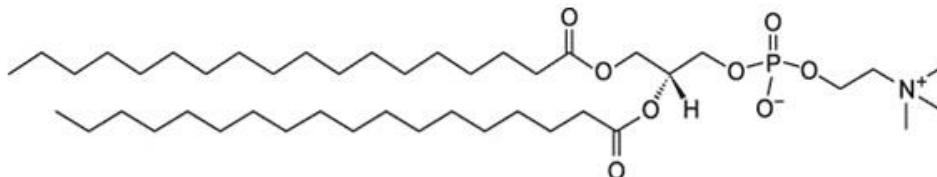


Design
Develop
Study

Biomaterials

A.

Amphiphilic

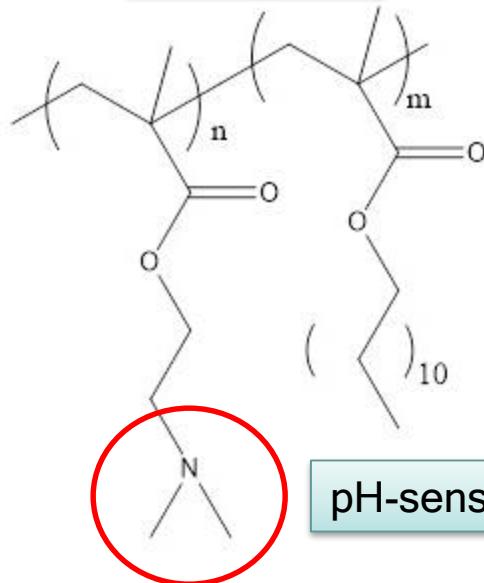


HSPC

L-a-phosphatidylcholine, hydrogenated (Soy)

B.

Amphiphilic



pH-sensitive

PDMAEMA-b-PLMA

poly(2-(dimethylamino)ethyl methacrylate)-b-poly(lauryl methacrylate)



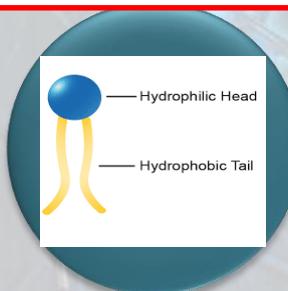
1: 70-30



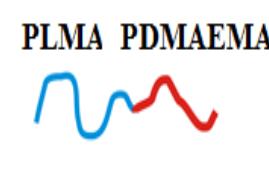
2: 60-40

Chimeric Liposome Preparation

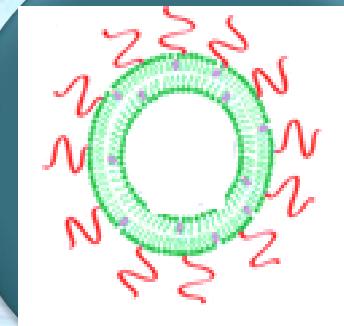
Phospholipid



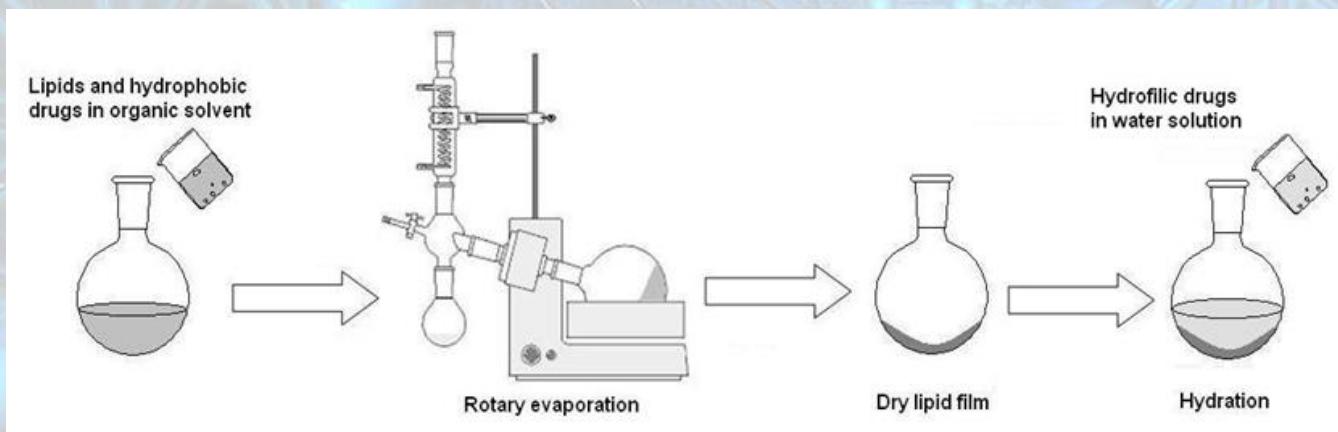
Block Copolymer



Polymer-Grafted Liposome

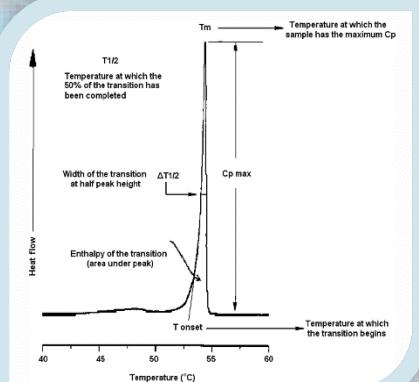


Thin-Film Hydration Method

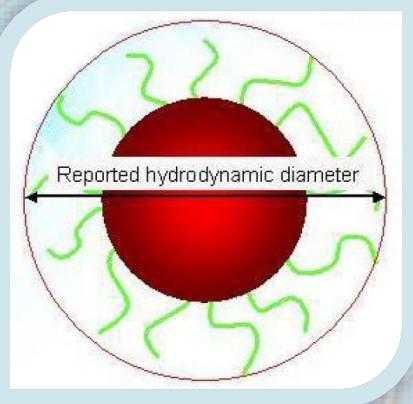


Methods of Analysis

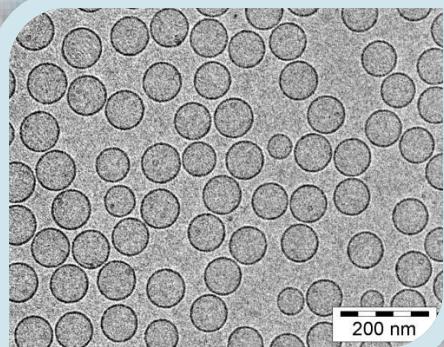
Thermal
Analysis
Techniques
(DSC)



Light
Scattering
Techniques
(DLS, ELS,
SLS)



Imaging
Techniques
(AFM, Cryo-
TEM)

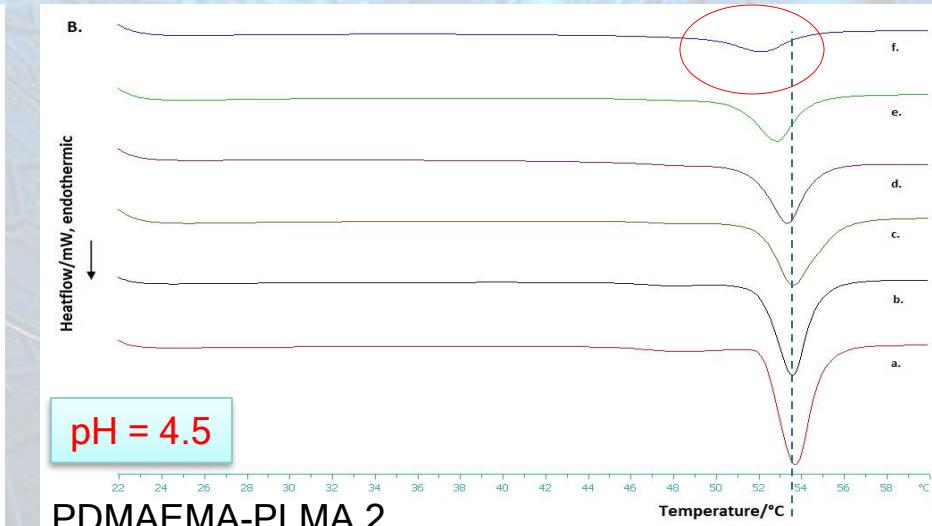
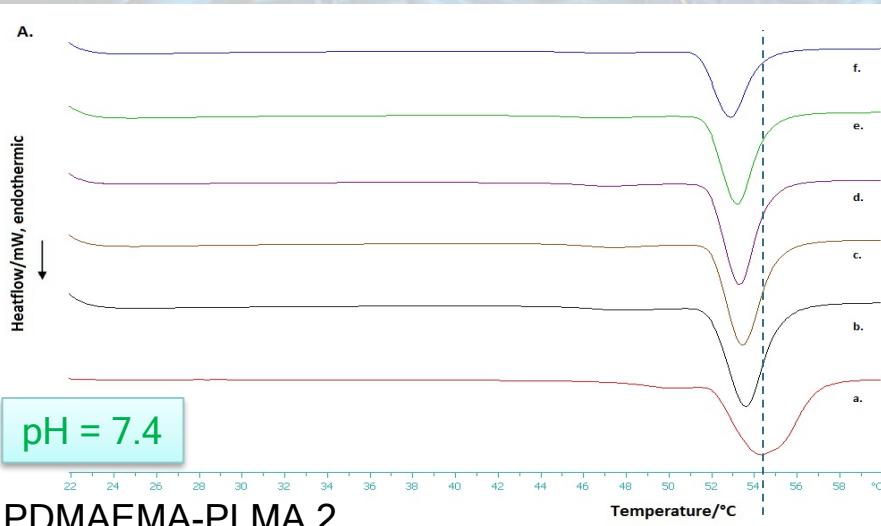
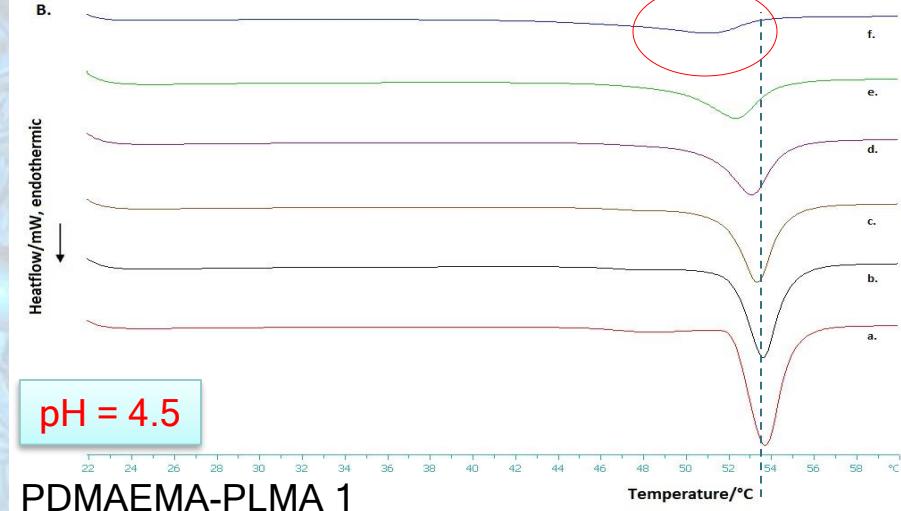
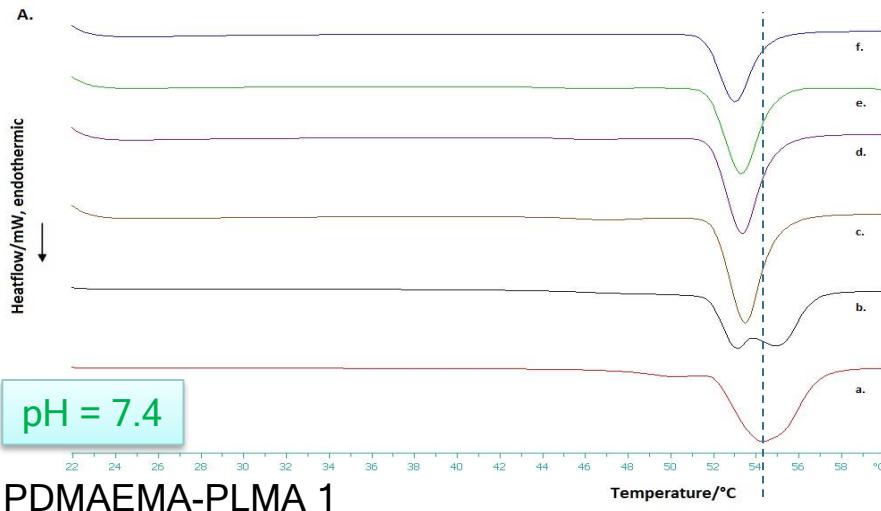


In Vitro
Screening
(MCF-10A)



Results

DSC



Results

DSC

Sample	Molar Ratio	Dispersion Medium	T _{onset,m} (°C)	T _m (°C)	ΔT _{1/2,m} (°C)	ΔH _m (J g ⁻¹)	T _{onset,s} (°C)	T _s (°C)	ΔT _{1/2,s} (°C)	ΔH _s (J g ⁻¹)
HSPC	-	PBS (pH=7.4)	52.1	54.3	3.07	50.2	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.02	PBS (pH=7.4)	51.8	53.1	3.64	46.9	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.05	PBS (pH=7.4)	52.0	53.5	1.65	48.2	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.1	PBS (pH=7.4)	51.9	53.3	1.68	44.3	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.2	PBS (pH=7.4)	51.8	53.3	1.74	39.6	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.5	PBS (pH=7.4)	51.6	53.0	1.65	30.8	-	-	-	-
HSPC	-	pH=4.5	52.2	53.8	1.62	59.4	45.6	48.2	3.17	2.8
HSPC:PDMAEMA-b-PLMA 1	9:0.02	pH=4.5	52.1	53.6	1.61	58.7	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.05	pH=4.5	51.7	53.3	1.71	53.3	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.1	pH=4.5	50.8	53.1	2.27	46.9	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.2	pH=4.5	49.2	52.3	2.93	42.2	-	-	-	-
HSPC:PDMAEMA-b-PLMA 1	9:0.5	pH=4.5	45.2	51.0	5.01	24.1	-	-	-	-

Sample	Molar Ratio	Dispersion Medium	T _{onset,m} (°C)	T _m (°C)	ΔT _{1/2,m} (°C)	ΔH _m (J g ⁻¹)	T _{onset,s} (°C)	T _s (°C)	ΔT _{1/2,s} (°C)	ΔH _s (J g ⁻¹)
HSPC	-	PBS (pH=7.4)	52.1	54.3	3.07	50.2	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.02	PBS (pH=7.4)	52.0	53.6	1.87	48.4	45.1	47.7	3.16	1.8
HSPC:PDMAEMA-b-PLMA 2	9:0.05	PBS (pH=7.4)	52.0	53.5	1.77	46.4	44.9	47.3	2.91	2.2
HSPC:PDMAEMA-b-PLMA 2	9:0.1	PBS (pH=7.4)	51.9	53.3	1.61	43.1	44.8	47.1	2.61	1.7
HSPC:PDMAEMA-b-PLMA 2	9:0.2	PBS (pH=7.4)	51.8	53.3	1.66	38.8	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.5	PBS (pH=7.4)	51.4	53.0	1.70	29.3	-	-	-	-
HSPC	-	pH=4.5	52.2	53.8	1.62	59.4	45.6	48.2	3.17	2.8
HSPC:PDMAEMA-b-PLMA 2	9:0.02	pH=4.5	52.0	53.6	1.59	57.7	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.05	pH=4.5	52.0	53.6	2.14	52.0	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.1	pH=4.5	51.6	53.3	1.83	42.8	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.2	pH=4.5	50.7	52.8	2.14	37.4	-	-	-	-
HSPC:PDMAEMA-b-PLMA 2	9:0.5	pH=4.5	49.1	52.2	3.06	22.4	-	-	-	-



Results

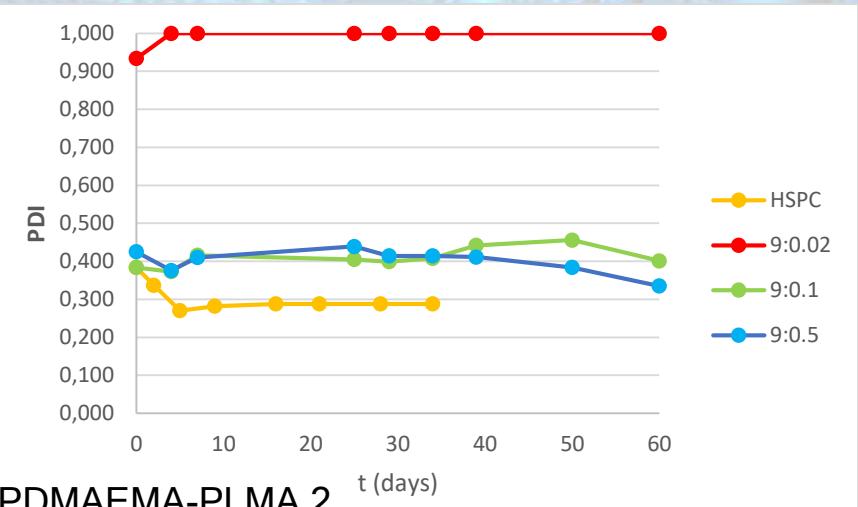
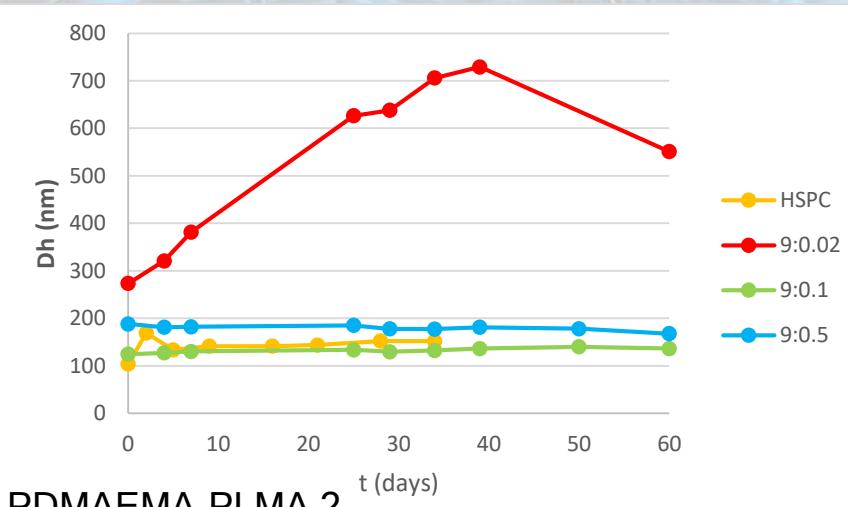
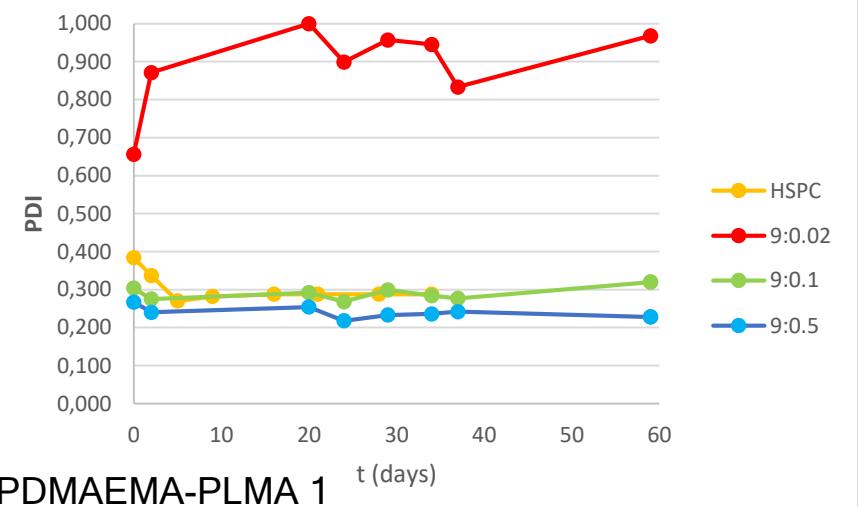
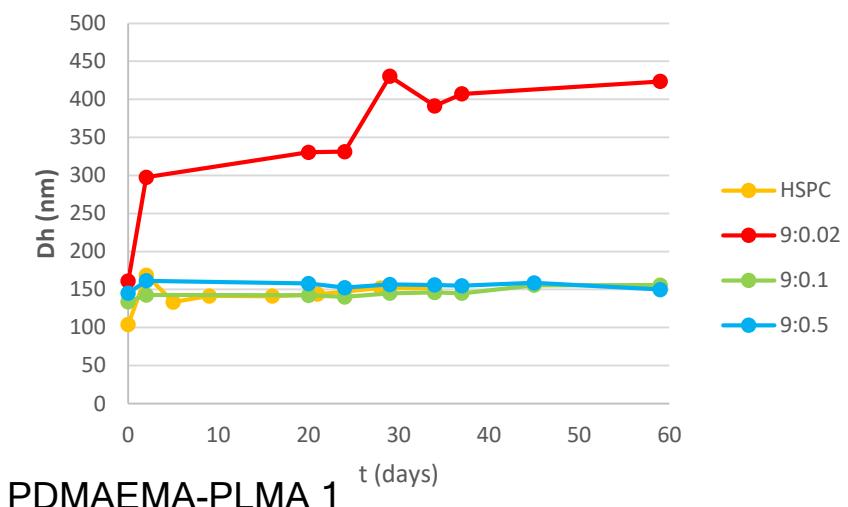
Physicochemical Properties

Sample	Molar Ratio	Dispersion Medium	D _h (nm)	SD	PDI	SD	ζ-pot (mV)	SD	R _g /R _h
HSPC	-	PBS (pH=7.4)	104.0	0.4	0.384	0.020	2.2	0.6	1.00
HSPC : PDMAEMA-b-PLMA 1	9:0.02	PBS (pH=7.4)	161.2	0.7	0.656	0.017	12.6	3.9	1.07
HSPC : PDMAEMA-b-PLMA 1	9:0.1	PBS (pH=7.4)	134.0	1.0	0.304	0.003	11.0	6.9	0.76
HSPC : PDMAEMA-b-PLMA 1	9:0.5	PBS (pH=7.4)	145.2	0.4	0.267	0.007	19.0	5.2	0.90
HSPC : PDMAEMA-b-PLMA 2	9:0.02	PBS (pH=7.4)	273.4	19.2	0.934	0.114	13.7	0.4	1.01
HSPC : PDMAEMA-b-PLMA 2	9:0.1	PBS (pH=7.4)	124.6	1.0	0.384	0.021	20.7	0.4	1.12
HSPC : PDMAEMA-b-PLMA 2	9:0.5	PBS (pH=7.4)	188.2	2.9	0.425	0.012	10.6	4.8	0.95

Sample	Molar Ratio	Dispersion Medium	D _h (nm)	SD	PDI	SD	ζ-pot (mV)	SD	R _g /R _h
HSPC	-	pH=4.5	107.6	2.4	0.367	0.010	0.0	3.3	-
HSPC : PDMAEMA-b-PLMA 1	9:0.02	pH=4.5	151.5	1.9	0.558	0.032	18.7	0.6	-
HSPC : PDMAEMA-b-PLMA 1	9:0.1	pH=4.5	120.6	0.9	0.211	0.018	11.4	22.6	-
HSPC : PDMAEMA-b-PLMA 1	9:0.5	pH=4.5	127.8	1.0	0.255	0.013	20.6	3.3	-
HSPC : PDMAEMA-b-PLMA 2	9:0.02	pH=4.5	168.4	5.4	0.725	0.110	4.9	12.4	-
HSPC : PDMAEMA-b-PLMA 2	9:0.1	pH=4.5	107.2	0.4	0.284	0.015	15.6	1.4	-
HSPC : PDMAEMA-b-PLMA 2	9:0.5	pH=4.5	163.4	1.7	0.434	0.037	19.3	2.4	-

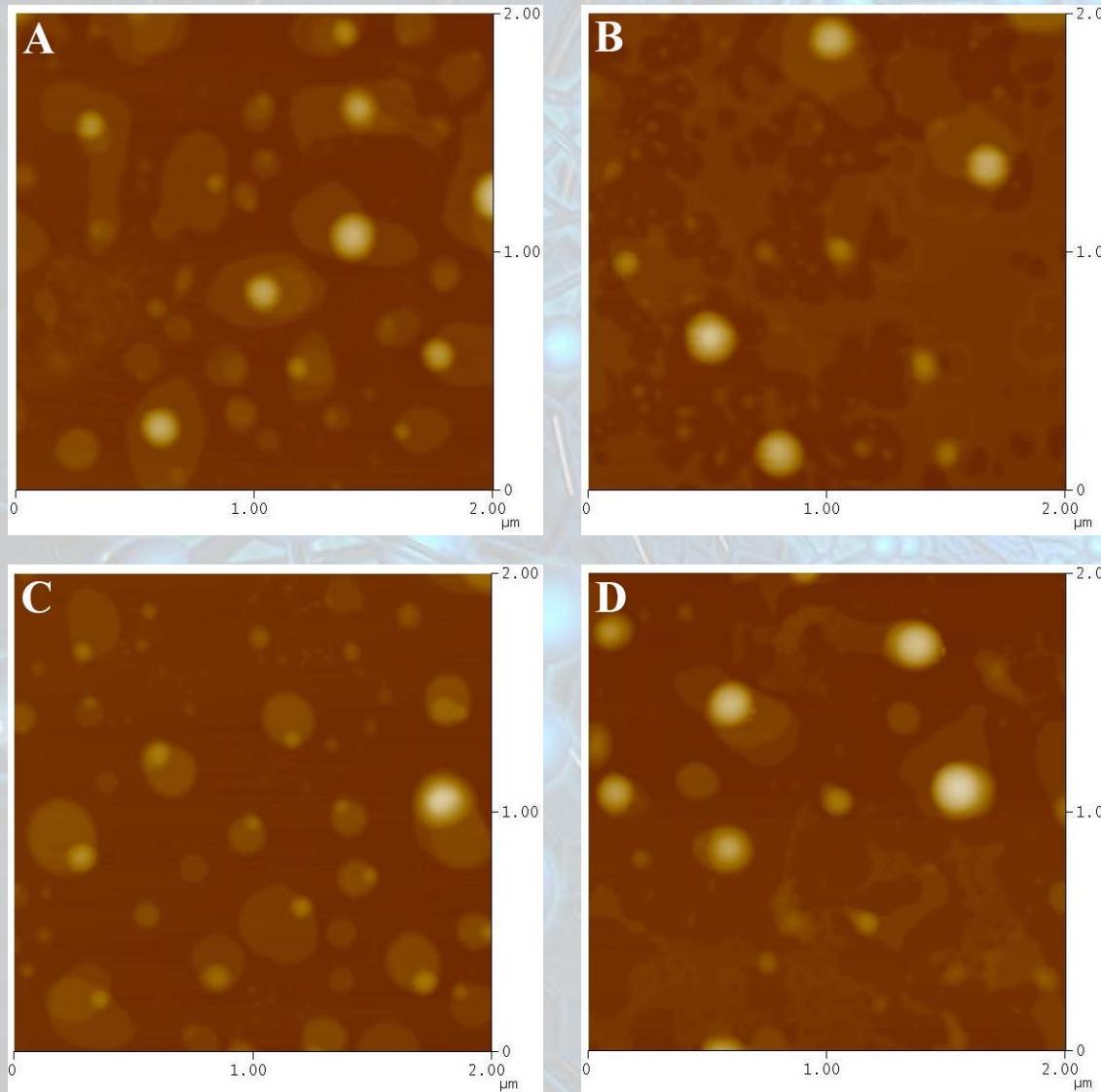
Results

Colloidal Stability



Results

Morphology



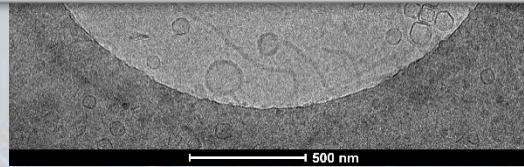
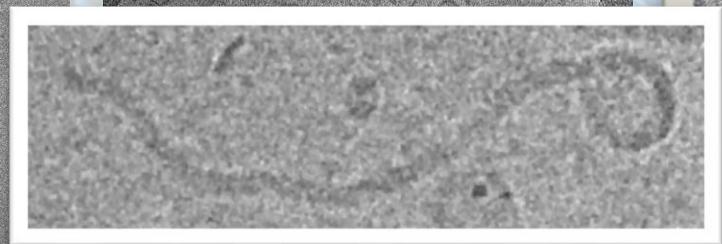
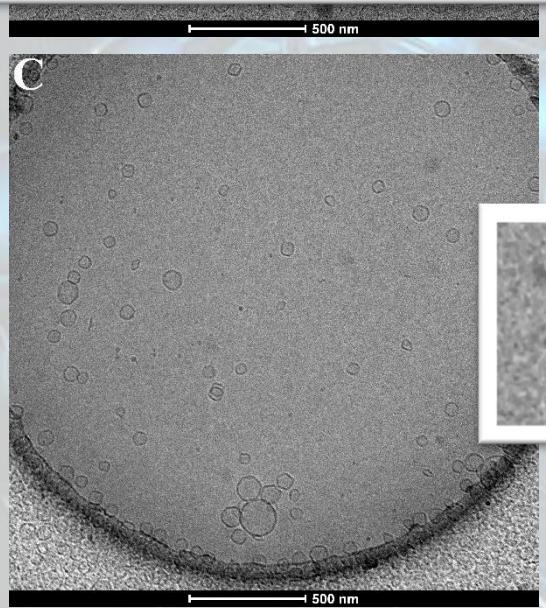
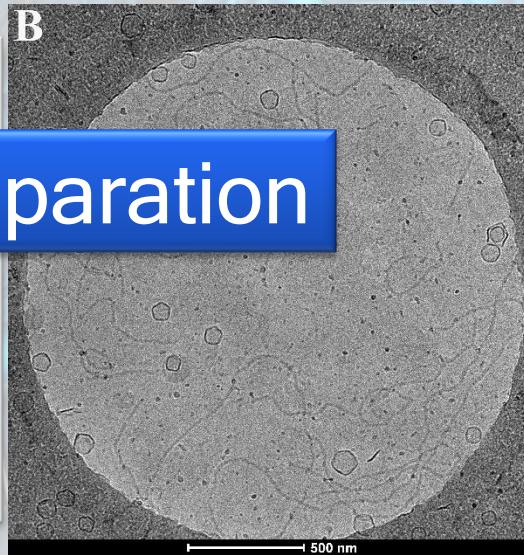
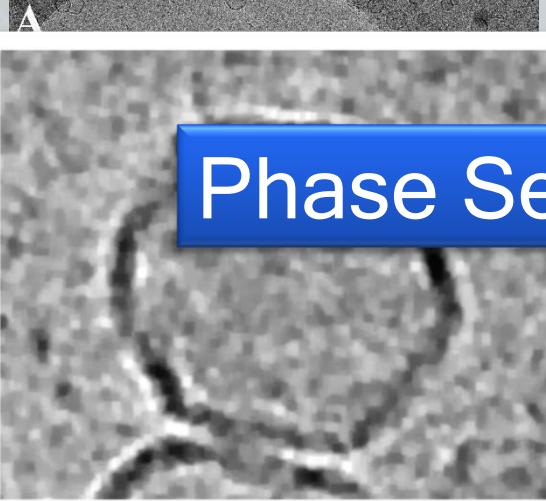
AFM Images of
Chimeric Systems

**HSPC:PDMAEMA-b-PLMA 1
9:0.1 + 9:0.5 (A + B)**

**HSPC:PDMAEMA-b-PLMA 2
9:0.1 + 9:0.5 (C + D)**

Results

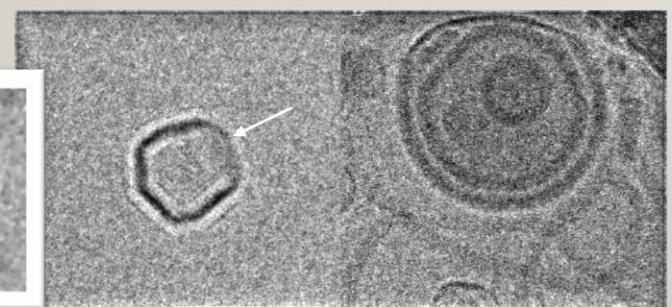
Morphology



Cryo-TEM Images of
Chimeric Systems

HSPC:PDMAEMA-b-PLMA 1
9:0.1 + 9:0.5 (A + B)

HSPC:PDMAEMA-b-PLMA 2
9:0.1 + 9:0.5 (C + D)



Results

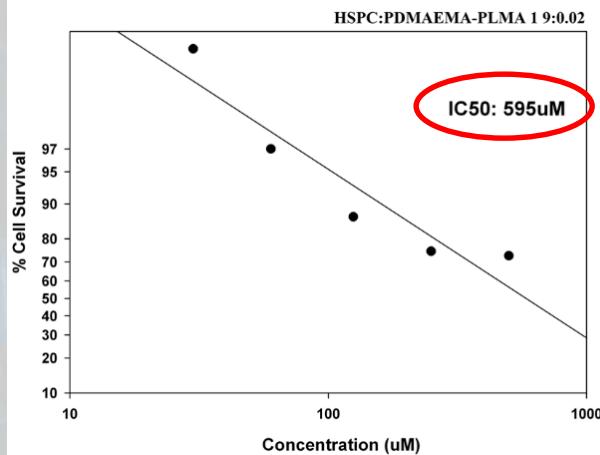
Morphology

Sample	Molar Ratio	Type of Objects Observed		Wall Thickness (nm)	Diameter (nm)	Quantity (%)
HSPC:PDMAEMA-b-PLMA 1	9:0.1	Liposomes	Homogeneous membrane	6.1	69.3	94.8
			Heterogeneous membrane			0.2
		Polymersomes	Homogeneous membrane	12.8	116.1	3.9
			Heterogeneous membrane			0.1
		Disk-like micelles		26.3	117.3	1
		Worm-like micelles		-	-	0
	9:0.5	Liposomes	Homogeneous membrane	5.8	80.1	85.8
			Heterogeneous membrane			0.2
		Polymersomes	Homogeneous membrane	13.1	131.7	6.9
			Heterogeneous membrane			0.1
		Disk-like micelles		31.5	115.4	2
		Worm-like micelles		13.2	> 1000	5
HSPC:PDMAEMA-b-PLMA 2	9:0.1	Liposomes	Homogeneous membrane	5.7	69.9	91.8
			Heterogeneous membrane			0.2
		Polymersomes	Homogeneous membrane	13.7	154.3	3.8
			Heterogeneous membrane			0.2
		Disk-like micelles		35.0	147.6	2
		Worm-like micelles		14.9	~ 800	2
	9:0.5	Liposomes	Homogeneous membrane	5.8	75.5	50.5
			Heterogeneous membrane			0.5
		Polymersomes	Homogeneous membrane	15.1	149.4	24.6
			Heterogeneous membrane			0.4
		Disk-like micelles		38.3	128.2	3
		Worm-like micelles		15.3	> 1000	21

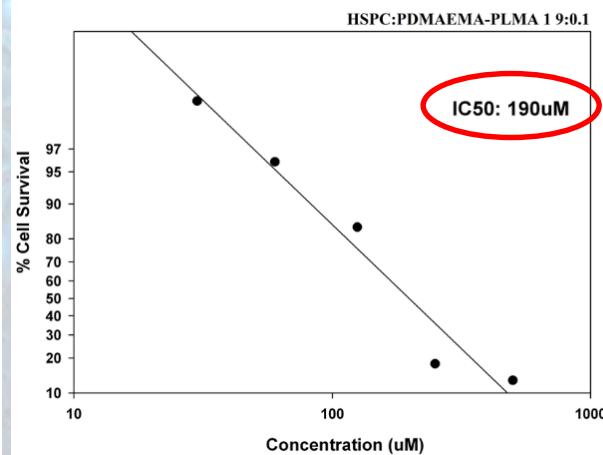
Results

Toxicity

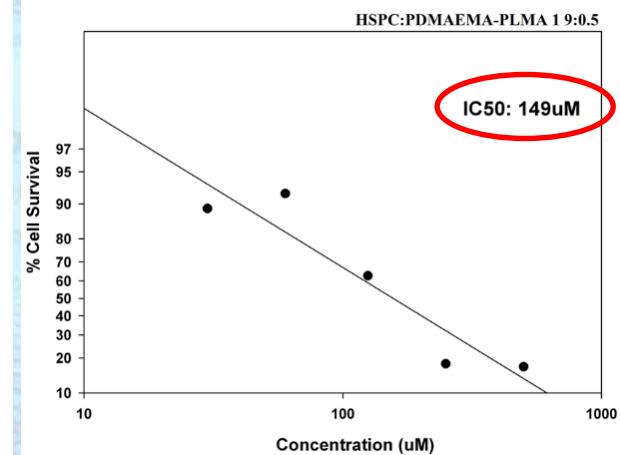
HSPC:PDMAEMA-PLMA 1 9:0.02



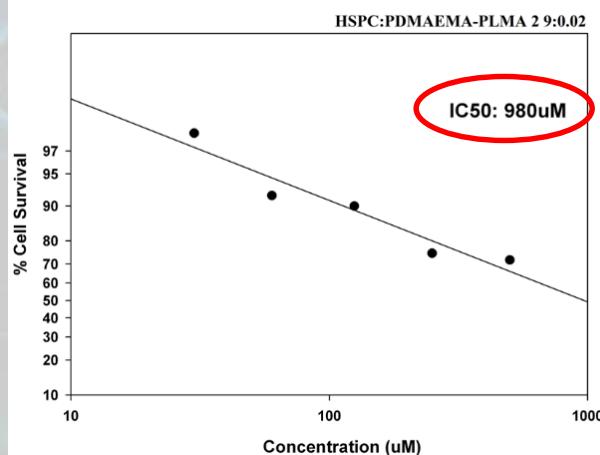
HSPC:PDMAEMA-PLMA 1 9:0.1



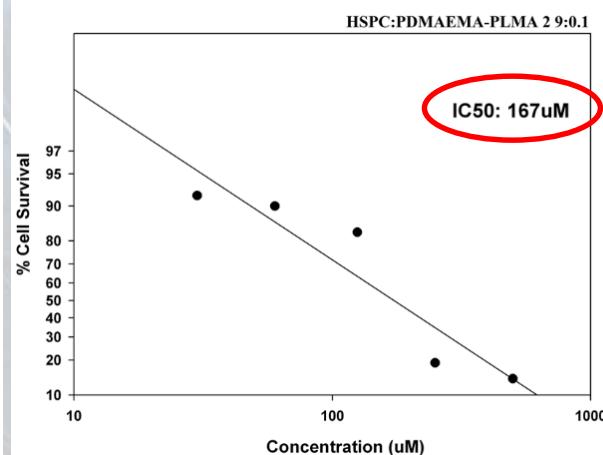
HSPC:PDMAEMA-PLMA 1 9:0.5



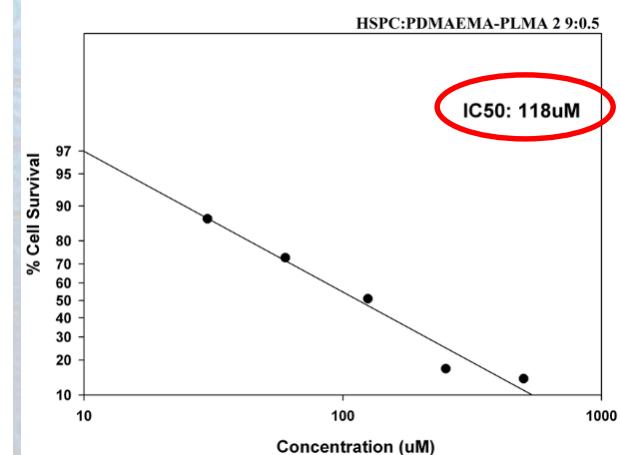
HSPC:PDMAEMA-PLMA 2 9:0.02



HSPC:PDMAEMA-PLMA 2 9:0.1



HSPC:PDMAEMA-PLMA 2 9:0.5



Results

Preparation of Optimization

- pH = 7.0

Sample	Molar Ratio	Dispersion Medium	D _h (nm)	SD	PDI	SD	ζ-pot (mV)	SD
HSPC	-	PBS (pH=7.0)	119.1	1.0	0.615	0.003	-	-
HSPC : PDMAEMA-b-PLMA 1	9:0.02	PBS (pH=7.0)	-	-	-	-	-	-
HSPC : PDMAEMA-b-PLMA 1	9:0.1	PBS (pH=7.0)	82.9	0.7	0.281	0.021	18.6	6.3
HSPC : PDMAEMA-b-PLMA 1	9:0.5	PBS (pH=7.0)	83.1	0.9	0.233	0.003	14.4	1.9
HSPC : PDMAEMA-b-PLMA 2	9:0.02	PBS (pH=7.0)	-	-	-	-	-	-
HSPC : PDMAEMA-b-PLMA 2	9:0.1	PBS (pH=7.0)	75.4	0.7	0.253	0.025	19.7	4.2
HSPC : PDMAEMA-b-PLMA 2	9:0.5	PBS (pH=7.0)	83.3	0.7	0.209	0.008	19.3	2.4

pH-Sensitive Differential Conformation
During Self-Assembly

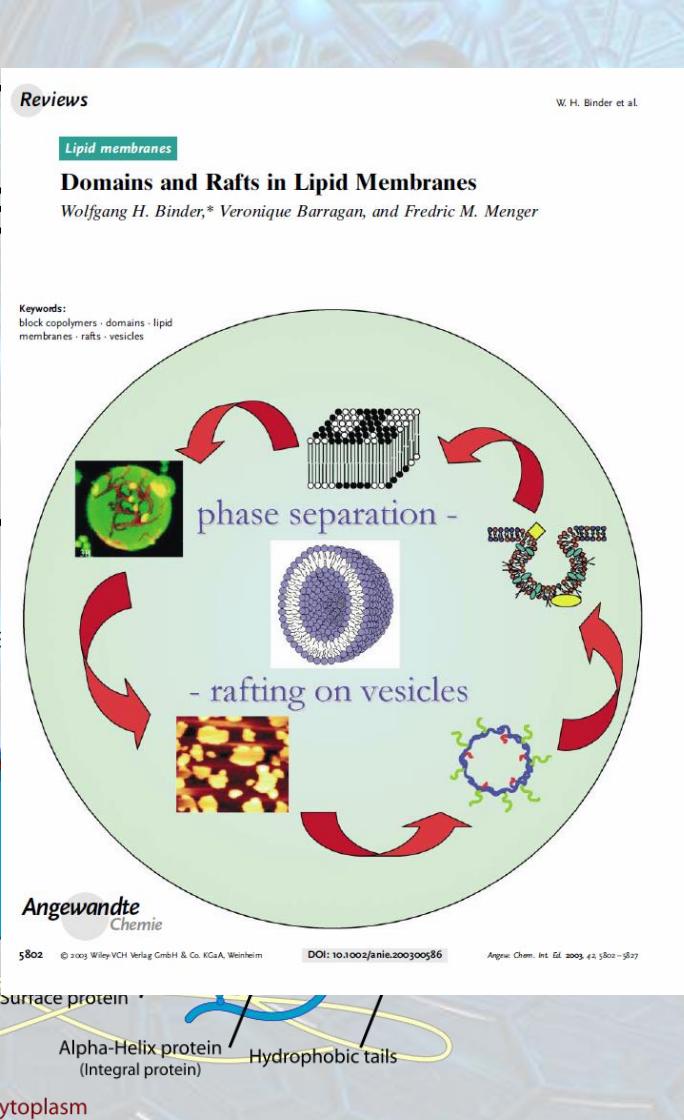
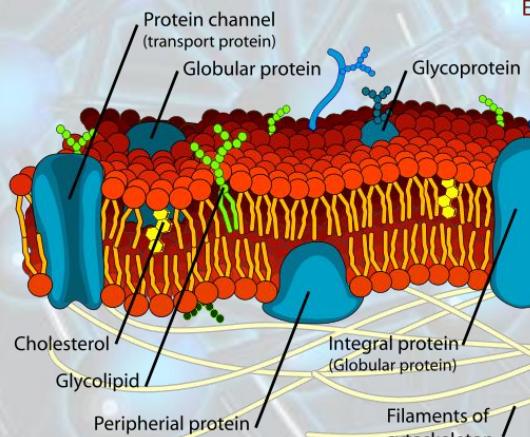
Hydration Forces (Proton Conc.)
Influence Even Pure HSPC

Conclusion

- The thermodynamic, physicochemical and morphology studies provide useful information for the biophysical behaviour of pH-responsive chimeric/mixed nanocarriers.
- Indicating which are promising advanced drug delivery nanosystems (aDDnSs).
- Protocol parameters important, due to lyotropism of biomaterials.
- Concentration and composition-dependent polymer effect.

Conclusion

- Suitable membrane phenomena
- Membrane separation diseases, the



(membrane hogenesis). From phase with various and rafts.

Future

- microDSC Studies on Liposomes, Prof. Fessas



- Incorporation of Immunomodulatory Proteins for Cancer Treatment
- In vivo Toxicity + Efficacy

Contributors

- **Nikolaos Naziris**
 - **Dr. Natassa Pippa**
 - **Prof. Demetzos Costas**
- } Section of Pharmaceutical Technology
Department of Pharmacy
National and Kapodistrian University of Athens
Greece
-
- **Varvara Chrysostomou**
 - **Dr. Stergios Pispas**
- } Theoretical and Physical Chemistry Institute
National Hellenic Research Foundation
Greece
-
- **Dr. Martin Libera**
 - **Dr. Barbara Trzebicka**
- } Centre of Polymer and Carbon Materials
Polish Academy of Sciences
Poland

Special Thanks to

- **Dr. Dimitris Stellas**

Biomedical Research Foundation, Academy of Athens, Athens, Greece

Vaccine Branch, Center for Cancer Research,
National Cancer Institute, National Institutes of Health,
Frederick Maryland, USA

NANOGLIO

- This work is carried out within the context of the project:



Nanotechnology Based Immunotherapy for Glioblastoma



PhD Scholarship



GSERT

**GENERAL SECRETARIAT FOR
RESEARCH AND TECHNOLOGY**



HELLENIC REPUBLIC
**Ministry of Education,
Research and Religious
Affairs**



HELLENIC REPUBLIC
**National and Kapodistrian
University of Athens**



ΕΛΙΔΕΚ.
Ελληνικό Ίδρυμα Έρευνας & Καινοτομίας

Related Literature

- Demetzos C.
Biophysics and Thermodynamics: The Scientific Building Blocks of Bio-inspired Drug Delivery Nano Systems
AAPS Pharm Sci Tech.
Current Nanomedicine, 2016, 6, 1-20

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

1 Costas Demetzos^{1,2}

Demetzos C.
Stimuli-responsive Drug Delivery Nanosystems: From Bench to Clinic.

REVIEW ARTICLE

Stimuli-responsive Drug Delivery Nanosystems: From Bench to Clinic

Nikolaos Naziris^a, Natassa Pippa^{a,b}, Stergios Pispas^{b,*} and Costas Demetzos^a

^aDepartment of Pharmaceutical Technology, Faculty of Pharmacy, Panepistimioupolis Zografou 15771, National and Kapodistrian University of Athens, Athens, Greece; ^bTheoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 11635, Athens, Greece

Irzepicka B.
Morphological diversity of block copolymer
J Nanopart Res.

- Naziris N, Pippa N, Pispas S, Demetzos C.
The thermal analysis of liposomal formulations and their effectiveness as drug and vaccine delivery systems
Liposomes: Historical, Clinical and Molecular

V, Pispas S, Demetzos C, Libera M,

Chapter

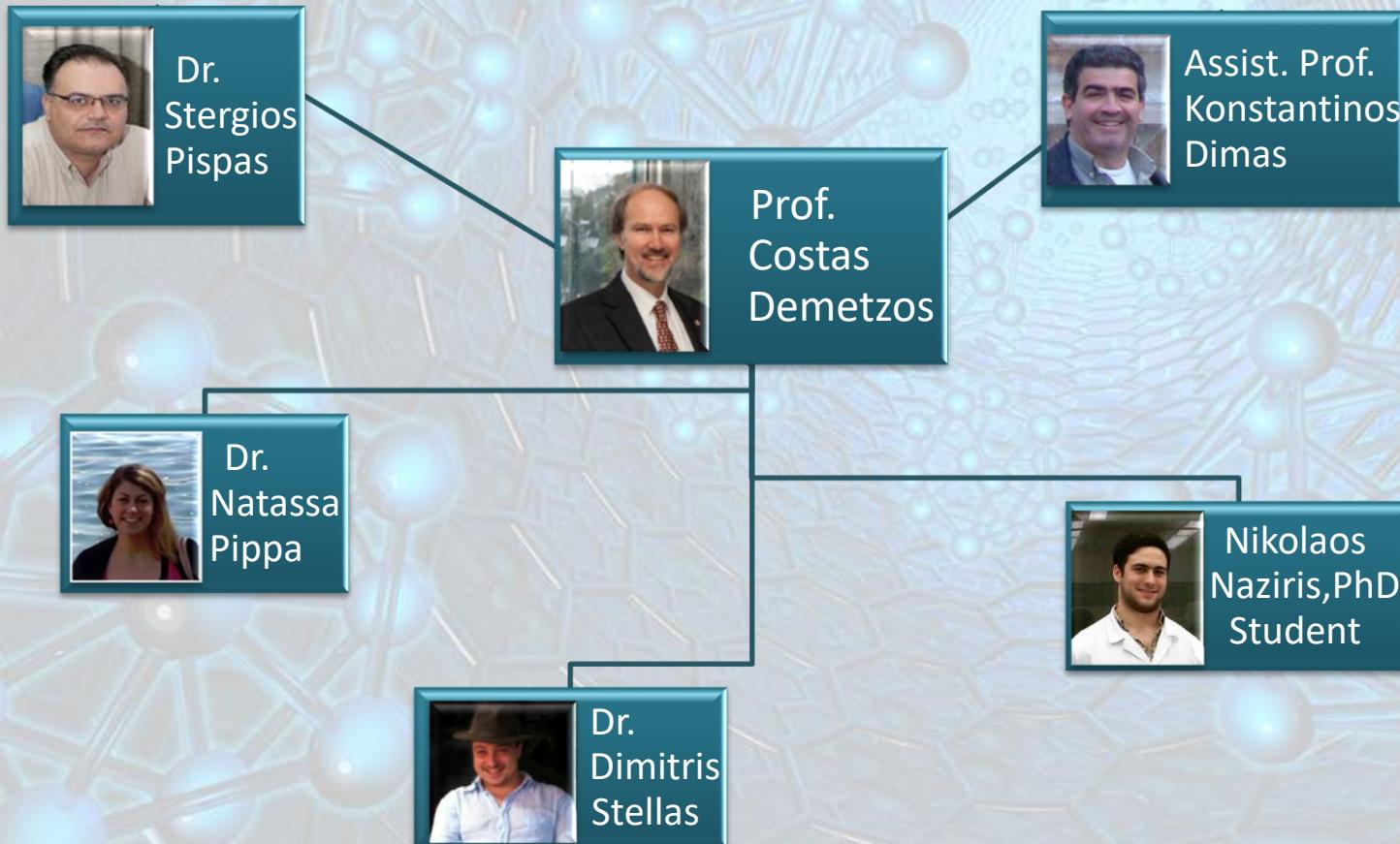
THE THERMAL ANALYSIS OF LIPOSOMAL FORMULATIONS AS AN ELEMENT TO EVALUATE THEIR EFFECTIVENESS AS DRUG AND VACCINE DELIVERY SYSTEMS

Nikolaos Naziris¹, Natassa Pippa^{1,2}, Stergios Pispas²
and Costas Demetzos¹

¹Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

²Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, Athens, Greece

Our Lab Team



Thank You

**Do you have
any questions?**

