Controlled release from advanced Drug Delivery nano Systems: the physicochemical, morphological and thermodynamic characteristics of the vehicle.

Natassa Pippa, Stergios Pispas, Costas Demetzos

ILS 2015 MEETING

LIPOSOME IN DRUG AND VACCINE DELIVERY

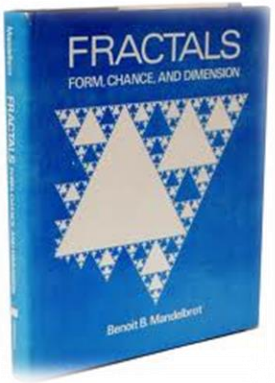
19-22 December 2015

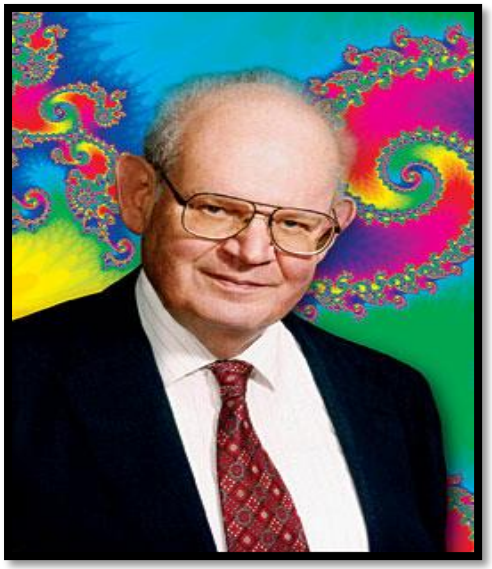
UCL School of Pharmacy, London

Contents:

- Fractal geometry
  - The fractal concepts in pharmaceutical sciences
- Drug Delivery Systems
- Polymers in Pharmaceutics
- The fractal dimensionality of nanoparticles
  - Examples
  - Advantages and Benefits

Fractal geometry:





- 1975
- Introduced the term “fractal” which is from the Latin word *fractus*, meaning broken

- Iterative
- Self-similar
- Non-integer dimension (fraction)

“Clouds are not spheres, mountains are not cones, coastlines are not circles, and bark is not smooth, nor does lightening travel in a straight line.”

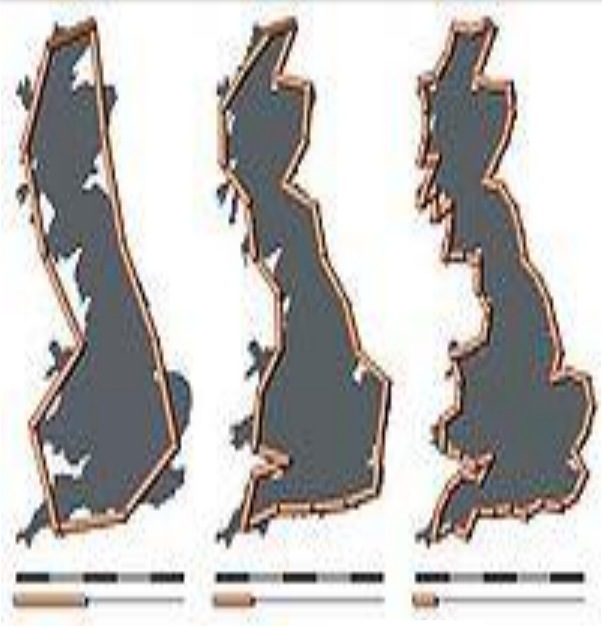
Benoit Mandelbrot

The fractal dimension= not integer dimension

Brain

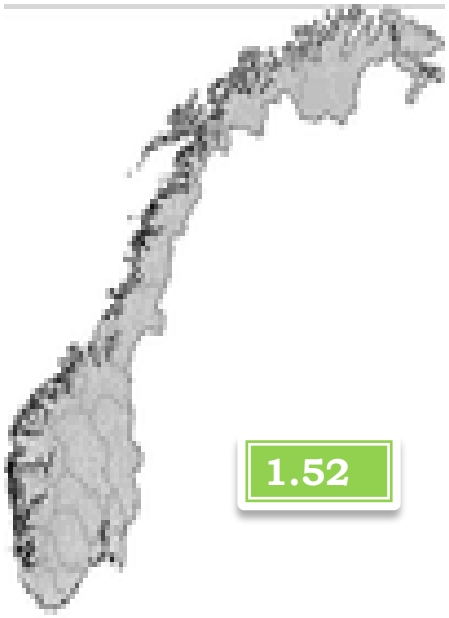
2.79

Coastlines of Great Britain



1.25

Coastlines of Norway



1.52

Cauliflower

2.33

Broccoli

2.66

Paper Balls

2.50

Fractal geometry applied as a frequent mathematical formalism in several areas of the pharmaceutical research and practice, from the formulation of drugs to *in vitro* and *in vivo* studies.

International Journal of Pharmaceutics 456 (2013) 340–352

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Review

On the ubiquitous presence of fractals and fractal concepts in pharmaceutical sciences: A review

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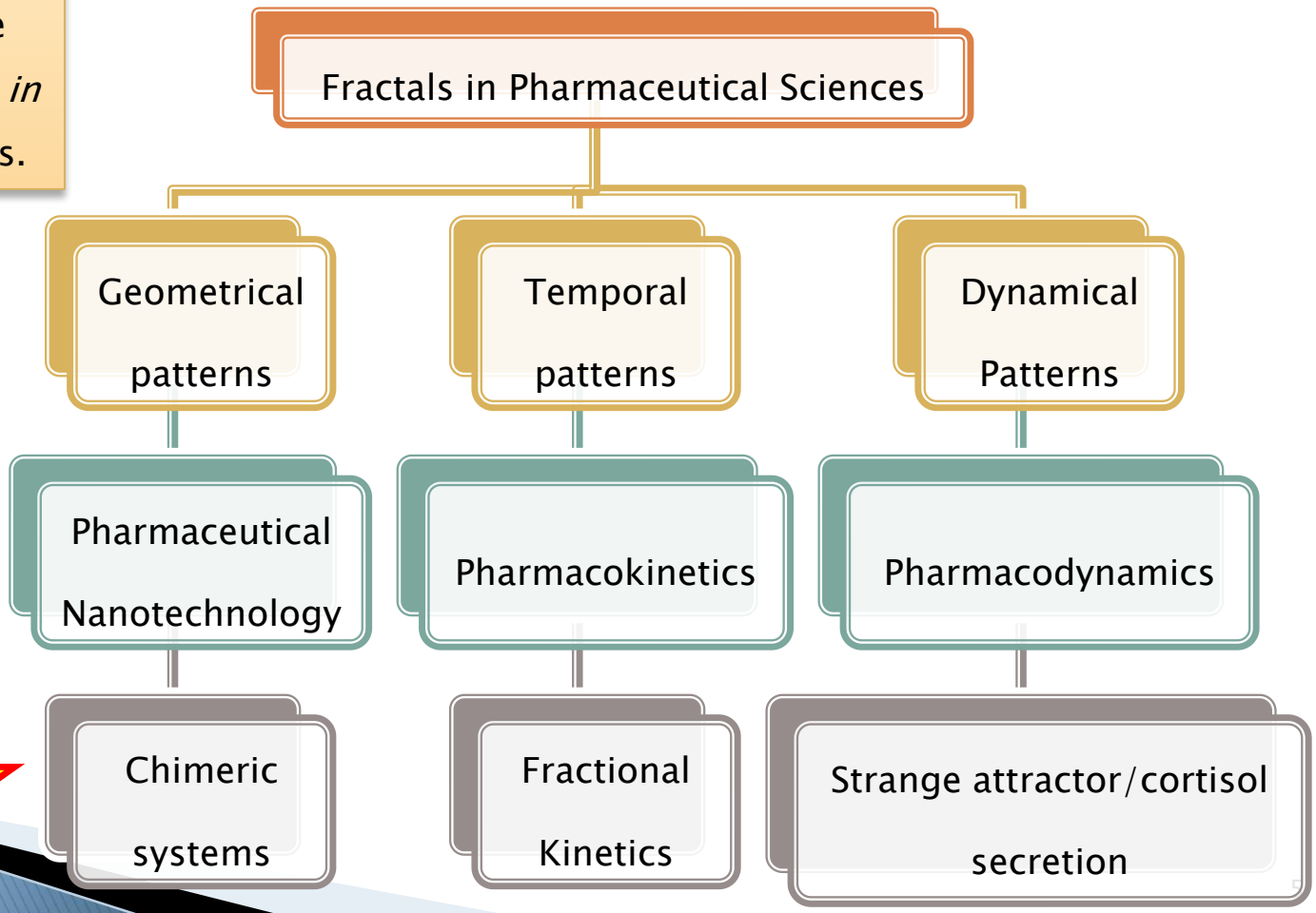
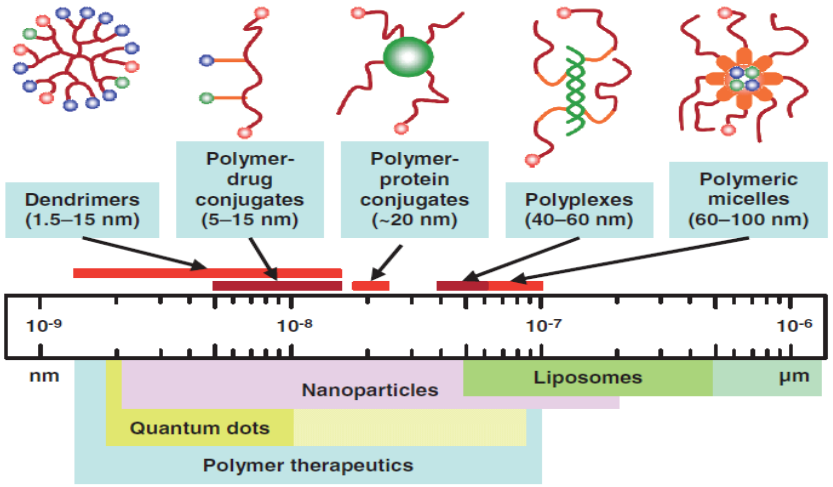


Drug Delivery Systems

According to Rowland et al., (2012): *"A Drug Delivery System (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body."*



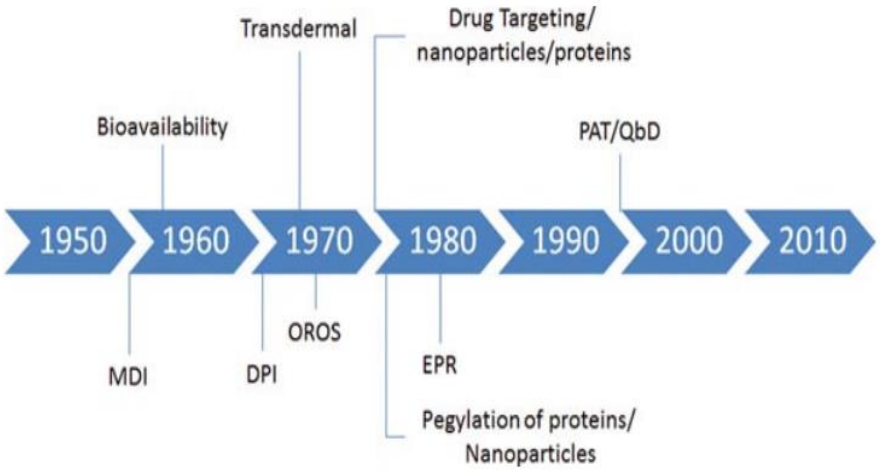
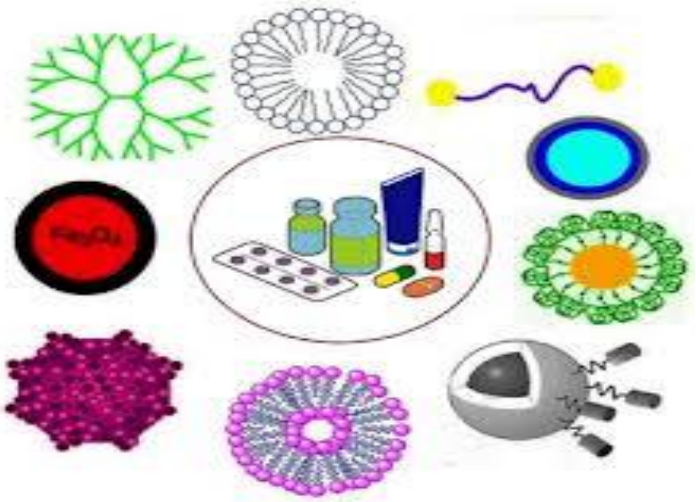
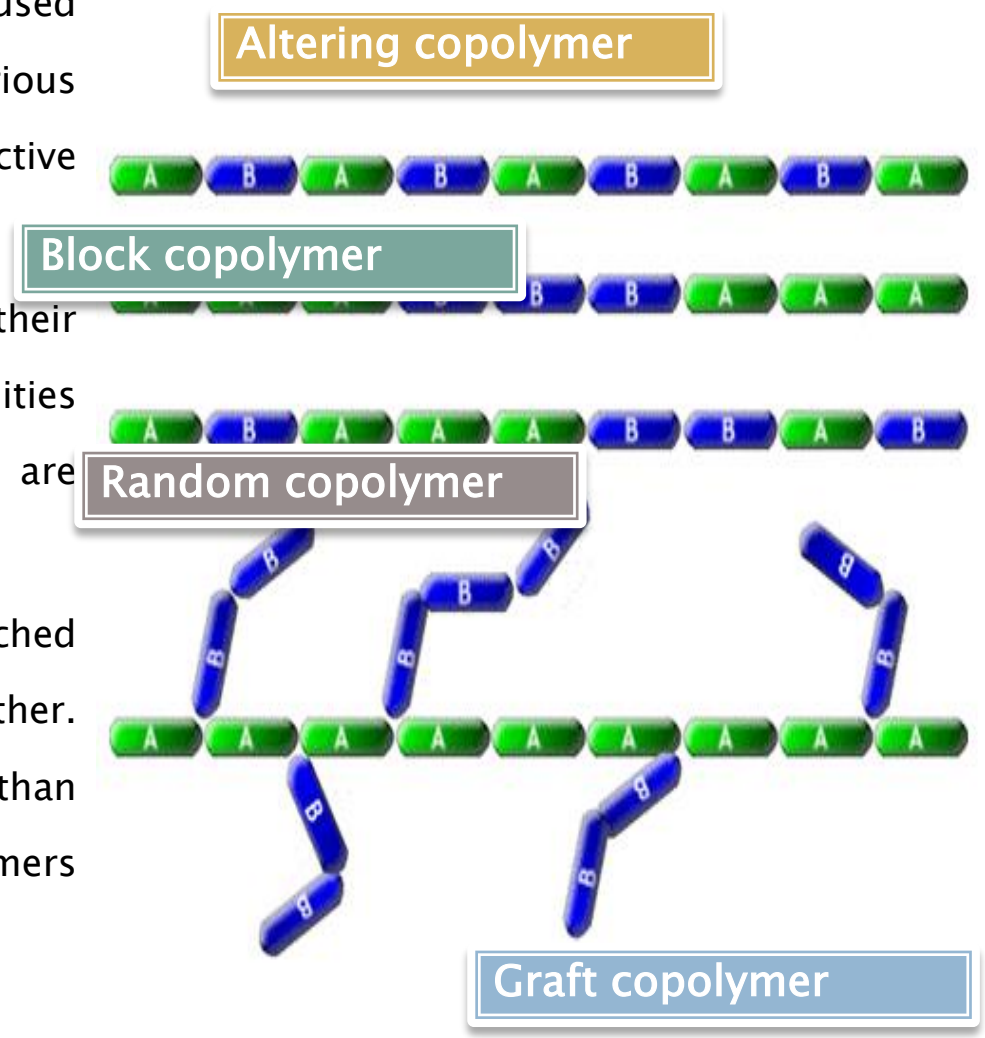


Figure 4. Timeline of introduction of some key concepts and developments in formulation sciences. MDI = metered dose inhaler, DPI = dry powder inhalation, OROS = osmotic release oral system, ERP = enhanced permeability and retention effect, PAT/QbD = process analytical technology/quality by design.

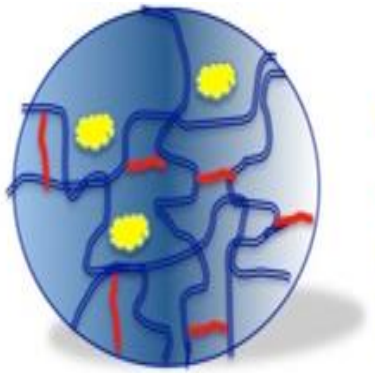


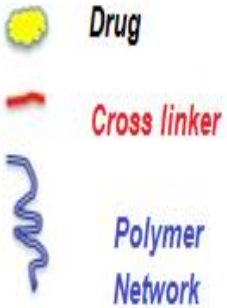
Polymers in Pharmaceutics:

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.

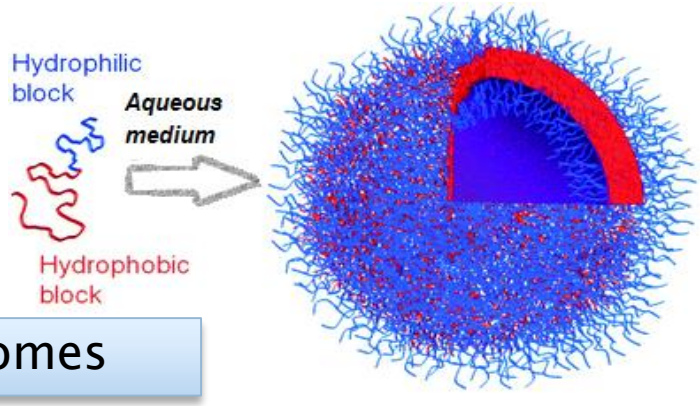


Polymers in Drug Delivery:

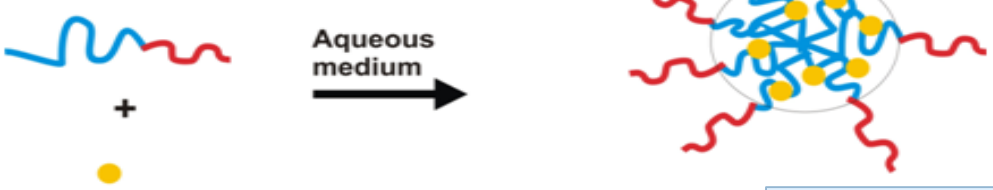




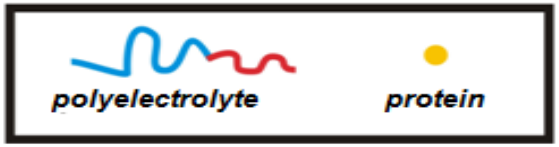
Nanogels



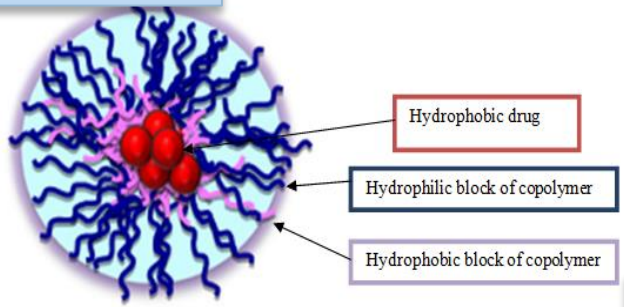
Polymersomes

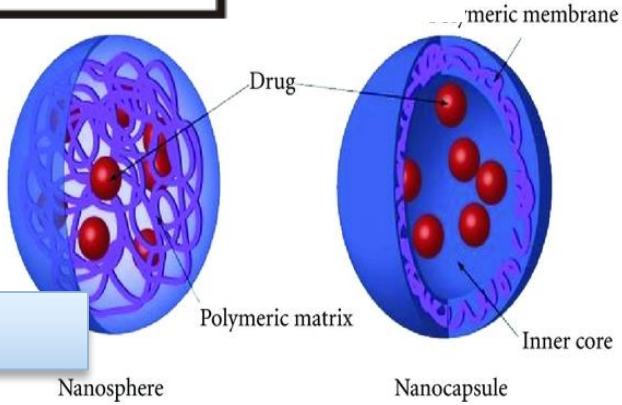


Polyelectrolyte complexes



Micelles



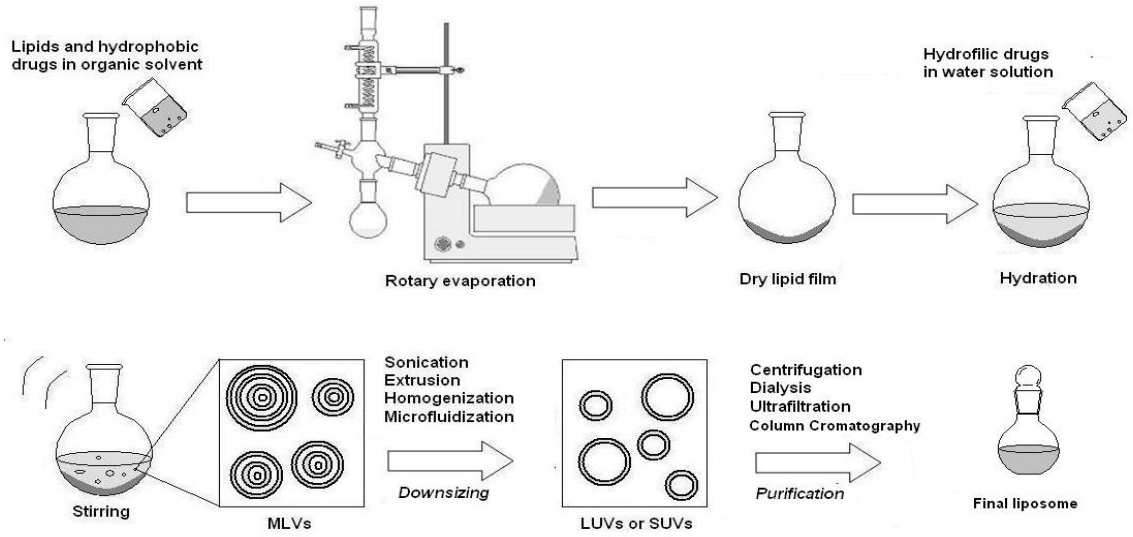


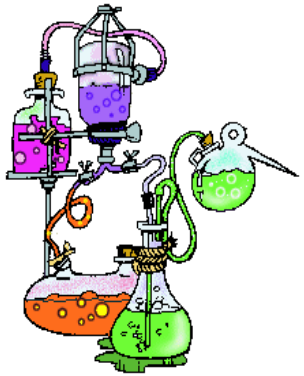
Nanospheres

The purposes of this study:

➢The goal of this study is to design and develop novel chimeric nanoassemblies based on **LIPIDS** and **POLYMERS** that can be utilized as chimeric advanced Drug Delivery nano Systems (chi-aDDnSs).

Thin-film hydration method.

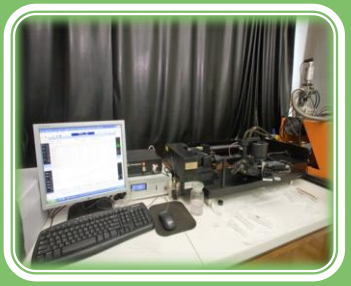




Methods and Techniques:

Dynamic and Electrophoretic Light Scattering

- Size and Size distribution
- Z-potential



Static Light Scattering

- Fractal dimension
- Rg/Rh ratio (external morphology)

Encapsulation Efficiency and Drug release

- Indomethacin: Model drug
- Fractal dimension of nanocarriers during drug release

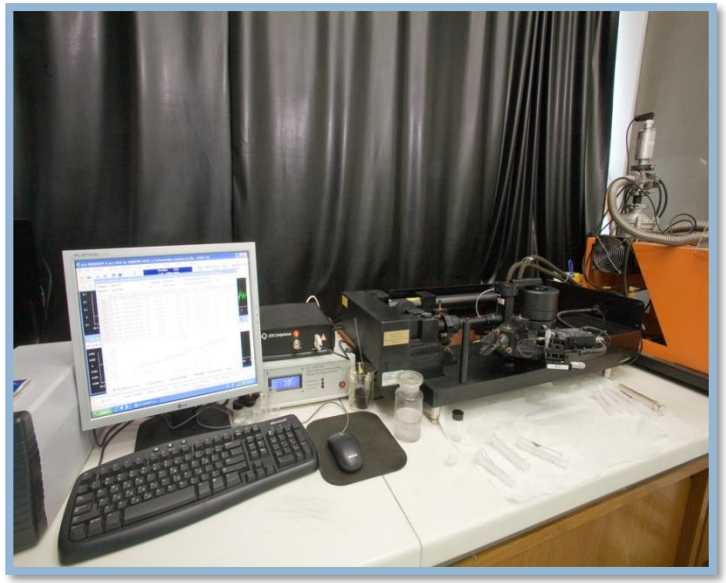
The techniques for the determination of fractal dimension.

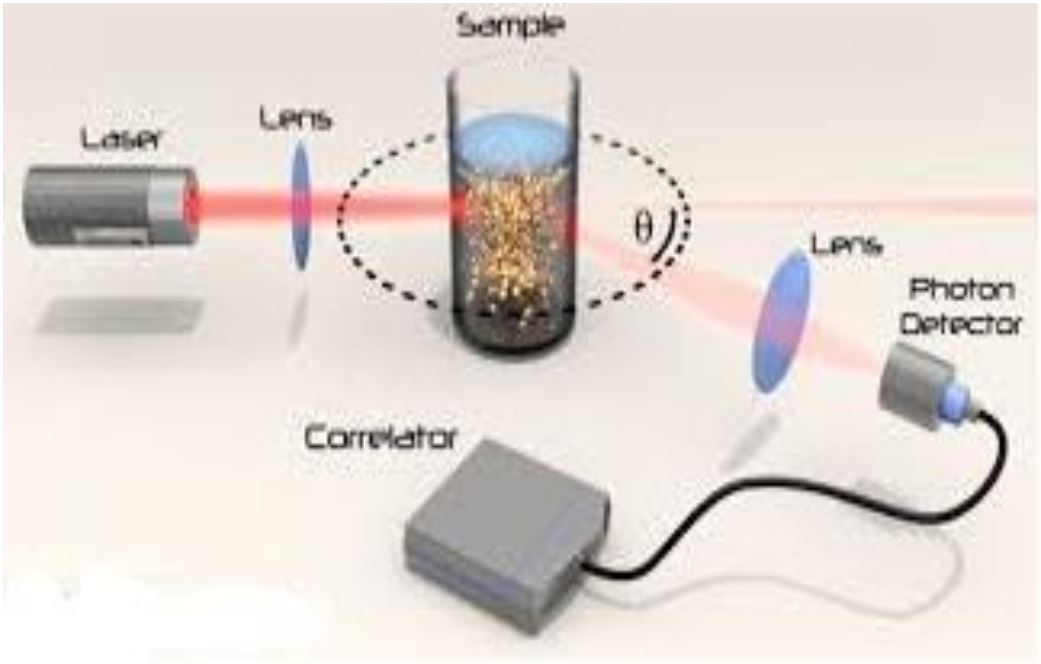
- Complete delineation of physicochemical characteristics
- Quantification of the morphology of nanoparticles in situ (in dispersion/solution)

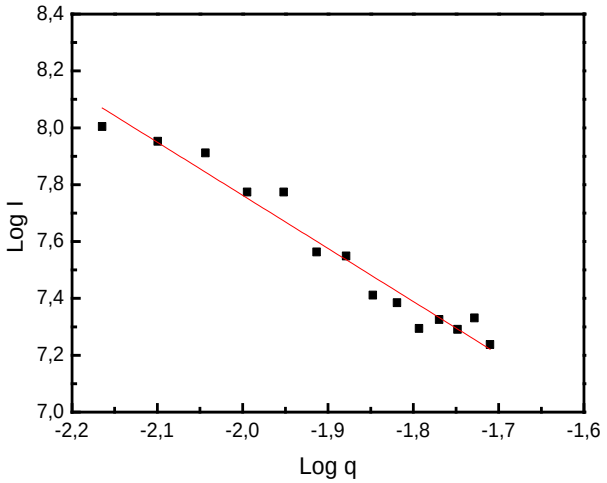
Techniques
Static Light Scattering (SLS)
Small-Angle-X-Ray Scattering
Small-Angle neutron scattering
Dynamic Light Scattering
Wide-Angle X-ray Diffraction
Dynamic Rheological measurements
Sedimentation
Confocal Scanning Laser Microscopy
Electron microscopy

Light scattering:

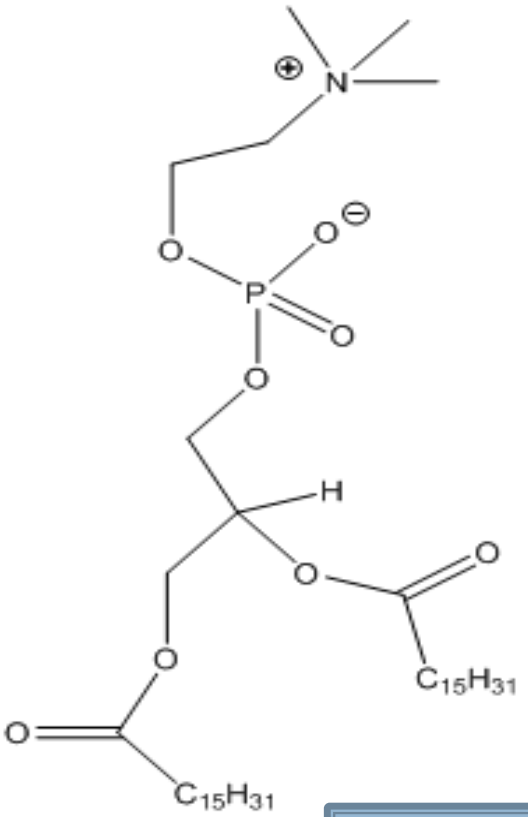
- Light scattering has been extensively used in the study of the fractal dimension of nanoparticles and aggregates.



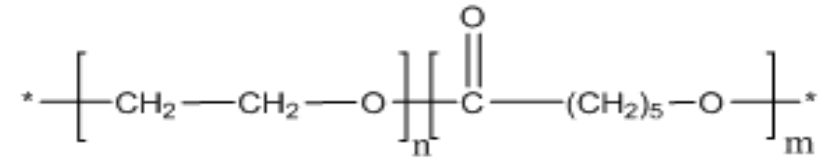


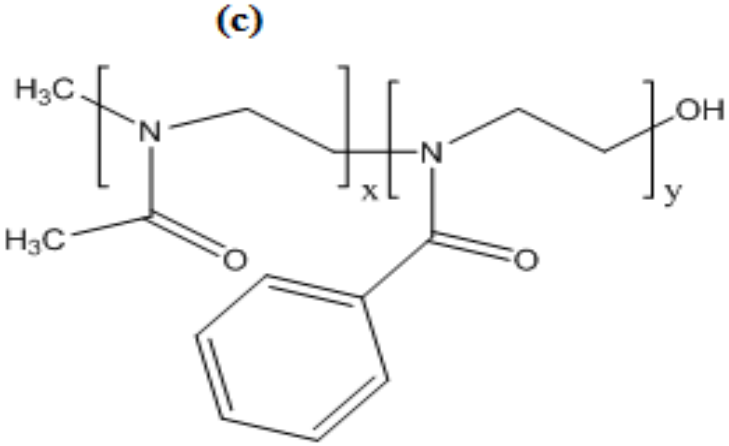


Log I vs. Log q plot for DPPC:PEO-b-PCL (9:1 molar ratio) chimeric nanoassemblies in HPLC-grade water.

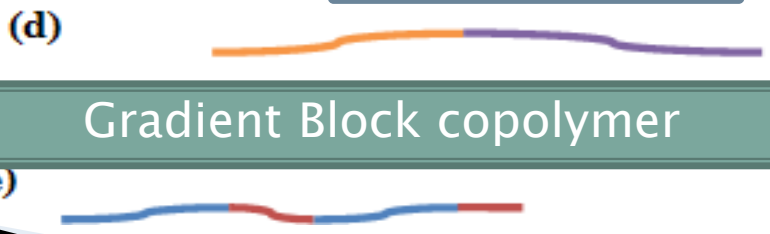


Block copolymer





Gradient Block copolymer

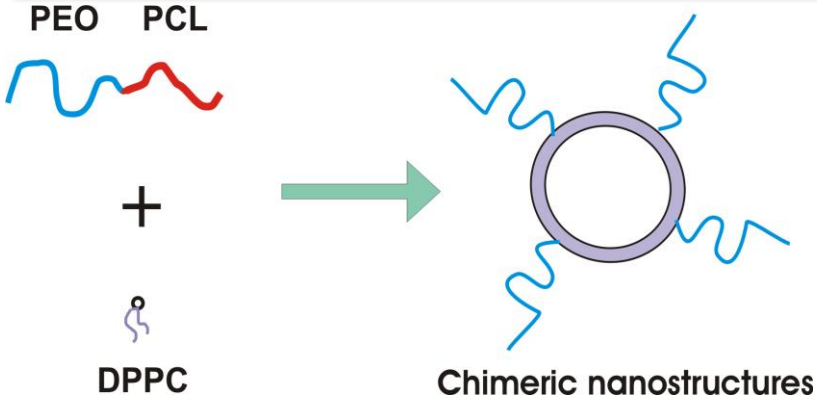


Gradient Block copolymer

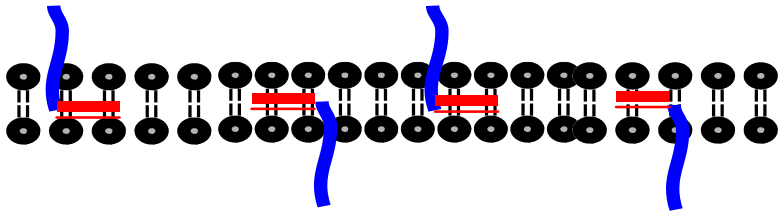
Chemical structures of (a) DPPC lipid (b) the block copolymer PEO-b-PCL, (c) the gradient block copolymer MPOx, and the macromolecular architecture of (d) PEO-b-PCL and (e) MPOx employed in this study.

Structure properties of stealth liposomes constructed from block and gradient copolymers and DPPC lipids.

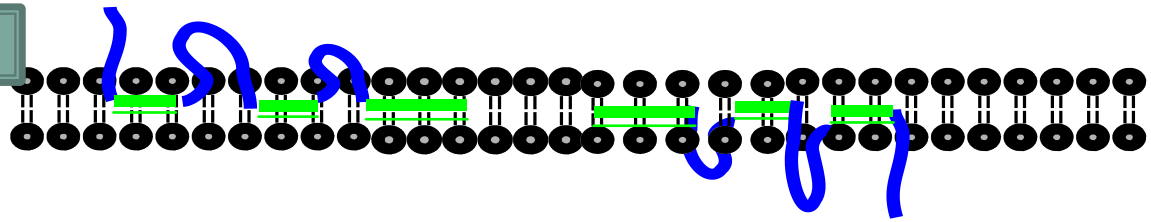
The gradient copolymer chain is expected to have several entry and exit points in the lipid membrane, in contrast to lipid–hydrophilic polymer conjugates and amphiphilic diblock copolymers, where the hydrophobic part is incorporated into the lipid membrane, and the hydrophilic polymer chain is anchored on the membrane.



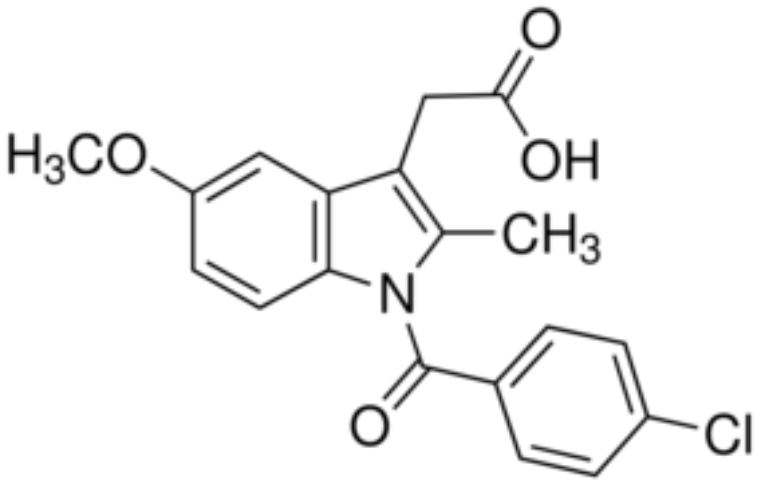
Block copolymer



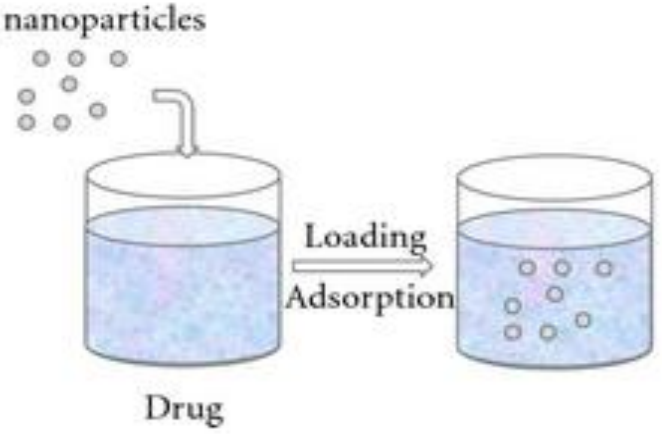
Gradient block copolymer



Indomethacin: Model drug for this study

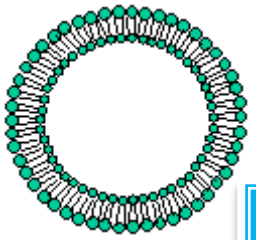


$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug encapsulated in the formulation}}{\text{Total amount of drug in the formulation}} \times 100$$



Li Fractal morphology of liposomes:

Conventional liposomes

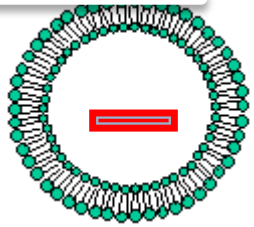


2.51

Anionic liposomes

Cationic liposomes

1.76

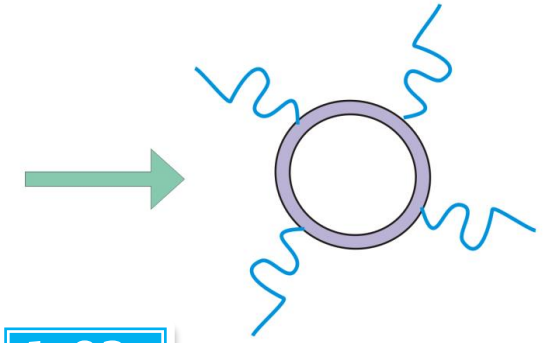


1.81

PEO PCL

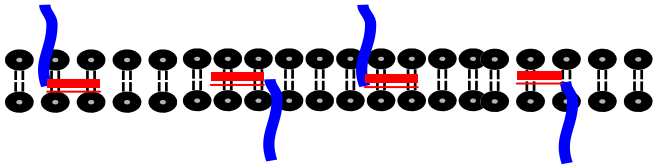
\+

DPPC

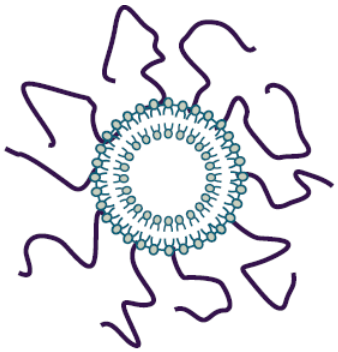


1.83

Chimeric nanostructures

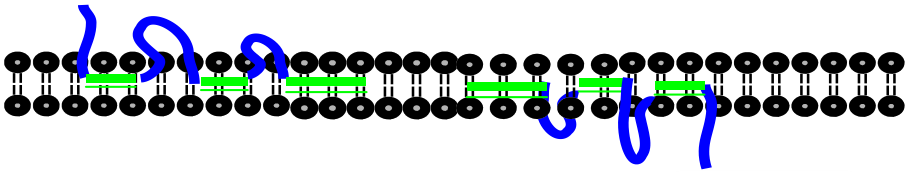


PEGylated (stealth) liposomes



1.98

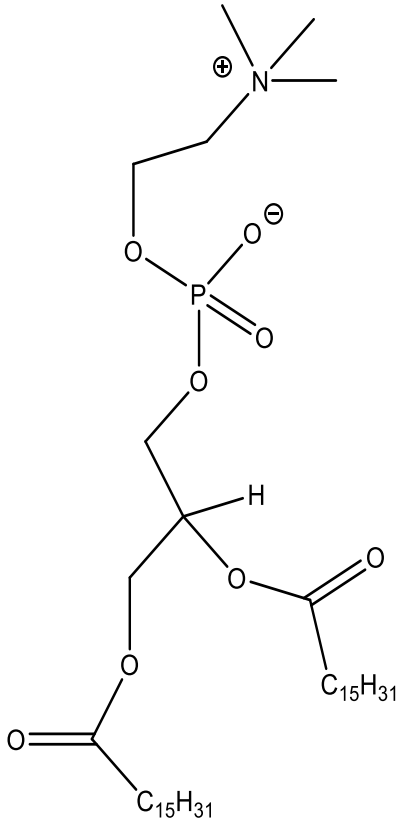
Polymer grafted liposomes

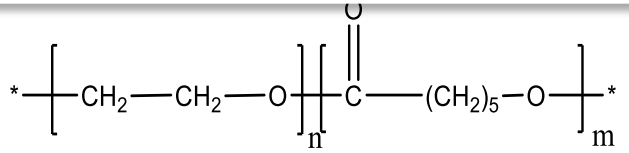


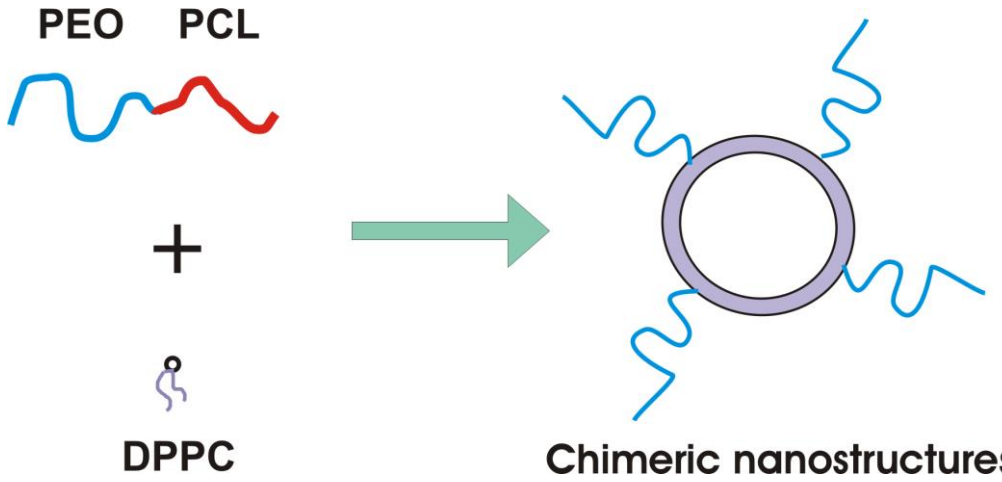
2.46

DPPC:PEO-b-PCL chimeric/mixed nanocarriers:

Chemical structures of lipid DPPC (left) and the block copolymer, PEO-b-PCL, employed in this study (right).





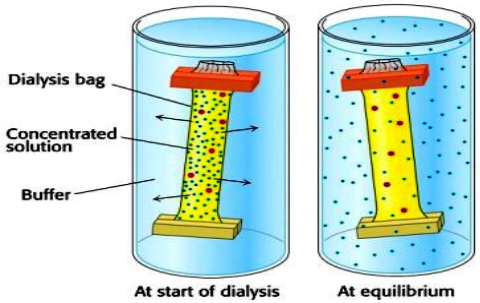


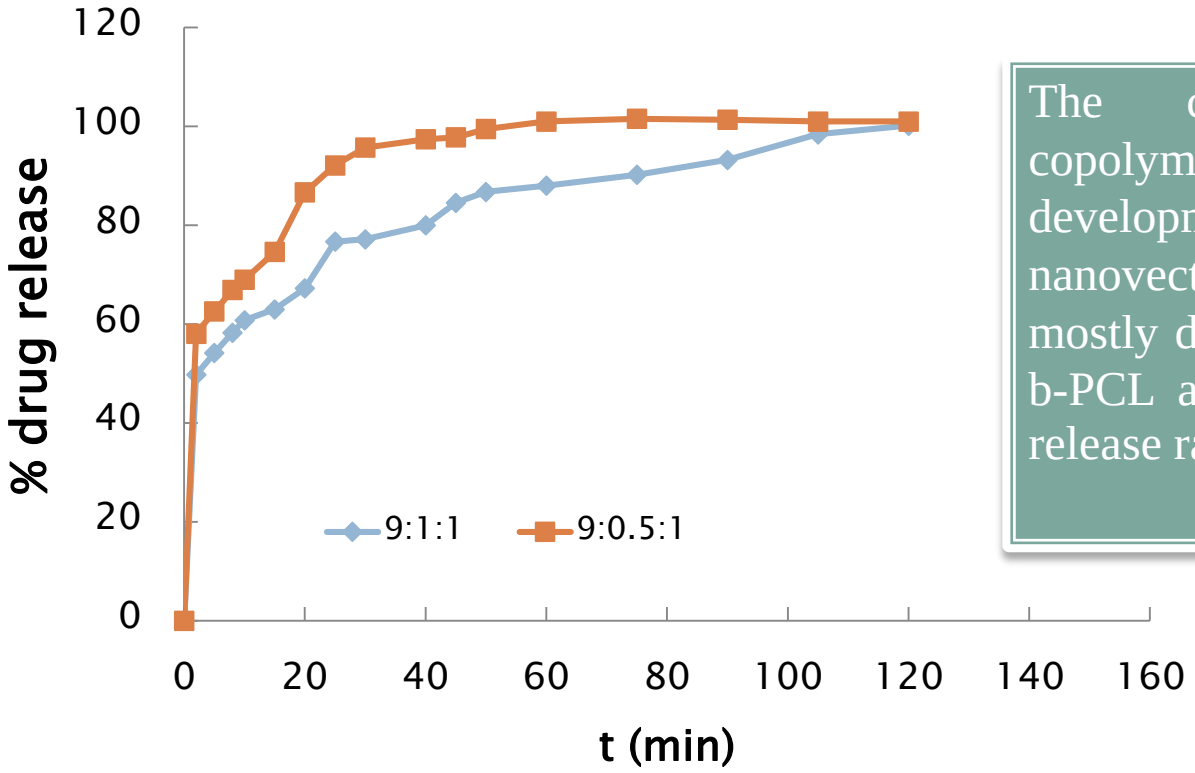
Structure and drug encapsulating properties of novel self-assembled chimeric nanostructures constructed from biocompatible PEO-b-PCL copolymer and lipids are presented.

Sample	Dispersion Medium	R_h(nm)	PD.I.	ζ-potential (mV)	d_f
DPPC liposomes	HPLC-grade water	62.5	0.61	1.0	2.5_1
DPPC:PEO-b-PCL (9:0.1 molar ratio)	HPLC-grade water	44.8	0.35	-0.9	2.4_2
DPPC:PEO-b-PCL (9:0.5 molar ratio)	HPLC-grade water	40.6	0.25	-8.6	2.5_6
DPPC:PEO-b-PCL (9:1 molar ratio)	HPLC-grade water	41.2	0.27	-9.3	1.8_3
DPPC λιποσώματα	PBS	95.8	0.70	0.7	2.5_5
DPPC:PEO-b-PCL (9:0.1 molar ratio)	PBS	46.9	0.32	0.3	2.4_8
DPPC:PEO-b-PCL (9:0.5 molar ratio)	PBS	44.8	0.28	0.1	2.5_8
DPPC:PEO-b-PCL (9:1 molar ratio)	PBS	44.7	0.31	-3.4	1.8_4

Sample	Dispersion Medium	R_h (nm)	PD.I.	d_f	ζ-potential (mV)	% Encapsulation Efficiency
DPPC:PEO-b-PCL:IND (9:0.1:1 molar ratio)	HPLC-grade water	32.5	0.22	2.5_6	-4.1	10.2
DPPC:PEO-b-PCL:IND (9:0.5:1 molar ratio)	HPLC-grade water	34.1	0.41	2.4_6	-1.4	11.5
DPPC:PEO-b-PCL:IND (9:1:1 molar ratio)	HPLC-grade water	38.4	0.33	1.9_0	-15.3	13.5
DPPC:PEO-b-PCL:IND (9:0.1:1 molar ratio)	PBS	44.2	0.27	1.9_6	-6.8	13.2
DPPC:PEO-b-PCL:IND (9:0.5:1 molar ratio)	PBS	39.7	0.25	1.9_9	-1.8	22.6
DPPC:PEO-b-PCL:IND (9:1:1 molar ratio)	PBS	38.8	0.29	1.9_1	-3.1	30.5

Drug release studies

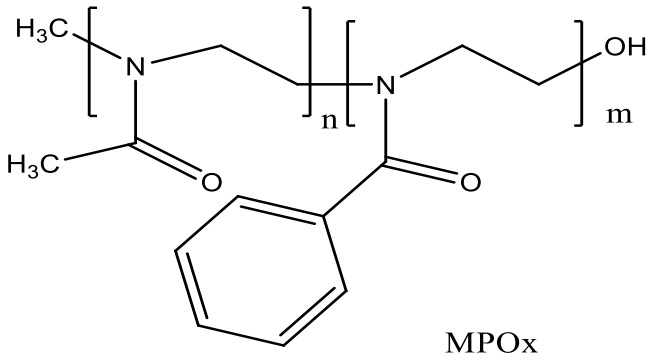




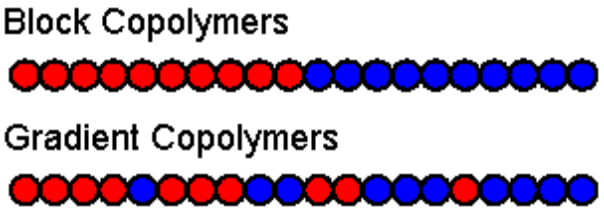
The combination of block copolymers with liposomes for the development of a novel chimeric nanovector appears very promising, mostly due to the fact that the PEO-b-PCL acts as a modulator for the release rate of the IND.

It is observed that the *in vitro* release of the drug from the prepared chimeric nanostructures is quite fast especially for the mixed nanovectors prepared with the lower ratio of block copolymer.

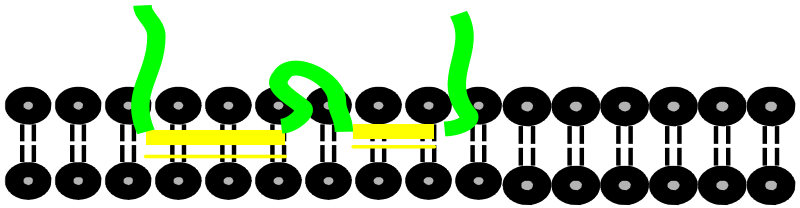
DPPC:MPOx chimeric/mixed nanocarriers:



MPOx



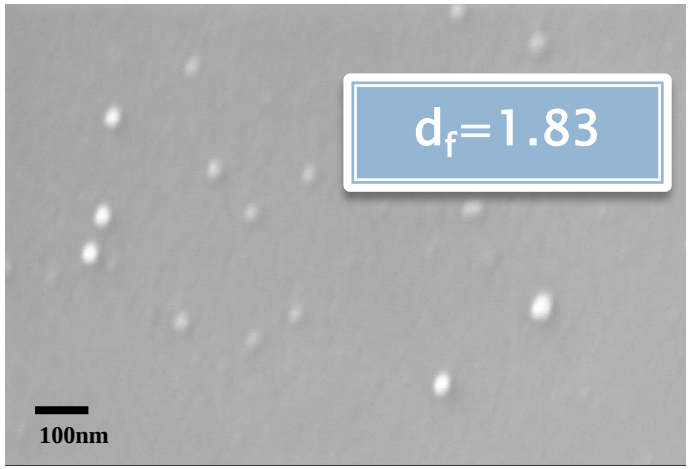
poly(2-methyl-2-oxazoline-grad-2 phenyl-2-oxazoline) gradient block copolymers

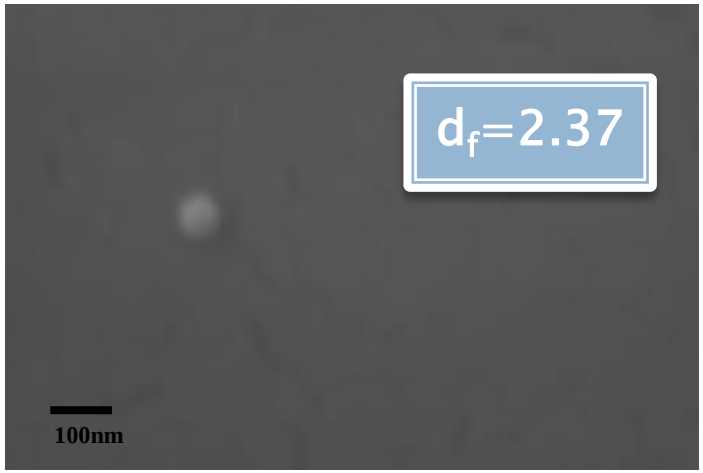


Poly(2-oxazoline)s and their copolymer are characterized as bioinspired materials due to the ***pseudopeptide nature*** of the oxazoline segments, while poly(2-methyl-2-oxazoline) is proposed as an alternative to PEG in terms of biocompatibility and stealth properties and their solvation and self-assembly behavior were extensively studied (Hoogenboom et al. 2007, 2008, Kempe et al. 2009; Schlaad et al. 2010; Barz et al. 2011; Dasa and Hong 2011; Lambermont-Thijs et al. 2011).

Sample	Dispersion Medium	R_h(nm)	PD.I.	ζ-potential (mV)	d_f
DPPC liposomes	HPCL-grade water	62.5	0.61	1.0	2.5_1
DPPC:MPOx-1 (9:0.1)	HPCL-grade water	66.0	0.53	0.9	2.4_5
DPPC:MPOx-1 (9:0.5)	HPCL-grade water	60.4	0.60	2.5	2.3_8
DPPC:MPOx-1 (9:1)	HPCL-grade water	61.8	0.60	3.7	2.3_7
DPPC:MPOx-1 (9:2)	HPCL-grade water	50.3	0.49	1.1	2.0_8
DPPC:MPOx-1 (9:3)	HPCL-grade water	55.8	0.59	0.7	2.1_6
DPPC liposomes	PBS	95.9	0.70	0.7	2.5_5
DPPC:MPOx-1 (9:0.1)	PBS	55.3	0.59	0	2.4_1
DPPC:MPOx-1 (9:0.5)	PBS	61.6	0.63	0.9	2.3_1
DPPC:MPOx-1 (9:1)	PBS	75.4	0.68	1.5	2.4_5
DPPC:MPOx-1 (9:2)	PBS	54.0	0.51	-3.1	2.0_8
DPPC:MPOx-1 (9:3)	PBS	52.3	0.51	-3.5	2.0_8

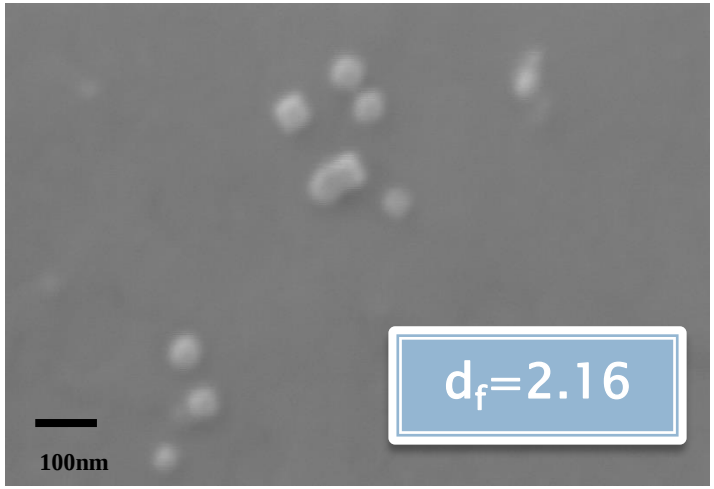
Sample	Dispersion Medium	R_h (nm)	PD.I.	d_f	ζ-potential (mV)	% Encapsulation Efficiency
DPPC:MPOx-1:IND (9:0.1:1)	HPLC-grade water	99.5	0.67	2.2_8	-0.4	13.2
DPPC:MPOx-1:IND (9:0.5:1)	HPLC-grade water	67.1	0.45	2.5_6	-0.2	13.7
DPPC:MPOx-1:IND (9:1:1)	HPLC-grade water	58.4	0.35	2.1_2	-8.5	8.3
DPPC:MPOx-1:IND (9:2:1)	HPLC-grade water	78.0	0.42	2.0_7	+0.1	7.6
DPPC:MPOx-1:IND (9:3:1)	HPLC-grade water	80.3	0.38	2.2_9	-1.1	13.6
DPPC:MPOx-1:IND (9:0.1:1)	PBS	205.4	0.55	2.3_8	+0.3	11.1
DPPC:MPOx-1:IND (9:0.5:1)	PBS	75.0	0.59	2.3_7	-3.1	18.7
DPPC:MPOx-1:IND (9:1:1)	PBS	60.4	0.52	2.2_6	-2.6	22.9
DPPC:MPOx-1:IND (9:2:1)	PBS	61.8	0.53	2.1_8	-1.0	13.8
DPPC:MPOx-1:IND (9:3:1)	PBS	65.5	0.48	2.4_7	-1.1	10.8





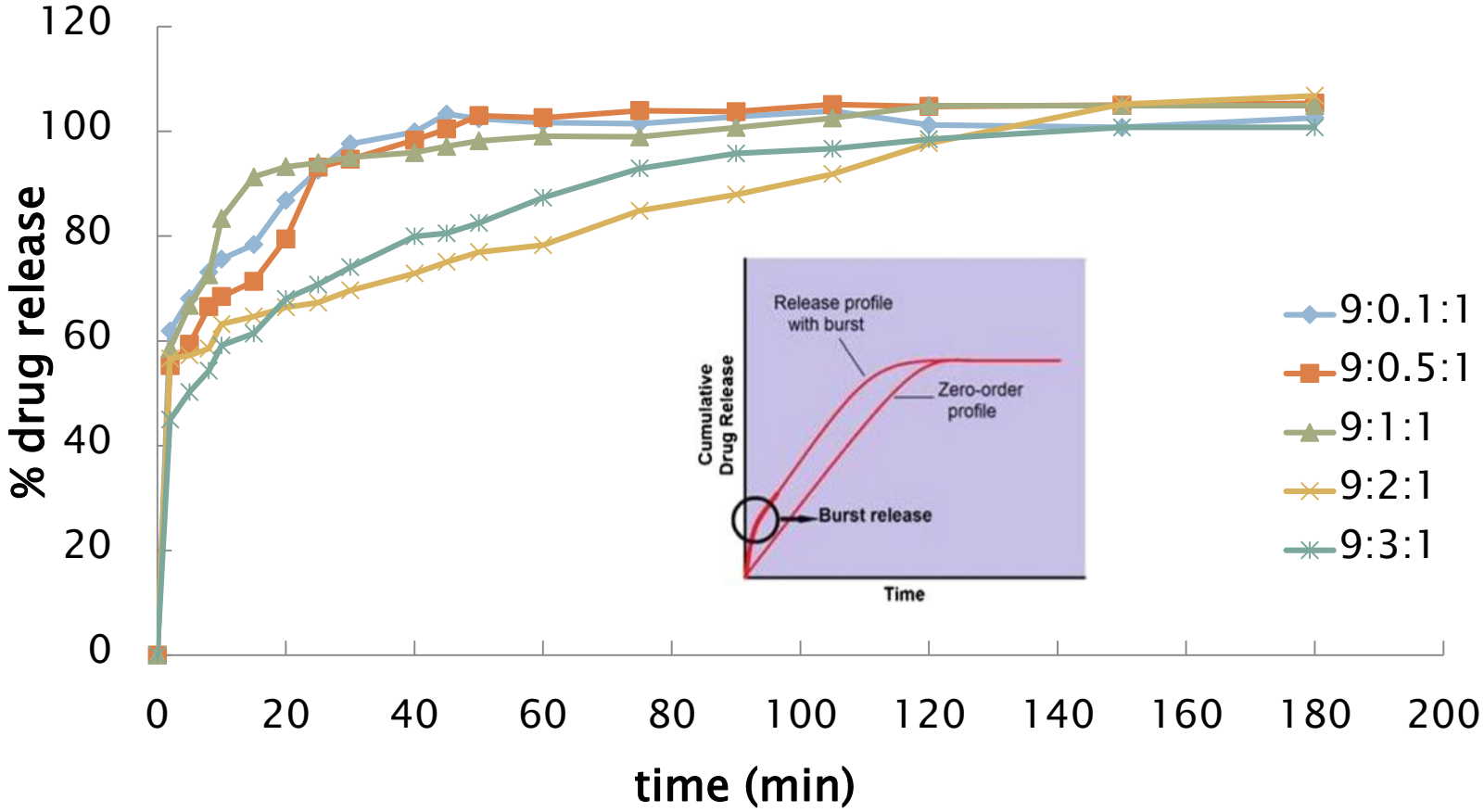
SEM images **a.** DPPC:PEO-b-PCL 1 (9:1 molar ratio)

The shape of chimeric nanoparticles

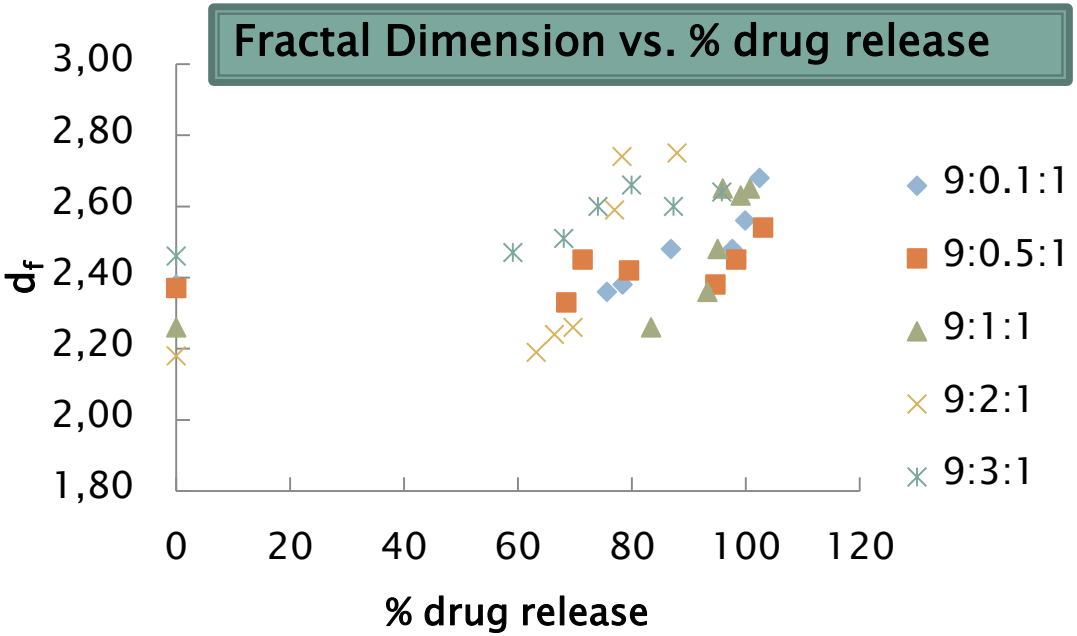


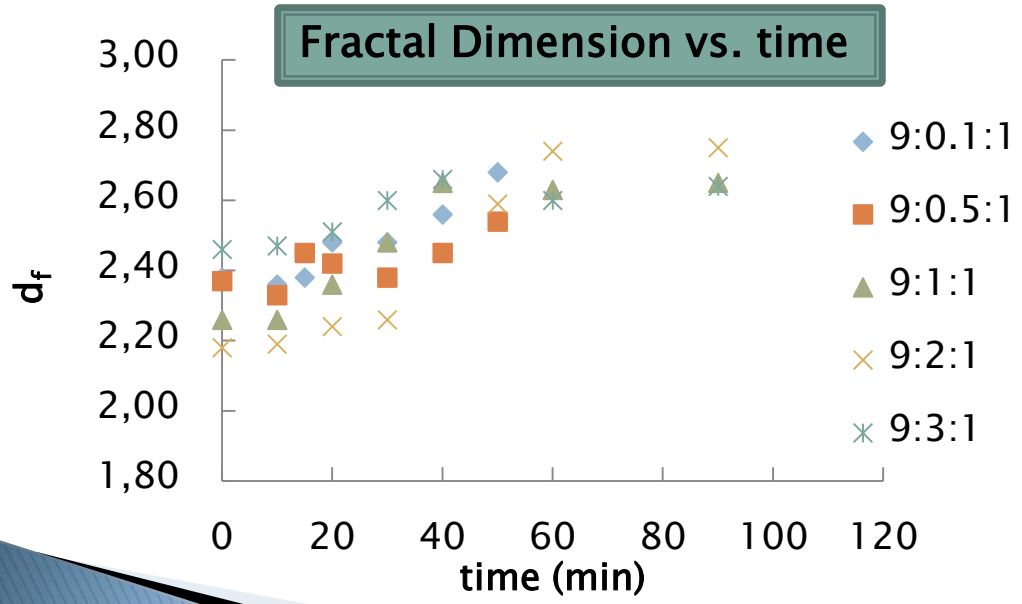
SEM images DPPC:MPOx 1 **a.** 9:1 and **b.** 9:3 molar ratio

Drug release studies



The *in vitro* release of the IND is faster from the polymer grafted liposomes with the lower ratio of gradient block copolymer. This phenomenology could serve as a guide factor for the preparation and development of formulations with the desired release profile, modulating the release rate of the IND via the ratio of the components, improving the release profile of the incorporating drug.





There is a strong interplay between the morphology of the liposomes, as expressed by d_f values and the rate of release of IND from the mixed liposomal nanovectors. An increase of d_f values during the release of IND was observed.

higher d_f values correspond to lower rates of release, while as the release progresses the morphology of the liposome is self-reassembled and d_f increases.

A time delay is observed between each release snapshot and the corresponding change of d_f which reflects the time of reorganization of the morphology of the nanostructure.

Publications for mixed/chimeric liposomes:

Journal of Liposome Research, 2012; 22(1): 55–61
© 2012 Informa Healthcare USA, Inc.
ISSN 0898-2104 print/ISSN 1532-2394 online
DOI: 10.3109/08982104.2011.590142

RESEARCH ARTICLE

The formalism of fractal aggregation phenomena of colloidal drug delivery systems

Natassa Pippa[1], Costas Demetzos[1,*], and Emmanuel Danezis[2]

International Journal of Pharmaceutics 430 (2012) 65–73
Contents lists available at SciVerse ScienceDirect
International Journal of Pharmaceutics
journal homepage: www.elsevier.com/locate/ijpharm

The fractal hologram and elucidation of the structure of liposomal carriers in aqueous and biological media

Costas Demetzos[a,*]

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

RESEARCH PAPER

Journal of Liposome Research
ISSN
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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

RESEARCH ARTICLE

The imaging and the fracta… Delivery nano Systems: the… polymeric guest

Natassa Pippa[1,2], Stergios Pispas[2], and Costas Demetzos[1]

…ology Balance: A Study of Stealth Liposomes …ia Fractal Analysis and Drug Encapsulation

…latassa Pippa · Faidra Psarommati · Stergios Pispas · Costas Demetzos

International Journal of Pharmaceutics 437 (2012) 264–274
Contents lists available at SciVerse ScienceDirect
International Journal of Pharmaceutics
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Pharmaceutical Nanotechnology

The delineation of the morphology of charged liposomal vectors via a fractal analysis in aqueous and biological media: Physicochemical and self-assembly studies

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International Journal of Pharmaceutics 465 (2014) 63–69
Contents lists available at ScienceDirect
International Journal of Pharmaceutics
journal homepage: www.elsevier.com/locate/ijpharm

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

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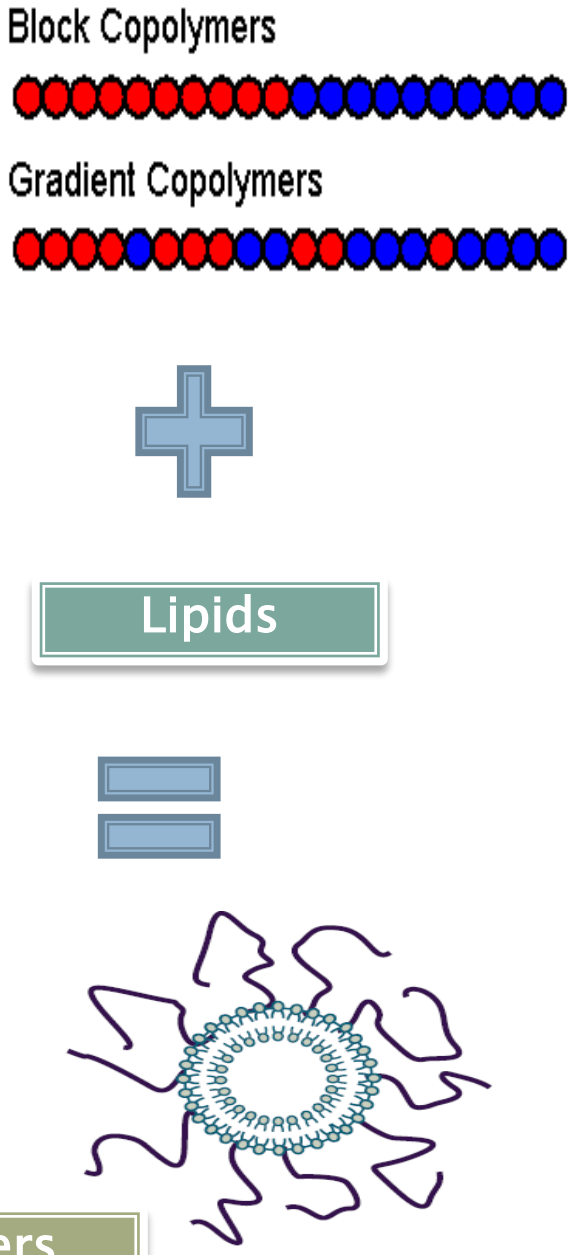
[b] Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, Athens, Greece

Conclusions:

- The combination of block and gradient block copolymers with lipid for the development of a novel mixed nanovector appears very promising, **mostly due to the fact that the polymeric guest acts as a modulator for the release rate of the encapsulated drug.**
- *The interdependence of shape and morphology of prepared polymer-grafted liposomes (as quantified via fractal dimension) and the rate of the drug release.*
- The fractal morphology of the polymer grafted liposomes affects the drug release and must be taken into account to develop liposomal drug **with complete knowledge of their structural properties due to the strong interplay between the morphology of the liposomes and the rate of release of the incorporated drug.**



Acknowledgments:

Prof. Costas Demetzos

Dr. Stergios Pispas

Dr. Aristides Dokoumetzidis

Thank you for your kind attention!

Ευχαριστώ πολύ για την προσοχή σας!