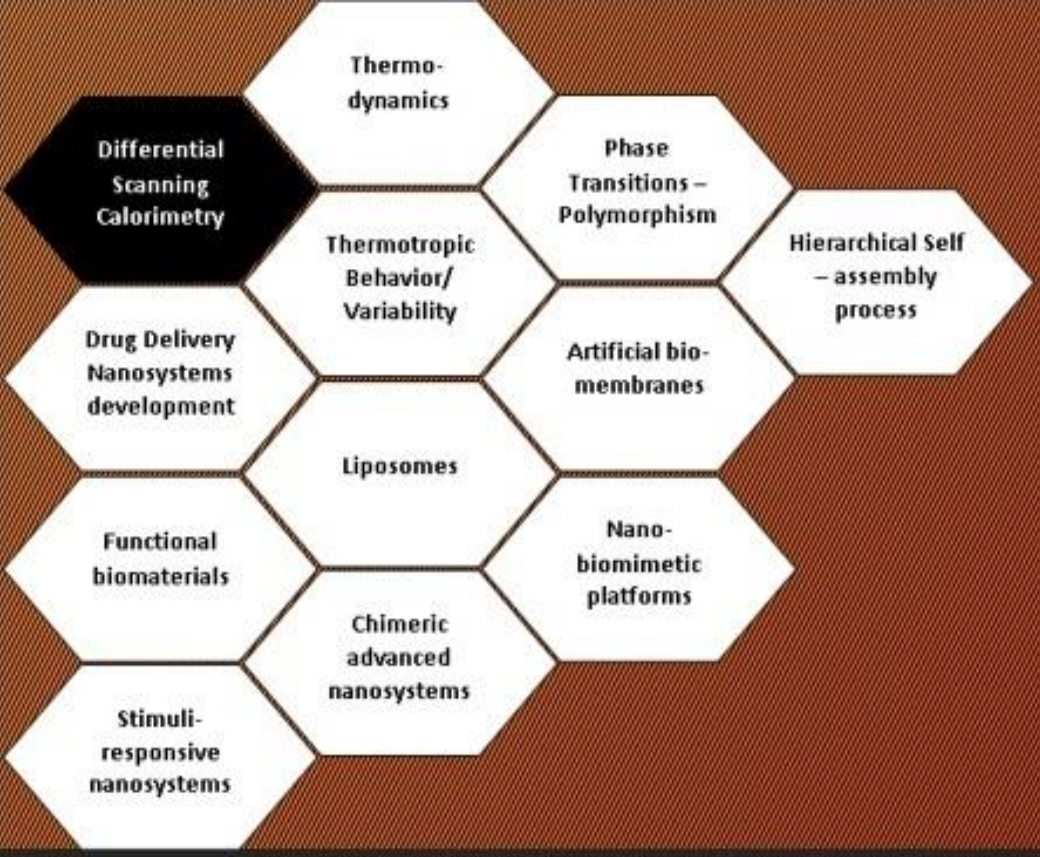


# Thermodynamics of Artificial Biomembranes

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## ❖ INTRODUCTION

**The macroscopic properties of matter promote extensive discussions among scientists and the classical thermodynamics is considered as a powerful tool of the state of equilibrium. Heat is referred to as the energy in transit and is accepted as the form of energy that can transit from one place to another because of difference in the temperature between the two places. This statement is important and not considered as obvious, since scientists confused temperature with heat two centuries ago.**

**Another important issue is the thermal state of a system. A system is a part of the universe that comprises of well-defined and measurable variables. The systems based on their ability to exchange energy with the surroundings, can be classified as, isolated, closed and open systems. The thermodynamic state of a system is an important aspect by which it could be classified as in an equilibrium (all parts of the system are at the same temperature) or in a non-equilibrium state.**

**Thermal analysis techniques and thermodynamics of complex systems are important fields of research. Simulation studies on biological templates (such as biological membranes), based on thermodynamics, are considered as impressive, because they are elements that allow comparison between the biological functions and those of artificial bio-membranes.**

**Moreover, studies on artificial bio-networks of high complexity, that are classified as biomimetic systems, have been used in order to design and to develop micro or nano ($10^{-9}$ m) macromolecular self-assembled vehicles, in order to transport pharmaco- or bio molecules to the target tissue of human organism. Techniques such as Thermal Analysis and especially Differential Scanning Calorimetry (DSC) could be used as important technical tools for studying the behaviour and functionality of biomimetic systems and to project their properties biological systems. We have to point out that the environmental physicochemical conditions and the internal physicochemical properties of the biomimetic systems affect their behaviour and their functionality.**

**The phase transitions of complex biomimetic systems and their thermotropic changes are impressive scientific topics and dozens of laboratories worldwide deal with such approaches.**

**By corelating the thermodynamics and the thermotropic behaviour of artificial biomimetic networks with that of living ones, we could be closer to understand the biophysical basis of human diseases. However, the term '*biophysical disease factor'* is an emerging field which can contribute to a better understanding of the rationale of diseases and subsequently lead to the rise of new and innovative therapeutic approaches. The phase transitions of a system are considered as non-equilibrium processes and the phenomenon of polymorphism of matter is of importance and should be extensively studied. The polymorphisms have different energetic content and the most thermodynamically stable polymorphic form is that with the lowest free energy. The rate of transition from unstable to stable polymorphic forms is very slow and practically unimportant for further studies. The critical point is: which polymorphic form behaves as in a metastable state and how we can recognize this metastability process, which is very critical, because of the transition from a non-equilibrium state to another one. This final statement could be the critical point that should be introduced to the development process of medicines in the pharmaceutical science, even as a debate.**

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**The laboratory of Pharmaceutical Nanotechnology of the Department of Pharmacy in the National and Kapodistrian University of Athens, in the recent years (almost during the last 20 years) has been oriented towards the study of the Thermodynamics of complex artificial bio-networks and systems that are able to serve as vehicles, in order to transport bioactive (pharmaco- and bio-molecules) cargos to target tissues. However, we focus our research on the study of the thermotropic behaviour and the phase transitions of non-equilibrium processes (metastable phases) of semi-complex -in their composition- artificial biosystems, like liposomes, dendrimers, polymeric particles or mixed/chimeric systems that are in nano dimension. In particular, we perform:**

- **study of the thermotropic variability of nano- and micro-drug delivery systems, based on the changes of their thermodynamic variables**
- **design and development of artificial biomimetic membranes and thermodynamical studies of their behaviour in different micro-environmental stimuli**
- **combination of different kinds of biomaterials (from the same or different biomaterial categories) in the same nanosystem (mixed nanosystems) (development of advanced hybrid or chimeric nanosystems respectively), investigation of their cooperativity, as well as evaluation of the thermotropic behavior, complexity and functionality of the whole resultant nanosystem**
- **development of nanovesicular biomimetic lipidic systems such as liposomes that are able to respond to external stimuli, like changes of temperature, pH and ionic strength**
- **evaluation of the results regarding the thermotropic behaviour of nanoparticulate systems and design of innovative nanobiomimetic platforms for drug delivery**
- **evaluation of the results and correlation with experimental results of other methods, for the thoroughgoing characterization of the prepared nanosystems (for example, correlation of the thermotropic behavior of the nanosystems with their physicochemical stability and their content release profile)**
- **design and study of the hierarchical self-assembly of biomolecules, for the development of nanosystems and nanostructures, based on their thermodynamic variability**

**Based on the above activities, our laboratory has published almost 40 research peer-review papers in international scientific journals and has announced a huge number (more than 100) of oral and poster presentation in international congresses. Dozens of PhD and Master Theses and Undergraduate Dissertations have been completed on the above scientific topics.**

**The present volume deals with the synopsis of the lasting effort of the members of the laboratory of Pharmaceutical Nanotechnology over the last 20 years to study**

**and highlight the key role of Thermodynamics in the development of drug delivery and biomimetic systems, in collaboration with partners from all over the world.**

**By reading the published papers of this volume, the reader can distinguish peer-review articles that deal with:**

- **review of past works on the Thermodynamics of nanosystems**
- **research on the design and development of artificial biomimetic cell membranes, which are able to simulate cell membranes, with functions and behaviour that are based on their metastability process**
- **interactions of biomimetic nanosystems, like liposomes with incorporated bioactive molecules or even drugs**
- **evaluation of the interactions between biomaterials, in order to rationally design innovative drug delivery biomimetic nanocarriers**
- **design and development of conventional and mixed/chimeric lipidic drug delivery nanosystems, empty or with incorporated drug and their physicochemical properties, such as stability in biological and non-biological media, as well as their responsiveness in external physical and chemical stimuli**

**Summing up,**

**I would like to emphasize the great importance of Thermodynamics of complex systems in non-equilibrium state and their correlation with the living systems and networks. The correlation of the thermotropic behaviour of artificial biomimetic systems and networks with the living cells' membrane functions could be the driving force to learn more about nature's *'encrypted code'*, in order to rationally design effective drug delivery systems and finally, effective and safe medicines. It could also help us understand the biophysical mechanisms and *'anomalies'* by which human diseases are born.**

**Costas Demetzos**

**Professor**

**National & Kapodistrian University of Athens**

## ❖ Articles

1. *Ether Phospholipid-AZT Conjugates Possessing Anti-HIV and Antitumor Cell Activity. Synthesis, Conformational Analysis, and Study of Their Thermal Effects on Membrane Bilayers* . T. Mavromoustakos, T. Calogeropoulou, M. Koufaki, A. Kolocouris, I. Daliani, C. Demetzos, Z. Meng, A. Makriyannis, J. Balzarini, and E. De Clercq, *J. Med. Chem*. **2001**, 44, 1702-1709
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3. *In vitro cytotaxic/cytotoxic activity of anionic liposomes containing vinblastine against leukemic human cell lines .* H. Maswadeh, C. Demetzos, K. Dimas, Y. Loukas, A. Georgopoulos, T. Mavromoustakos, G.Th. Ppapaioannou, *J. Pharmacy and Pharmacology*, **2002**, 54:189–196.
4. *A molecular basis explanation of the dynamic and thermal* effects of vinblastine sulfate upon dipalmitoylphosphatidylcholine bilayer membranes, H. Maswadeh , C. Demetzos , I. Daliani , I. Kyrikou , T. Mavromoustakos , A. Tsortos, G. Nounesis , *Biochimica et Biophysica Acta*, 1567, **2002**, 49–55
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8. *Phase transitions of lipids and liposomes,* S. Hatziantoniou, C. Demetzos, M. Wagner*, User Com,* 1, 16-19,**2005**
9. *A comparative study of cholesterol and sclareol, a bioactive labdane type diterpene on phospholipid bilayers,* I. Kyrikou, S. Hatziantoniou, A. Georgopoulos, T. Mavromoustakow, C. Demetzos, *Chemistry and Physics of Lipids*, 134, **2005**, 123-135
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## ❖ Articles with abstracts

1. *Ether Phospholipid-AZT Conjugates Possessing Anti-HIV and Antitumor Cell Activity. Synthesis, Conformational Analysis, and Study of Their Thermal Effects on Membrane Bilayers* . T. Mavromoustakos, T. Calogeropoulou, M. Koufaki, A. Kolocouris, I. Daliani, C. Demetzos, Z. Meng, A. Makriyannis, J. Balzarini, and E. De Clercq, *J. Med. Chem*. **2001**, 44, 1702-1709

The 1-O-hexadecyl-2-O-methyl-sn-glyceryl phosphodiester AZT 4 and hexadecyl-phosphodiester AZT 5 derivatives were synthesized and found to be active against HIV-1, HIV-2, and tumour cell proliferation. Compared to AZT, compound 4 possessed ca. 10-fold lower anti-HIV activity and ca. 10-fold higher anti-tumour cell activity. Compound 5 was 10-fold less potent than compound 4 in both biological tests. In an attempt to correlate biological activity of compounds 4 and 5 with structure, their conformational and thermal effects on membrane bilayers were compared using a combination of NMR spectroscopy, computational analysis, and Differential Scanning Calorimetry. The obtained results showed that compound 4 adopts a compact conformation in which the alkyl chain, the 2-methoxyglyceryl functionality, and the methyl group of thymine are in spatial proximity, while analogue 5 possesses a less compact conformation of the nucleoside base and the alkyl chain. The presence of the 2-methoxyglyceryl group in compound 4 may augment its potency by inducing a turn of the alkyl chain stabilized by hydrophobic interactions. The DSC scans show that conjugate 4 affects less effectively the thermotropic properties of model membrane bilayers than compound 5. This may be attributed to the fact that compound 4 is incorporated in a compact conformation and does not perturb significantly the trans:gauche isomerization of the membrane phospholipids. In contrast, conjugate 5 may enter with a less compact conformation and perturb more the membrane bilayers.

2. *Structure elucidation, conformational analysis and thermal effects on membrane bilayers of an antimicrobial myricetin ether derivative*. C. Demetzos, D. Angelopoulou, A. Kolokouris, I. Daliani, T. Mavromoustakow*, J. Heterocyclic Chem.* 38, 703-710, **2001**.

The membrane perturbing 3,7,4',5'-tetramethyl ether of myricetin 1 was isolated from *Cistus monspeliensis* L. Its structure was elucidated and its conformational properties were explored using combination of 2D NMR spectroscopy and computational chemistry. (…) The Differential Scanning Calorimetry (DSC) results revealed that the degree of thermal effects exerted by the flavonoids at dipalmytoylphosphocholine (DPPC) bilayers followed the order 1>2>myricetin. Their antimicrobial activity against Gram positive followed the same order.

3. *In vitro cytotaxic/cytotoxic activity of anionic liposomes containing vinblastine against leukemic human cell lines .* H. Maswadeh, C. Demetzos, K. Dimas, Y. Loukas, A. Georgopoulos, T. Mavromoustakos, G.Th. Ppapaioannou, *J. Pharmacy and Pharmacology*, **2002**, 54:189–196.

Liposomes prepared from lipids dipalmitoylphosphatidylcholine (DPPC) and dipalmytoylphosphatidylglycerol (DPPG) with cholesterol were used to investigate the percentage of vinblastine encapsulation and influence of lipid composition on the retention properties of vinblastine in buffer as well as culture medium. Differential scanning calorimetry (DSC) was applied to study the effect of cholesterol on the phase transition temperature and on the DH the two liposome formulations. The cytotoxic and cytostatic activity of the liposome-encapsulated vinblastine was also examined against leukemic human cell lines. The results showed that encapsulation of vinblastine into liposomes was greater than 98% with a drug to lipid ratio of 0.13-0.18 . The major improvement in vinblastine retention time in buffer as well as in culture medium was achieve by employing DPPG. The DSC data showed that vinblastine exerted a more retexturing effect in DPPC-cholesterol bilayers in DPPG-cholesterol bilayers and this may explain their lower retention time. The 50% growth-inhibiting (GI 50)

and cytostatic (TGI) activity of liposomal vinblastine did not seem to be affected by the type of the liposome while the 50% cytotoxic activity (LC50) was affected in four out of the six cell lines tested. The parameters GI50, TGI and LC50 were estimated according to the instruction given by the NCI.

4. *A molecular basis explanation of the dynamic and thermal* effects of vinblastine sulfate upon dipalmitoylphosphatidylcholine bilayer membranes, H. Maswadeh , C. Demetzos , I. Daliani , I. Kyrikou , T. Mavromoustakos , A. Tsortos, G. Nounesis , *Biochimica et Biophysica Acta*, 1567, **2002**, 49–55

Differential scanning calorimetry has been employed to study the thermal effects of vinblastine sulphate upon aqueous, single and multiple bilayer dispersions of 1,2-dipalmitoyl-3-sn-phosphatidylcholine (DPPC). The calorimetric results summarized to an increase in the gel to liquid–crystalline phase transition enthalpy and the abolishment of the Lh V(gel phase) to Ph V(ripple phase) pretransition for the uni- and multilamellar dispersions, as well as an increase in the transition temperature Tm and the transition cooperativity for single bilayer DPPC/ vinblastine mixed vesicles, are consistent with an induced, partially interdigitated, gel phase. Computational analysis has been successfully applied to clarify the intermolecular effects and verify the feasibility of the proposed interdigitation for the vinblastine sulphate molecules and also for the ursodeoxycholic acid (UDCAH) and bromocylated taxanes, which have been shown to induce an interdigitated gel phase in DPPC bilayers.

5. *The effects of vinblastine sulfate on dipalmitoylphosphatidylcholine single and multiple bilayer membranes,* H. Maswadeh, C. Demetzos, I. Daliani, T. Mavromoustakos, G. Nounesis, A. Tsortos*, Drug Discovery and Design: Medical Aspects, J. Matsoukas and T. Mavromoustakos, Eds, IOS Press, **2002**.*

The thermal and dynamic effects of vinblastine encapsulated in large uni- and multilamellar vesicles upon the phospholipid membranes have been studied for Diparmytoylphosphocholine (DPPC). The hydrophilic vinblastine sulphate molecules have been encapsulated using p H gradient technique. MAS 13C NMR spectroscopy at various temperatures and a combination of high-precision differential scanning calorimetry techniques have been employed to study the effects upon the lipid phase transition sequence and especially upon the gel/crystalline phase transition. The calorimetric and spectroscopic results demonstrate that the encapsulation of vinblastine results in the abolishment of the Lb' to Pb' pretransition and most importantly in an increase of the molecular cooperativity of the single-bilayer phospholipid membranes along with an increase in the total enthalpy change (ΔH) for the main lipid phase transition. Computational analysis points out the intermolecular interactions between vinblastine and DPPC bilayers that can explain the thermograms and the NMR spectra.

6. *Encapsulation of naturally occurring flavonoids into liposomes: physicochemical properties and biological activity against human cancer cell lines*, M. Goniotaki, S. Hatziantoniou, K. Dimas, M. Wagner and C. Demetzos, *Journal of Pharmacy and Pharmacology*, **2004**, 56: 1217–1224.

Liposomes consisting of egg phosphatidylcholine were prepared by a thin-film hydration method followed by sonication and were used to investigate the percentage encapsulation of four flavonoids (quercetin, rutin, isoscutellarein and isoscutellarein diglycoside). The lipid recovery and the flavonoid-to-lipid molar ratio were measured using high-performance thin-layer chromatography/ flame ionization detection and UV-vis spectroscopy. Differential scanning calorimetry was used to study the effect of the flavonoids on the phase transition temperature and on the enthalpy of the main phase transition of dipalmitoylphosphatidylcholine bilayers, and their ability to influence the membrane fluidity. The final liposomal formulation incorporating flavonoids, as well as free flavonoids, were tested for their activity against human cancer cell lines using the sulforhodamine B assay. The results showed that the encapsulation efficiency varied from 95% (0.21 flavonoid-to-lipid molar ratio) to 37.5% (0.09 flavonoid-to-lipid molar ratio) for isoscutellarein and its glycoside, respectively. The differential scanning calorimetry data showed close thermal and dynamic effects depending on the structure of the flavonoids, and suggest that there is a relationship between flavonoid molecular structure and the

interaction with model membranes. Liposomal isoscutellarein showed improved growth inhibiting activity against all cell lines tested in comparison with that of its free form, which was inactive (>100M).

7. *The modulation of thermal properties of vinblastine by cholesterol in membrane bilayers*, I. Kyrikou, I. Daliani, T. Mavromoustakow, H. Maswhadeh, C. Demetzos, S. Hatziantoniou, S. Giatrellis, G. Nounesis*. Biochimica et Biophysica Acta*, 1661 , **2004**, 1–8.

It has been shown that the partitioning of vinblastine in (….) DPPC single and multiple bilayer dispersion induces partial interdigitation of the lipid alkyl chains. (….) differential scanning calorimetry (DSC) has been employed to investigate the role of lipid molecular characteristics such as the alkyl chain length and the polarity of the head group, as well as the impact of cholesterol upon vinblastine-induced interdigitation. It is found that vinblastine does not induce interdigitation in lipids with either shorter or longer alkyl chains than DPPC, or phase behaviour of the lipid/vinblastine bilayer system. Preliminary studies show that properties directly affect the encapsulation efficiency and the pharmacokinetics of liposomes.

8. *Phase transitions of lipids and liposomes,* S. Hatziantoniou, C. Demetzos, M. Wagner*, User Com,* 1, 16-19,**2005**

DSC studies on the interaction of flavonoids with model DPPC membranes and on the relationship of the flavonoid structure to the lipid environment showed that the efficiency of incorporation depends on the structure of flavonoid. The results indicate that the presence of a sugar group or even just a number of hydroxyl groups in different positions in the flavonoid structure, plays a role in the incorporation of flavonoids in liposomes and the interaction of flavonoids with DPPC membranes. The liposome formulations with flavonoids were also tested for their activity against human cancer cell lines. In the case of quercetin, the formulation showed a lower degree of growth inhibition compared with the free quercetin; improved growth inhibition was however observed with the isoscutellarein formulation.

9. *A comparative study of cholesterol and sclareol, a bioactive labdane type diterpene on phospholipid bilayers,* I. Kyrikou, S. Hatziantoniou, A. Georgopoulos, T. Mavromoustakow, C. Demetzos, *Chemistry and Physics of Lipids*, 134, **2005**, 123-135

Sclareol (…) is a highly water-insoluble molecule that belongs to the labdane -type of diterpenes and is characterized as a biologically active molecule, due to its cytotoxic and cytostatic effects against human leukemic cell lines. (…) Differential Scanning Calorimetry (DSC) was applied to compare the thermal changes caused by either cholesterol or sclareol when are incorporated in DPPC bilayers. The results showed that sclareol is incorporated into phospholipid model membranes and mimics the thermal effects of cholesterol especially at concentrations up to X sclareol = 9.1 mol%. These effects can be summarized as the abolition of pre-transition, lowering of the main phase transition and reduction of the enthalpy change (ΔH) of the gel to liquid-crystalline phase transition of DPPC bilayers. (….)

10. *Labdane-type diterpenes: thermal effects on phospholipid bilayers, incorporation into liposomes and biological activity*, C. Matsingou, S. Hatziantoniou, A. Georgopoulos, K. Dimas, A. Terzis, C. Demetzos, *Chemistry and Physics of Lipids*, 138**, 2005**, 1-11.

Labd-13(E)-ene-8a, 15-diol (1) and its active derivative labd-13 (E)-ene-8a, 15-yl acetate, 2, are water insoluble biological active molecules and their structures were elucidated using NMR and X-ray techniques. Differential Scanning Calorimetry (DSC) was applied to study the thermal effects of 1 and 2 on DPPC lipid bilayers. (…) The effect of 1 and 2 on DPPC bilayers caused abolition of the pre-transition temperature, lowering the main phase transition and reduction of the transition enthalpy only in the presence of cholesterol. (…)

11. *Effect of bioactive curcumin derivative on DPPC memebrane : ADSC and RAMAN spectroscopy study,* K. Gardikis, S. Hatziantoniou, K. Viras, C. Demetzos*, Thermochimica Acta,* 447, **2006**, 1-4.

Interactions of dimethoxycurcumin (1), a lipophilic bioactive curcumin derivative with (….) DPPC were investigated. The thermodynamic changes cause by 1 and its location into the DPPC lipid bilayers were monitored by Differential Scanning Calorimetry and RAMAN spectroscopy. (….) The results of these studies provide information on the membrane integrity and physicochemical properties that are essential for the rational design lipidic drug delivery systems.

12. *Interactions of dendrimers with model lipid membranes assessed by DSC and RAMAN spectroscopy*, K. Gardikis, S. Hatziantoniou , K. Viras, M. Wagner and C. Demetzos , M.R. Mozafari (ed.), Nanocarrier Technologies: Frontiers of Nanotherapy,207–220, © **2006** Springer. Printed in the Netherlands.

The aim of the present work was to study the interaction between PAMAM generation 4 (G4) dendrimer with model lipid membranes (DPPC) for designing new controlled released systems for bioactive molecules by combining dendrimer and liposomal technologies. Thermal analysis and Raman spectroscopy were applied to assess the thermodynamic changes caused by PAMAM G4 (polyamidoamines) dendrimer and to specify the exact location of this dendrimer into the DPPC lipid bilayer. DSC thermograms indicated that the maximum percent of PAMAM G4 that can be incorporated in the DPPC membrane without deranging its integrity is 5%. The Raman intensity ratios I 2935 / 2880 and I 1090/1130 cm 1 showed the degree of the fluidity of the lipid bilayer, while the absorption at 715 cm 1 showed a strong interaction of PAMAM G4 with the polar head group of phospholipids. The results showed that the incorporation of the PAMAM G4 dendrimer in DPPC bilayers causes a concentration dependent increase of the membrane fluidity and they interact strongly with both the lipophilic part and the polar head group of the phospholipids. Additionally, due to the current weak knowledge of how dendrimers interact with lipidic membranes these results may justify the tendency of dendrimers to disrupt biological membranes

13. *A DSC and RAMAN spectroscopy on the effect of PAMAM dendrimer on DPPC model lipid membranes,* K. Gardikis, S. Hatziantoniou, K. Viras, M. Wagner, C. Demetzos, *International Journal of Pharmaceutics*, 318, **2006**, 118-123.

The interactions between PAMAM (…) dendrimer generation 4 (G4) and 3.5 (G3.5) with model lipid membranes composed of (….) DPPC, has been investigated. Differential Scanning Calorimetry (DSC) and RAMAN spectroscopy have been applied to to asses the thermodynamic changes caused by PAMAM G4 and G3.5 and to specify the exact location of these dendrimers into DPPC lipid bilayers. DSC thermograms indicated that the maximum percentage of PAMAM G4 and G3.5 that can be incorporated in the DPPC membrane without deranging its integrity were 5% and 3% respectively. (…) The findings from this study could also prove helpful to rationally design new liposomal drug carriers for bioactive molecules by combining dendrimers and liposomal technologies.

14. *The role of the anticancer drug vinorelbine in lipid bilayers using differential scanning calorimetry and molecular modelling,* C. Koukoulitsa, I. Kyrikou, C. Demetzos, T. Mavromoustakos*, Chemistry and Physics of Lipids, 144, **2006**, 85-95.*

Differential Scanning Calorimetry (DSC) has been employed to investigate the thermal effects caused by the anticancer alkaloid drug vinorelbine in (…) DPPC lipid bilayers. The total enthalpy increased by the presence of the drug molecule, indicating a partial interdigitation of the lipid alkyl chains. The presence of cholesterol in DPPC bilayers including vinorelbine induced an obstruction of the interdigitation, since cholesterol interrupts the upraise of enthalpy change. Vinorelbine's interdigitation ability and stabilization properties with the active site of the receptor have been compared with those of similar in structure amphiphilic and bulky alkaloid, vinblastine. The obtained results may in part explain their similar mechanism of action but different bioactivity.

15. *Synthesis, liposomal formulation and thermal effects on phospholipid bilayers of leuprolide*, V. Saroglou S. Hatziantoniou M. Smyrniotakis I. Kyrikou T. Mavromoustakos, A. Zompra, V. Magafa, P. Cordopatis and C. Demetzos, *Journal of Peptide Science*, 12, **2006**, 43-50.

A novel liposomal formulation was developed for the encapsulation of the oligopeptide leuprolide (GlpHisTrpSerTyr-DLeuLeuArgProNHEt), a potent analogue of gonadotropin releasing hormone used in the treatment of advanced prostate cancer, endometriosis and precocious puberty. Leuprolide was synthesized using solid phase methodology on a {3-[(ethyl-Fmoc-amino)methyl]-1-indol-1-yl}-acetyl AM resin and Fmoc/tBu chemistry. The new liposomal formulation, called 'liposomes in liposomes' is composed of egg phosphatidylcholine:dipalmitoylphosphatidylglycerol in a molar ratio of 98.91:1.09 (internal liposomes) and egg phosphatidylcholine:dipalmitoylphosphatidylglycerol:cholesterol in a molar ratio of 68.71:0.76:30.53 (external liposomes). It offers high encapsulation efficiency (73.8% for leuprolide); it can provide new delivery characteristics and it may have possible advantages in future applications regarding the encapsulation and delivery of bioactive peptides to target tissues. Furthermore, the physicochemical characteristics (size distribution and ζ-potential) of the liposomal formulations and the thermal effects on leuprolide in model lipidic bilayers composed of dipalmitoylphosphatidylcholine were studied using differential scanning calorimetry. Finally, the dynamic effects of leuprolide in an egg phosphatidylcholine/cholesterol system were examined using solid state 13C MAS NMR spectroscopy.

16. *Calorimetric study on the induction of interdigitated phase in hydrated DPPC bilayers by bioactive labdanes and correlation to their liposomal stability. The role of chemical structure,* C. Matsingou, C. Demetzos*, Chemistry and Physics of Lipids, 145, **2007**, 45-62.*

Labd-7, 13-dien-15-ol (1), labd-13-ene-8a, 15-dio (2) and labd-14-ene-8,13-diol (sclareol) have been found to exhibit cytotoxic and cytostatic effects. Their partitioning in to phospholipid bilayers may induce membrane structure modifications, crucial in the development of liposomes.DSC was used to elucidate the profile of modifications induced in DPPC bilayers by incorporating increasing concentrations of labdanes. All labdanes strongly affect the bilayer organization in a concentration dependent manner in terms of a decrease of the cooperativity, the fluidization and partially destabilization of the gel phase, the induction of a lateral phase separation and the possible existence of interdigitated domains in the bilayer (…).

17. *Effect of the Nature of the 3b-Substitution in Manoyl Oxides on the Thermotropic Behavior of DPPC Lipid Bilayer and on DPPC Liposomes,* C. Matsingou, C. Demetzos, Journal of Liposome Research, 17:89–105, **2007.**

Functionalized manoyl oxide derivatives have been proved over the years to evoke several biological responses. Among them, 3 β -hydroxy-manoyl oxide (1) and 3 β -acetoxy-manoyl oxide (2) have been shown to exhibit in vitro antimicrobial and cytotoxic activity, while Nimidazole-3 β -thiocarbonyl ester of manoyl oxide (3) was found to exhibit potent cytotoxic effect. Their partitioning into phospholipid bilayers may lead to membrane structure modifications that are crucial in liposome development as they may influence their maintenance and integrity. DSC was used to study the modifications induced in DPPC bilayers by incorporating increasing concentrations of the three manoyl oxide derivatives. All derivatives were found to strongly affect the bilayer structural organization in terms of a decrease of the cooperativity, the fluidization and partially destabilization of the gel phase and the induction of a lateral phase separation in clustering domains. Derivatives 1 and 3 were incorporated into DPPC liposomes and their physicochemical stability was monitored at 4 ° C. The stability of liposomes was strongly influenced by the presence of 1 and 3 at any molar ratio studied. DPPC/1 liposomes were found to retain its stability for 48 h at low concentration of 10% mol, while at higher concentrations up to 30% mol they collapsed into aggregated material. In all cases DPPC/3 liposomes were found unstable and sticky aggregated structures precipitated from the bulk suspension.

18. *The perturbing effect of cholesterol on the interaction between labdanes and DPPC bilayers,* C. Matsingou, C. Demetzos, *Thermochmica Acta*, 452, *2007*, 116-123.

Differential Scanning Calorimetry (DSC) was used to study the effect of cholesterol on the perturbation of DPPC bilayers induced by eight bioactive structurally related labdanes isolated from the resin 'ladano' of Cistus criticus subsp. Criticus (Cistaceae) or semi-synthesized from the mother compound. Labdanes themselves induced profound modifications in DPPC bilayers organization and thermotropic properties that were altered when cholesterol was incorporated in equimolar amounts to the labdanes (…).

19. *Differential Scanning Calorimetry (DSC): A Tool to Study the Thermal Behavior of Lipid Bilayers and Liposomal Stability*, C. Demetzos, *Journal of Liposome Research*, 18:159–173**, 2008.**

Thermodynamical techniques are applied for determining the thermal stress of medicinal compounds of the excipients as well as their interactions during the formulation process. The physicochemical properties and the stability of the medicinal products could be measured as a function of temperature or time using thermal analysis. Differential Scanning Calorimetry (DSC) is a suitable thermal analysis technique for determining the purity, the polymorphic forms and the melting point of a sample in the Pharmaceutical Industry. It is also considered as a tool to study the thermal behaviour of lipid bilayers and of lipidic drug delivery systems, like liposomes by measuring thermodynamic parameters (i.e. ΔH and Tm), which affect the stability of the liposomal suspension under given storage conditions.

20. *Lipids of membranes: chemistry, biological role and applications as drug carriers,* S. Hatziantoniou, C. Demetzos*, Studies in Natural Products Chemistry Ed. Atta-ur-Rahman,* 34,173-2002, **2008**.

The lipids are a wide and heterogeneous class of natural products. They are considered as essential biomolecules for the structure and function in living cells. (…)This review article deals with the structure and classification of the main lipid classes that are taking part in the membrane structure (….). This report underlines the thermodynamic and thermotropic properties of model lipidic membranes, their formation to lipidic drug carriers, their encapsulation efficiency and their physicochemical stability (…)

21. Solid lipid nanoparticles and nanoemulsions containing ceramides: Preparation and physicochemical characterization, G. Deli, S. Hatziantoniou, Y. Nikas, C. Demetzos, *Journal of Liposome Research*, **2009**; 19(3): 180–188.

Nanoemulsions (NEs) and solid lipid nanoparticles (SLNs) are widely used colloidal carriers for bioactive compounds. They are used in therapeutic, diagnostic, and cosmetic formulations. Ceramides are main components of the stratum carenum and are essential for the efficient barrier function. Their very high lipophilicity renders them difficult to incorporate in an acceptable formulation. The aim of this work was to investigate the possibility of using the benefits of nanotechnology in the efficient topical delivery of ceramides formulated as NEs or SLNs. The physicochemical characteristics of such carriers incorporating ceramides were investigated and their stability over time was assessed. Their morphology was examined under a scanning electron microscope and the interactions of their components were studied by differential scanning calorimetry. The results showed that the nanoemulsions can incorporate a high percentage (48.4% of total lipids by weight) of ceramides giving more homogeneous particle distributions of spherical shaped nanoparticles and they maintained their characteristics over time. On the contrary, SLNs' incorporation of ceramide higher than 10.8% of total lipids by weight led to the formation of rod-like nanoparticles deteriorating the homogeneity of the particle distribution, as depicted on the high polydispersity indexes of the corresponding formulations. The results demonstrate that NEs may be the more suitable carrier, compared to SLNs.

22. *Thermodynamic and structural characterization of liposoma-locked in-dendrimers as drug carriers*, K. Gardikis, S. Hatziantoniou, M. Signorelli, M.

Pusceddu, M. Micha-Skretta, A. Schraldi, C. Demetzos, D. Fessas, *Colloids and Surfaces B: Biointrfaces*, 81, **2010**, 11-19.

A new liposomal -locked in Dendrimer (LLD) formed by DPPC-DPPG and RAMAN 3.5 incorporating the anticancer drug DOX was studied by means of spectroscopic and DSC investigations. (…) The thermodynamic interpretation of the DSC data allowed a better understanding of the physicochemical factors that justify this behaviour that makes these LLDs very promising as a new class of Modulatory Liposomal Controlled Release System (MLCRS) that could lead to drug formulations with higher safety and efficacy profiles.

23. *New Drug Delivery Nanosystem combining liposomal and dendrimeric technology (Liposomal Locked-In-Dendrimers) for cancer therapy,* K. Gardikis, S. Hatziantoniou, M. Bucos, D. Fessas, M. Signorelli, T. Felekis, M. Zervou, C. Skrettas, B. R. Steele, M. Ionov, M. Micha-SKretta, B. Klajnert, M. Bryszewska, C. Demetzos, *Journal of Pharmaceutical Sciences*, 99, ( 8), **2010**.

Liposomal locked-in dendrimers (LLDs) the combination of liposomes and dendrimers in one formulation, represents a relatively new term in the drug carrier technology. (…) The loading of DOX as well as its in vitro release rate from all system was determined and the interactions of liposomes with dendrimers was assessed by thermal analysis and fluorescence spectroscopy. The results were very promising in terms of drug encapsulation and release rate factors that can alter the therapeutic profile of a drug with low therapeutic index as DOX.

24. *Effect of amyloid beta peptides Ab1–28 and Ab25–40 on model lipid membranes,* M. Ionov, B. Klajnert, K. Gardiki, S. Hatziantoniou, B. Palecz, B. Salakhutdinov, J. Cladera, Maria Zamaraeva, C. Demetzos, M. Bryszewska, *J Therm Anal Calorim* , **2010**, 99:741–747.

To investigate the molecular interaction of amyloid beta peptides Ab1–28 or Ab25–40 with model lipid membranes differential scanning calorimetry (DSC) and DPH and TMA DPH fluorescence anisotropy approaches were used. The main transition temperature (Tm) and enthalpy change (DH) of model lipid membranes composed of DMPC/DPPG on addition of Ab25–40 or Ab25–40 at 10:1 (w/w) phospholipid/peptide ratio either non aggregated or previously aggregated were examined. The effect of Ab1–28 and Ab25–40 on the membrane fluidity of liposomes made of DMPC/DPPG (98:2 w/w) was determined by fluorescence anisotropy of incorporated DPH and TMA DPH. The results of this study provide information that Ab1–28 preferentially interacts with the hydrophilic part of the model membranes, while Ab25–40 rather locates itself in the hydrophobic core of the bilayer where it reduces the order of the phospholipids packing.

25. *The effect of aminoglycoside antibiotics on the thermodynamic properties of liposomal vesicles,* Y. Jia, H. Joly, D.M.Leek, C. Demetzos, A. Omri*, Journal of Liposome Research,* 20 (1), 84-96, **2010**

Liposomes are ideal drug-delivery systems because they can alter the pharmacokinetic characteristics and biodistribution profile of the incorporated bioactive molecule. The effect of the aminoglycoside antibiotics, gentamicin, tobramycin and amikacin on the thermodynamic properties of multilamellar vesicles composed of DPPC was studied by using differential scanning calorimetry (DSC), electron paramagnetic resonance (EPR) and 31P nuclear magnetic resonance (NMR) spectroscopy. (…) In conclusion, the molecular organization and thermotropic properties of the multilamellar DPPC vesicles were dependent on the osmotic gradient and structure of aminoglycoside.

26. *A New Chimeric Drug Delivery Nano System (chi-aDDnS) Composed of PAMAM G 3.5 Dendrimer and Liposomes as Doxorubicin's Carrier. In Vitro Pharmacological Studies*, K. Gardikis, D. Fessas, M. Signorelli, K. Dimas, C. Tsimplouli, M. Ionov, C. Demetzos, *Journal of Nanoscience and Nanotechnology*, 11, 3764–3772, **2011.**

Chimeric advanced Drug Delivery nano Systems (chi-aDDnSs) could be defined as mixed nanosystems due to the combination process of nanobiomaterials and can offer advantages as drug carriers. The role of the release modulator from the liposomal system is undertaken by the dendrimer molecules leading to new pharmacokinetic and, probably, pharmacological properties of the chimeric system. In this work, a conventional DOPC/DPPG liposomal system and a new chiaDDnS composed of liposomes (DOPC/DPPG) incorporating PAMAM G3,5 has been developed, Doxorubicin (Dox) was loaded in the systems and the final formulations were lyophilized. The physicochemical (spectroscopic and calorimetric) investigation concerning the chi-aDDnS, revealed a strong interaction between both lipophilic and hydrophilic parts of the liposomal membrane and the dendrimer, with the induction of multiple energetic states. These states are probably the basis of higher Dox encapsulation and slower release rate compared to the respective conventional liposome. These results, in conjunction with the increase in TI observed in two investigated cancer cell lines (i.e., MB231 and MCF7), compared to the respective conventional liposomal system and to the free Dox, make this new chi-aDDnS the basic candidate for further in vivo investigations.

27. *Interactions of cationic phosphorus dendrimers (CPD) with charged and neutral lipid membranes,* M. Ionov, K. Gardikis, D. Wrobel, S. Hatziantoniou, H. Mourelatou, J-P. Majoral, B. Klajnert, M. Bryzewska, C. Demetzos*, Colloids and Surfaces B: Biointerfaces,* 82, **2011**, 8-12*.*

(…) The aim of the present work was to study the interactions of cationic phosphorus-containing dendrimers (CPD), with model lipid membranes with no charge or bearing surface charge. The interactions of two generations of phosphorus dendrimers on the thermotropic behaviour of model lipid membranes composed of DMPC (uncharged)or DMPC/DPPG (negatively charged) were studied using differential scanning calorimetry (DSC). The results of this study showed that CPDs can alter the thermotropic behaviour of the bilayer by reducing the cooperativity of phospholipids and this effect strongly depends on membrane surface charge (…).

28. *Interactions of phosphorus-containing dendrimers with liposomes,* D. Wrobel, M. Ionov, K. Gardikis, C. Demetzos, J-P. Majoral, B. Palecz, B. Klajnert, M. Bryszewska, *Biochimica et Biophysica Acta*, 1811, **2011**, 221-226.

The influence of cationic phosphorus-containing dendrimers generation 3 and 4 on model DMPC or DPPC lipid membranes was studied. Measurements of fluorescence anisotropy and differential scanning calorimetry (DSC) were applied to assess changes in lipid bilayers parameters, including fluidity, anisotropy and phase transition temperature. (…). The results suggest that dendrimers can interact both with the hydrophobic part and the polar head-group region of the phospholipid bilayers.

29. *Effect of Phosphorus dendrimers on DMPC lipid membranes,* M. Ionov, D. Wrobel, K. Gardikis, S. Hatziantoniou, C. Demetzos, J-P. Majoral, B. Klajnert, M. Bryszewska*, Chemistry and Physics of Lipids, 165, **2011**, 408-413.*

Large unilamellar liposomes and multilamellar vesicles consisting of DMPC interacted with cationic phosphorus containing dendrimers CPDs G3 and G4. DSC and z-potential measurements have shown that liposomal-dendrimers molecular recognition probably occurs due to the interaction between the complementary surface groups. Calorimetric studies indicate that the enthalpy on the transition of the lipids that interact with CPDs is dependent on the dendrimer generation (…).

30. *Preparation and Thermal Behaviour of Liposomal Nanoparticles Incorporating Bioactive Labdane Epimers,* N. Pippa, S. Hatziantoniou, E. A. Mourelatou, Juan M. Amaro-Luis, Darly Villalobos-Osorio, C. Demetzos*, Advanced Science Letters*, 5, 1–6, **2012.**

Lipophilic labdane type epimers 1 (5H,9H,13H,10Me-labda-8-17diol) and 2 (5H,9H,13H,10Me labda-8-17diol) were isolated from *Oxylobus glanduliferus*. Their partitioning into model lipid membranes composed of Dipalmitoylphosphatidylcholine (DPPC) / Dipalmitoylphosphatidylglycerol (DPPG) (9:1 molar ratio) may induce membrane structure modifications. Differential Scanning Calorimetry (DSC)

was used for elucidating the thermodynamic profile of the modifications induced in DPPC/DPPG bilayers after incorporating increasing concentrations of 1 or 2 by measuring calorimetric parameters. The epimers 1 and 2 showed thermal effects on the lipid bilayers composed of DPPC/DPPG in a manner depending on the concentration of 1 or 2. The decrease of the lipid bilayer cooperativity and the existence of interdigitated domains in the DPPC/DPPG bilayer were observed, studied and discussed. The different stereochemistry of the C-13 of 1 and 2 affected the structural order of lipid bilayers. The epimers 1 and 2 were incorporated into liposomal Small Unilamellar Vesicles (SUVs) composed of DPPC/DPPG. Their physicochemical stability was monitored over time under storage at 4 C while the physicochemical stability of liposomes was strongly influenced by the negative charge of the liposomal vesicles.

31. *The Thermotropic Behaviour of Chimeric Liposomes as the Mechanistic Explanation of Drug Release* N. Pippa, S. Pispas, K. Gardikis, C. Demetzos, *Pharmakeftik*i, 25, III, 94-99, **2013.**

In this work, we report on the self-assembly behaviour of chimeric nanosystems consisting of dipalmitoylphosphatidylcholine (DPPC) and poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2oxazoline) (MPOx) gradient copolymers with different composition in phosphate buffer saline (PBS). Indomethacin (IND) was successfully incorporated into these nanocarriers and drug release rate was depended on the composition of MPOx component. We observed that there is a strong interplay between the cooperativity of the biomaterials comprising the prepared chimeric liposomes, as expressed by thermotropic characteristics and thermograms and the rate of release of IND from the chimeric nanovectors. Namely, higher cooperativity corresponds to lower release rates. In conclusion, thermotropic behaviour of chimeric liposomal membranes is the mechanistic explanation of the release rate of lipophilic/ amphiphilic drugs.

32. *The physicochemical/thermodynamic balance of advanced drug liposomal delivery systems*, N. Pippa, K. Gardikis, S. Pispas, C. Demetzos, *J Therm Anal Calorim* DOI 10.1007/s10973-013-3406-7, **2014**.

The aim of this work is to study the morphological characteristics via fractal analysis and the alterations of the thermotropic behaviour of dipalmitoylphosphatidylcholine (DPPC) liposomes, caused by the incorporation of cholesterol, poly(amidoamine) (PAMAM) dendrimer, and MPOx (poly (2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline)) gradient block copolymer (9:1 molar ratio). A gamut of light scattering techniques and differential scanning calorimetry were used in order to extract information on the morphological (in different dispersion media) and thermodynamic characteristics of liposomal drug nanocarriers, respectively. The vesicles' structure of liposomes has a different thermodynamic content, which corresponds to a different thermotropic behaviour, in comparison to pure lipid bilayers. The observed metastable phase only for DPPC liposomes has been considered as a ''physical impurity'', which leads to ''physical incompatibility'' and consequently promotes the aggregation of DPPC liposomes in aqueous media. The incorporation of biomaterials such as PAMAM G4 and MPOx, caused alterations in the thermotropic behaviour of DPPC liposomes affecting only the main transition specific enthalpy DH. All the other calorimetric parameters remained unaltered. These findings supported the hypothesis that the exceptional stability and transition cooperativity of the chimeric liposomal membrane might be due to the reduction of the vesicle size with the smaller membrane curvature that is indicated by the fractal dimensionality of the system. In conclusion, the results from the thermal analysis of the liposomal systems were in line with the picture of their structural characteristics, as indicated by the interplay between physicochemical and thermodynamical parameters, which determines their fractal morphology.

33. *Special Chapter on Current Advancement of Thermal Analysis with Special Focus on Bio- and Pharmaceutical Researches from the Mediterranians*, C. Demetzos, J Therm Anal Calorim DOI 10.1007/s10973-013-3616-z, **2014**.

This is a Special Chapter of the Journal of Thermal Analysis and Calorimetry comprising 18 peer-reviewed articles on recent advances in the field of Thermal Analysis, suitable for everyone interested in this field. These advances are related to the thermal processes occurring in materials contributing in

a wide spectrum of everyday life activities. All the articles are considered as high impact and scientists from different scientific fields were welcomed to submit their research for evaluation. The topics covered in this Special Chapter include Engineering, Drug development, Cultural Heritage, Biology, and Miscellaneous. I have to point out that the presented articles in this Special Chapter on Biology and Pharmaceutics are of great interest and highly appreciated. The interactions of these scientific fields (i.e., biology and pharmaceutics) with sciences out of their conventional collaborative environment (i.e., chemistry, pharmacology, toxicology, etc.) such as material sciences, physics, etc., promote elements like thermal analysis techniques that can be used in order to control the thermodynamic behaviour of biomaterials used for producing drug or for studying microorganisms. Thermodynamics of drug delivery systems is considered as important tool for their design and development, which contributes to the laboratory research and to the scale up process in the Pharmaceutical Industry, respectively. The Mediterranean scientists participating in the Mediterranean Thermal Analysis Societies, [Grupo Especializado de Calorimetria y Analisis Termico (GECAT), Associazone Italiana de Calorimetria e Analisi Termica (AICAT), Grupo Interdivisionale di Calorimetria e Analiso Termica (GICAT), Grupos de Calorimetria e Analise Termica do Porto (CATPOR), Israeli Group for Thermal Analysis and Calorimetry (IGTAC), and Hellenic Society of Thermal Analysis (HSTA)] efficiently contribute to this Special Chapter by providing new knowledge in the field of thermodynamics and calorimetry and by offering their research for publication with regard to this issue. I hope that this Special Chapter will provide and stimulate the recent research advances in Thermal Analysis including articles that come from post-graduate students as well as from the well-known scientists in the field of thermal analysis. Finally, I would like to express my gratitude to all colleagues who submitted scientific work for publication in this Special Chapter, for their careful preparation and collaboration. I am grateful to the Honorary Editor–in Chief Prof. Judith Simon who trusts me to be the Guest Editor of this Special Chapter in Journal of Thermal Analysis and Calorimetry.

34. *The interplay between the rate of release from polymer grafted liposomes and their fractal morphology* N. Pippa, A. Dokoumetzidis, S. Pispas, C. Demetzos, *International Journal of Pharmaceutics*, 465, **2014**, 63-69.

The purposes of this study were to investigate the indomethacin (IND) release profile from dipalmytolphosphatidylcholine:poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline (DPPC: MPOx) (in different molar ratios) mixed liposomal nanovectors, to examine the relevance of power law using these experimental release data, and to detect the relationship of the fractal dimension (df) of nanovectors with the fraction of the IND release. The df of the mixed liposomes was determined by Static Light Scattering during the release of IND from the nanocontainers .I tis observed that the in vitro release of the drug from the prepared nanostructure is quite fast especially for the nanovectors prepared with the lower ratio of MPOx. The release kinetics was studied by regression analysis of drug concentrations in fractal matrices with respect to time. A power law, a piece-wise power law functions and Weibull distribution were fitted to the release data and the model parameters were estimated. Good fits were observed in all datasets analysed, while distinct regions of different release rates corresponding to different df values were described. The authors proposed that the fractal morphology of the mixed liposomes affects the drug release and must be taken into account to develop liposomal drug with complete knowledge of their structural properties.

35. *Biophysics and Thermodynamics*: The Scientific Building Blocks of Bio-inspired Drug Delivery Nano Systems, C. Demetzos, *AAPS PharmSciTech*, 16, (3), **2015** (# 2015) DOI: 10.1208/s12249-015-0321-1.

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.

36. *The metastable phases as modulators of biophysical behavior of liposomal membranes, The role of biomolecular sculpture of polymeric guest*, N. Pippa, S. Pispas, C. Demetzos, *J Therm Anal Calorim* **2015**, 120:937–945 DOI 10.1007/s10973-014-4116-5.

In this work, we investigate the alterations of the physicochemical, morphological, and thermotropic characteristics of conventional stealth and chimeric DPPC (dipalmitoylphosphatidylcholine) liposomes, caused by the incorporation of PEGylated lipid and block copolymers with different architectures and compositions. 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-3000] (DPPE-PEG 3000) is the PEGylated lipid, poly(ethylene oxide)-block-poly(e-caprolactone) (PEO-b-PCL) is the block copolymer, and poly(2-methyl-2oxazoline)-grad-poly(2-phenyl-2-oxazoline) (MPOx) is the gradient block copolymer, which are selected for preparing the liposomal systems. Light scattering techniques and differential scanning calorimetry (DSC) were used in order to extract information on the physicochemical/thermodynamic balance of the prepared liposomal systems. The physicochemical characteristics and the morphology via fractal analysis of these chimeric nanoassemblies were found to depend on the composition of the polymeric component, while DPPC liposomes were used for comparison reasons (reference system). The incorporation of polymeric components into liposomes promotes a structural rearrangement of lipid bilayers and affects their behaviour, as DSC experiments indicated. The fluidity, the inter vesicle interactions and the cooperativity of structural elements of liposomes were also changed significantly by polymer addition. It could be concluded that the different macromolecular architectures of the polymeric guest affect the thermotropic behaviour of liposomal membrane by producing new metastable phases, and consequently promote new insights in the field of biophysical concept for designing and developing chimeric advanced drug delivery nano systems (aDDnSs).

37. *Calorimetric study on pH-responsive block copolymer grafted lipid bilayers: rational design and development of liposomes*, N. Pippa, M. Chountoulesi, A. Kyrili, A. Meristoudi, S. Pispas, C. Demetzos, *Journal of Liposome Research*, 1–10, **2015** Taylor & Francis DOI: 10.3109/08982104.2015.107646.

This study is focused on chimeric advanced drug delivery nanosystems and specifically on pH-sensitive liposomes, combining lipids and pH-responsive amphiphilic block copolymers. Chimeric liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and two different forms of block copolymers, i.e. poly(n-butylacrylate)-b-poly(acrylic acid) (PnBA-b-PAA) at 70 and 85% content of PAA at six different molar ratios, each form respectively. PAA block exhibits pH-responsiveness, because of the regulative group of –COOH. –COOH is protonated under acidic pH (pKa ca. 4.2), while remains ionized under basic or neutral pH, leading to liposomes repulse and eventually stability. Lipid bilayers were prepared composed of DPPC and PnBA-b-PAA. Experiments were carried out using differential scanning calorimetry (DSC) in order to investigate their thermotropic properties. DSC indicated disappearance of pretransition at all chimeric lipid bilayers and slight thermotropic changes of the main transition temperature. Chimeric liposomes have been prepared and their physicochemical characteristics have been explored by measuring the size, size distribution and z-potential, owned to the presence of pH-responsive polymer. At percentages containing medium to high amounts of the polymer, chimeric liposomes were found to retain their size during the stability studies. These results were well correlated with those indicated in the DSC measurements of lipid bilayers incorporating polymers in order to explain their physicochemical behaviour. The incorporation of the appropriate amount of these novel pH-responsive block copolymers affects thus the cooperativity, the liposomal stabilization and imparts pH-responsiveness.

38. *Temperature-dependent drug release from DPPC:C12H25-PNIPAM-COOH liposomes: Control of the drug loading/release by modulation of the*

*nanocarriers' components,* N. Pippa, A. Meristoudi, S. Pispas, C. Demetzos*, International Journal of Pharmaceutics, 485, **2015**, 374-382.*

Novel polymer-modified thermosensitive liposomes were developed for the delivery of indomethacin in order to control its release profile. (…) The physicochemical/structure behaviour of these polymer-modified thermosensitive liposomes was found to depend on the PNIPAM: lipid molar ratio and the composition of the polymeric guest. The incorporation of PINIPAM has caused alterations in the thermotropic behaviour of DPPC liposomes as the differential scanning calorimetry (DSC) experiments revealed. (…)

39. A dual-stimuli-responsive polymer into phospholipid membranes. A thermotropic approach, I. Kolman, N. Pippa, A. Meristoudi, S. Pispas, C. Demetzos, *J Therm Anal Calorim,* **2016**, 123:2257–2271 DOI 10.1007/s10973-015-5080-4.

In this study, we investigate the thermotropic effects of diblock copolymer poly(N-isopropylacrylamide)block-poly(acrylic acid) (PNIPAM-b-PAA) on fully hydrated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) lipid bilayers and its ability to alter the membranes' organization, fluidity and phase behavior. The composition of the diblock copolymer and the nature of dispersion medium (pH and ionic strength) were also examined. For these purposes, pure DPPC lipid and polymer–lipid mixed systems ,hydrated in three different dispersion media (i.e., HPLC-grade water, phosphatebuffersalineandhydrochloricacidsolutionofpH4.5), were investigated by differential scanning calorimetry. Two compositions of PNIPAM-b-PAA with different molar ratio of the polymeric blocks were used. PNIPAM-b-PAA presents great scientific interest due to the combination of the special characteristics of its homopolymer components; it is dual responsive both in temperature and in pH changes. The incorporation of the PNIPAM-b-PAA into the DPPC bilayers causes particularly significant perturbations in their thermotropic behaviour, slightly different in each dispersion medium. The results indicated the ordering of the polymer guest near the polar head group surface probably by its PAA block and, on the other hand, the penetration of the PNIPAM block into the hydrophobic bilayer core, causing membrane disruption in a temperature-depended manner. We can conclude that the lipid–polymer interactions seem to be affected by the pH and the ionic strength of the hydration medium, as well as the polymer content incorporated in the DPPC bilayer. These studies could be a roadmap in order to rationally design and develop chimeric liposomes.

40. *Chimeric lipid/block copolymer nanovesicles: Physicochemical and biocompatibility evaluation,* N. Pippa, D. Stellas, A. Skandalis, S. Pispas, C. Demetzos, M.Libera, A. Marcinkowski, B. Trzebicka*, European Journal of Pharmaceutics and Biopharmaceutics,* 107, 295-309, **2016**.

Chimeric systems are mixed nanovectors composed by different in nature materials and exhibit new functionalities and properties. (…) Light scattering, differential scanning calorimetry (DSC) and imaging techniques (cryo-TEM, AFM) were employed in order to elucidate the structure and properties of the nanostructures, as well as the cooperativity between the components. DSC experiments showed considerable interaction of the block copolymer with the lipid bilayers and suggested an inhomogeneous distribution of the copolymer chains and lateral phase separation of the components. (…).

41. *Design and development of pH-responsive HSPC:C12H25-PAA chimeric liposomes,* N. Naziris, N. Pippa, A. Meristoudi, S. Pispas, C. Demetzos*, Journal of Liposome Res.,* 27 (2), 108-117, **2017.**

The application of stimuli-responsive medical particles has emerged, in which p H sensitive liposomes figure prominently. This study investigates the impact of the incorporation of different amounts of p H sensitive polymer (…) in (…) hydrogenated (soy) (HSPC) phospholipidic bilayers, with respect to biomimicry and functionality. (…) Our concern was to fully characterize, in a biophysical and thermodynamic manner, the mixed nanoassemblies arising from the combination of the two

biomaterials. As expected, thermodynamical findings are in line with physicochemical results and also explainthe loading and release profiles of IND.

42. *Design and development of pH -sensitive liposomes by evaluating the thermotropic behaviour of their chimeric bilayers,* A. Kyrili, M. Chountoulesi, N. Pippa, A. Meristoudi, S. Pispas, C. Demetzos, *J. Therm. Anal. Calorim.,* 127: 1381-1392, **2017**.

This study is focused on mixed/chimeric advanced drug delivery nanosystems and specifically on p H - sensitive liposomes, combining lipids and p H responsive amphiphilic block copolymers. (…) Experiments are carried out by using differential scanning calorimetry (DSC) in order to investigate their thermotropic properties. DSC indicated disappereance of the pretransitioneffect in all chimeric lipid bilayers, at both buffers and slight changes of the main transition temperature. (…).

43. *Lipid bilayers Incorporated violacein : Differential Scanning Calorimetry as an analytical tool for preformulation studies,* P. Loukopoulos, N. Pippa, C. Demetzos, A. Kanavouras*, Advanced Science, Engineering and Medicine,* 9, 1-9, **2017**.

Differential scanning calorimetry (DSC) has proven to be a useful tool revealing the thermodynamic changes of the liposomes when active substances have been incorporated. (…) The aim of this work is to study the alterations of the thermotropic behaviour of model lipid membranes, caused by the incorporation of violacein by using DSC technique. (…) DSC indicates disappearance of the pretransition effect in all lipid bilayers, at both buffers and slight changes of the main transition temperature (…). In conclusion, the results from DSC measurements provide useful information regarding the pre-formulation study for rationally preparing liposomal platforms to incorporate violacein.

44. *Design and development of multi-wall carbon nanotube-liposomes drug delivery platforms,* N. Pippa, D. Chronopoulos, D. Stellas, R. Fernandez-Pacheo, R. Arenal, C. Demetzos, N. Tagmatarchis*, Int. Journal of Pharmaceutics,* 528, 429-439, **2017**.

The aim of this study is to design and develop delivery platforms made of liposomes and multi-walled carbon nanotubes (MWCNTs). (…) By differential scanning calorimetry (DSC) . we studied the interaction between the DPPC and HSPC bilayers and MWCNTs. The presence of MWCNTs causes alterations of the size of the conventional HSPC and DPPC liposomes. The z-potential values of mixed nanocarriers are near zero. This observation indicates the effective incorporation of MWCNTs into the lipid bilayers of liposomes. (…)

45. *The thermal analysis of liposomal formulations as an element to evaluate their effectiveness as drug and vaccine delivery systems*. N. Naziris, N. Pippa, S. Pispas, C. Demetzos, NOVA book, **2018**

Liposomes are colloidal vesicles that are composed of amphiphilic molecules (i.e., phospholipids) and are considered as biocompatible and biodegradable drug and vaccine delivery nanosystems. Several analytical techniques are well established and are currently used for their physicochemical characterization. Because liposomes belong to the thermodynamically unstable dispersed systems, calorimetric analytical techniques and especially the Differential Scanning Calorimetry (DSC) have been applied to indicate whether a given process is spontaneous or not. The structural polymorphism of the liposomal formulations is a well known phenomenon and it has been recognized as a key point of the final product's quality and effectiveness. The aim of this chapter is to present the recent advances of liposomes in drug and vaccine delivery and shed light to the application of DSC to thermodynamic characterization of liposomal delivery platforms. Moreover, examples from the recent literature will be addressed, giving priority to metastable phases and to phase transitions processes.

## ❖ ANNEX

## ❖ Special articles on intelligent artificial bio-platforms

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46. *The Thermodynamics of Defect Formation in Self-Assembled Systems*. Colm T. O'Mahony, Richard A. Farrell, Tandra Goshal, Justin D. Holmes and Michael A. Morris **2011**. The Thermodynamics of Defect Formation in Self-Assembled Systems, Thermodynamics - Systems in Equilibrium and Non-Equilibrium, Dr. Juan Carlos Moreno PirajÃ¡n (Ed.), ISBN: 978-953-307-283-8, In Tech, Available from: http://www.intechopen.com/books/thermodynamics-systems-in-equilibrium-and-non-equilibrium/thethermodynamics-of-defect-formation-in-self-assembled-systems
47. *Energy and information correlation: towards sustainable e*nergy C.C. Chana, F.C. Chand and Dan Tub, *Journal of International Council on Electrical Engineering,* **2015,** 5, (1), 29–33.
48. *Innovative nano-vaccines: From bench to bedside.* N. Pippa, C. Demetzos, *Pharmakeftiki*, 29, IV, 8-10, **2017**
49. *Developing therapeutic drug delivery nanosystems from different lyotropic liquid crystalline mesophases*. M. Chountoulesi, N. Pippa, N. Tavernarakis, S. Pispas, C. Demetzos*, Pharmakeftiki*, 29, IV, 25-26, **2017**
50. *Physicochemical characteristics of liposomes and their lyotropism influence for protein-liposome interactions in vitro*, F. Papageorgiou, N. Pippa, N. Naziris, C. Demetzos, *Pharmakeftiki*, 29, IV, 27-28, **2017**
51. The Role of the Information/ Entropy Balance in Self-assembly. The Structural Hierarchy of Chimeric Drug Delivery Nanosystems. N. Naziris, N. Pippa, S. Pispas, C. Demetzos, *Pharmakeftiki*, 29, IV, 29-34, **2017**
52. *Morphological diversity of block copolymer/lipid chimeric nanostructures*. N. Naziris, N. Pippa, V. Chrysostomou, S. Pispas, C. Demetzos, M. Libera, B. Trzebicka*, J Nanopart Res* **2017**, 19:347 DOI 10.1007/s11051-017-4021-5
53. *The Formation of Chimeric Nanomorphologies, as a Reflection of Naturally Occurring Thermodynamic Processes*. N Naziris, C Demetzos, Conf. Series: *Journal of Physics*: Conf. Series 931, **2017**, 012028 doi :10.1088/1742-6596/931/1/012028
54. *Morphological diversity of block copolymer/lipid chimeric nanostructures, N. Naziris, N. Pippa, V. Chrisostomou, S. PIspas, C. Demetzos, M. Libera, B. Trzebicka, J. Nanopart. Res. 19: 347, 3-11,* ***2017***