SUMMARY OF SCIENTIFIC ACHIEVEMENTS IN RELATION TO HABILITATION PROCEDURE

LYOTROPIC LIQUID CRYSTALLINE PHASES FOR ACCOMMODATION AND CONTROLLED RELEASE OF DRUGS

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1. **Name and surname**

Ewa Nazaruk

2. **Education**

- 2003 MSc in chemistry, University of Warsaw, Faculty of Chemistry
- 2009 – PhD in chemistry (with honors), University of Warsaw, Faculty of Chemistry. Supervisor: prof. dr. hab. Renata Bilewicz. Title of the thesis, „Application of lipid liquid crystalline cubic phases to immobilization of enzymes on the electrode surfaces"

3. **Information on academic employment**

- 2012 - current Adjunct University of Warsaw, Warsaw, Poland
- 2010 - 2012; Assistant, University of Warsaw, Warsaw, Poland

4. **Indication of achievement resulting from art. 16 sec. 2, of march 14, 2003, of the act on university degrees and the university title and on university degrees and the university title in the field of fine arts (journal of laws 2016, no. 882, with later amendments, 2016, no 1311):**

a) *Title of scientific achievement*

"Lyotropic liquid crystalline phases for accommodation and controlled release of drugs"

b) List of scientific publications setting the basis for habilitation proceedings

[H1] E. Nazaruk, M. Szlęzak, E. Górecka, R. Bilewicz, Y.M. Osornio, P. Uebelhart, E.M. Landau, "Design and assembly of pH-sensitive lipidic cubic phase matrices for drug release" *Langmuir* 30 (5) **(2014)** 1383-1390.
IF$_{(2014)}$ = 4,457 Number of citations = 43

[H2] E. Nazaruk, P. Miszta, S. Filipek, E. Górecka, E. M. Landau, R. Bilewicz, "Lyotropic Cubic Phases for Drug Delivery: Diffusion and Sustained Release from the Mesophase Evaluated by Electrochemical Methods" *Langmuir*, 31 (46) (**2015**) 12753–12761.

IF$_{(2015)}$ = 3.993 Number of citations =28

[H3] E. Nazaruk, E. Górecka, Y.M. Osornio, E.M. Landau, R. Bilewicz, „Charged additives modify drug release rates from lipidic cubic phase carriers by modulating electrostatic interactions" *J. Electroanal. Chem.* 819 **(2018)** 269–274.

IF$_{(2018)}$ =3,235 Number of citations =1

[H4] E. Nazaruk ✉, A. Majkowska-Pilip, M. Godlewska, M. Salamończyk, D. Gawel, "Electrochemical and biological characterization of lyotropic liquid crystalline phases – retardation of drug release from hexagonal mesophases" *J. Electroanal. Chem.* 813 **(2018)** 208–215.

IF$_{(2018)}$=3.235 Number of citations =1

[H5] E. Nazaruk, E. Górecka, R. Bilewicz, "Enzymes and mediators hosted together in lipidic mesophases for the construction of biodevices" *J. Colloid Interface Sci.* 385 **(2012)** 130–136.

IF$_{(2012)}$= 3,172 Number of citations =14

[H6] E. Jabłonowska, E. Nazaruk, D. Matyszewska, C. Speziale, R. Mezzenga, E.M. Landau, R. Bilewicz, Interactions of Lipidic Cubic Phase Nanoparticles with Lipid Membranes, *Langmuir* 32 **(2016)** 9640–9648.

IF$_{(2016)}$= 3.833 Number of citations =5

[H7] E. Nazaruk, A. Majkowska-Pilip, R. Bilewicz, "Lipidic Cubic-Phase Nanoparticles—Cubosomes for Efficient Drug Delivery to Cancer Cells" *Chem. Plus. Chem.* 82 **(2017)** 570–575.

IF$_{(2017)}$ = 3.205 Number of citations = 8

[H8] M. Godlewska, A. Majkowska-Pilip, A. Stachurska, J. F. Biernat, D. Gaweł, E. Nazaruk ✉ "Voltammetric and biological studies of folate-targeted non-lamellar lipid mesophases" *Electrochimica Acta* 299 **(2019)** 1-11.

IF$_{(2018)}$=5,116 Number of citations =0

c) Summary of the scientific goals and major results of presented publications

The results obtained and described in the series of thematically related scientific papers are focused on the development and characterizing of new functional drug delivery systems based on the lyotropic liquid crystalline phases **[H1–H8]**. In my research, lipid liquid crystalline phases e.g. cubic (V2) or hexagonal (H2) phase and their nanoparticles are used as potential drug delivery systems. The main advantages of such lipid mesophases for the application in drug delivery systems are: the possibility of controlled drug release and of addressing the carrier. Lipid nanoparticles due to the high internal surface are able to accommodate large amount of drugs. They can be applied for the accommodation and delivery of drugs which are insoluble in water. Electrochemical methods were used to determine *i)* the effect of the lipid liquid crystalline matrix on the oxidative/reductive properties of drugs, *ii)* transport properties of drugs in the mesophase system, *iii)* release properties of drugs. In the presented papers I showed that the release rate of incorporated guest molecules can be controlled by i) pH **[H1]**, ii) structural parameters of mesophase system **[H2]**, iii) hydrophobic interactions **[H5]**, iv) electrostatic interactions **[H3]**, or v) phase transitions **[H4]**. The rate of drug transport can be regulated by the change of aqueous channel size of the hosting cubic phase **[H2]**. In certain conditions the mesophase system can be dispersed, forming nanoparticles that retain the structure of the parent bulk phase but possess lower viscosity **[H4, H6, H7, H8]**. The mechanism of interaction of cubosomes with lipid biomimetic and biological membranes of cells were characterized. At the air – water interface they were studied by the Langmuir technique allowing to follow interactions of cubosomes with phospholipid monolayers - model of one leaflet of the cell membrane **[H6]**. Biological analyses of the delivery potential of cubosomes and hexosomes revealed that such nanostructures are promising and could be further studied as sustained drug delivery systems in cancer therapy **[H5, H6, H8]**.

Introduction

The long-term use of conventional drugs may lead to damage of healthy cells and adverse side effects. To protect the healthy cells for the toxic side effects and to minimize adverse side effects of chemotherapeutics encapsulation of the drug molecule into drug delivery system (DDS) can be applied. The main problems connected with cancer treatment are poor bioavailability and insufficient accumulation of the drug into the tumor tissue. An advantageous system to use as DDS should have a high drug loading capacity to reduce quantity of matrix material needed for delivery and allow controlled release of the drug from the carrier. The main aim of a potential drug delivery system is to maintain the concentration of drug at a desired value as long as possible. Among various types of DDS lipidic nanoparticles conjugated with various drugs can be customized as drug vehicles. The lipidic formulation approach allows to minimize the side-effects of the toxic drug as compared to direct delivery of the drug alone and improves efficacy of low doses of drugs. Moreover, to selectively deliver anti-cancer drugs directly to the tumor site a ligand against a surface receptor usually overexpressed on cancer cells can be attached. To date the nanocarrier research area is dominated by the liposomes [1] but also lipidic liquid-crystalline materials (LCP) are perspective as drug carriers which I also show in my habilitation.

Lyotropic liquid crystals represents intermediate states of matter between a solid crystal and an isotropic liquid. Lipids forming LCP can adopt different structural arrangements, the most common are the inverted cubic, inverted hexagonal, sponge and micellar phases [2–4]. Certain amhiphilic lipids are able to form a variety of different lyotropic liquid crystalline systems that remain stable on dilution with water and which may control the release of drugs. The formation of different systems can be understood using the critical packing parameter (CPP) concept [5]. CPP is defined by the equation:

$$CPP = v/A_0 l_c$$

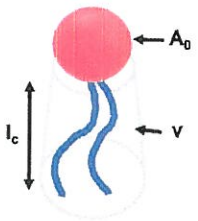


Figure 1 Lipid structure

, where v is the volume of the hydrophobic tail, A_0 is the polar headgroup area, and l_c is the length of the hydrophobic chain [Fig. 1].

Lamellar phases are formed by the cylindrically shaped molecules with CPP =1. The lamellar phase is characterized by zero curvature, since the cross-sections of the polar heads

and the lipophilic tails are similar. Lipid molecules with larger headgroups form normal phases, where the bilayer is curved towards the chain region, while the lipids with smaller headgoups form inverse type of architecture, where bilayer is curved toward the hydrophilic region (CPP >1) [Fig. 2].

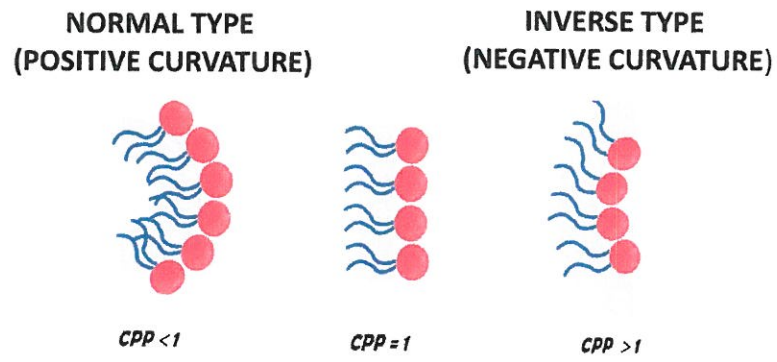


Figure 2 Aggregate morphology with different interfacial curvature.

For drug delivery application the inverted structures are of particular interest as they are generally stable in excess water. The most common lipid structures of inverse architecture, that may be formed are presented in order of increasing negative curvature in Figure 2. This includes the formation of 1D (lamellar) or 2D hexagonal (H2) phases, but also more complex 3D structures can be formed. The H2 phase consists of closed reverse micellar cylinders that are arranged in a 2D hexagonal lattice. 3D ordered structures include sponge phase (L_3), micellar cubic (I_2), and bicontinuous cubic phase (V2) structures (including the primitive (P, Im3m), diamond (D, Pn3m) and gyroid (G, Ia3d) cubic phases). The cubic phases comprise a curved bicontinuous lipid bilayer extending in 3D and two interpenetrating, but non-contacting, aqueous nano-channels.

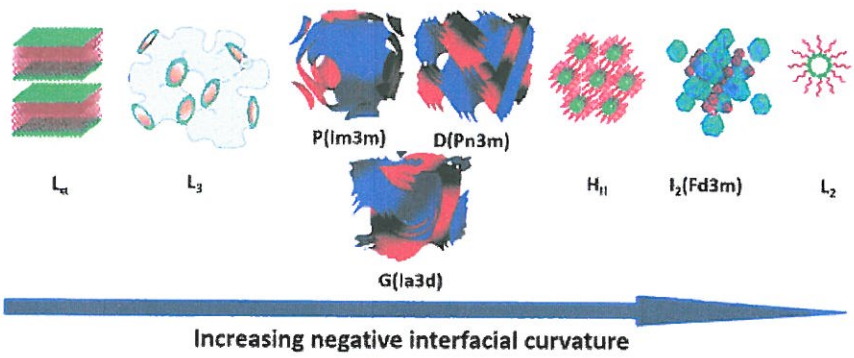


Figure 3 Shapes of inverted lipid crystals [6]

There are three different reversed bicontinuous cubic phases: Pn3m, Ia3d or Im3m. In those systems we have two interconnected water channels. The water channel connectivity is presented in Figure 4.

Figure 4 Scheme of the water channel connectivity

For the Im3m cubic phase water channels meet at 6-way (90°) junction, whereas for Pn3m phase water channel form 4-way (109.5°) junction. In cubic phase of Ia3d space group 3 water channels meet at angle 120°. The hexagonal phase is composed of cylindrical micelles packed in a hexagonal lattice. In contrast to the cubic phase, the water channels in the hexagonal phase are closed.

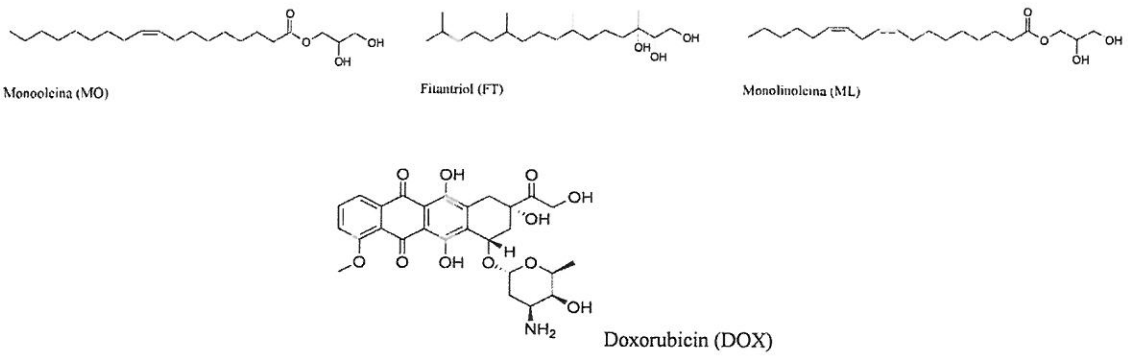
The properties of lipid mesophases that are useful for drug delivery systems application are that they are non-toxic, non-immunogenic and biocompatible. Lipid mesophases are advantageous materials for DDS since they are stable in excess of water and due to the high surface area of ca. $400m^2/g$ may provide a sufficient quantity of the drug substance. [7] Amphiphilic nature of LCP and their characteristic structure allows for the incorporation of a range of drugs of various polarities and molecular weight. The drug may associate with the water domain, the lipidic domain or the water–lipid interface of the self-assembled system. Release of the drugs may be adjusted and controlled by changing variables such as temperature, pressure, pH or composition. Also a diverse range of compounds may be added to functionalize the mesophase. This functionalization may change the curvature of lipid bilayer that may result in phase transition or changing the diameter of the water channel. Structural parameters can be tuned and targeted towards the specific structural requirements of the drug by changing curvature to affect the bilayer thickness, water channel diameter and unit cell size. The parameters that affect the symmetry of lipid liquid crystalline phases include chain length of the lipid used, splay, unsaturation within the chain and the size of the headgroup. Additives incorporated into the mesophase systems may alter the self-assembly of the lipid packing and induce changes in the lattice dimensions or even complete phases.

Major results of presented publications

Transport properties of drugs within cubic phase

Lipidic nanostructures are studied extensively as therapeutic and diagnostic agents and delivery systems due to their biocompatibility, ease of drug encapsulation and targeting. The characterization and control the transport properties of drugs within the drug carrier is a key factor to the development of efficient drug delivery system. Drug release usually is driven by the gradient of concentration between carrier and surrounding aqueous environment. Additional factors that influence the diffusion of an incorporated drug from the LCP are the size and polarity of the molecules. The parameter that is used to describe the ability of a drug to diffuse is the diffusion coefficient. Transport efficiency will be affected by the interactions between the drug and the lipid wall of the cubic phase.

Electrochemical methods were used to characterize the transport properties of doxorubicin in the three cubic phase systems. The transport properties of the anticancer drug doxorubicin was investigated in three liquid–crystalline lipidic systems: two monoacylglycerol-based LCPs composed of monoolein (MO) and monolinolein (ML), and one isoprenoid-type LCP based on phytantriol (PT). **[H1]** Structures of the compound used are presented in Scheme 1.



Scheme 1. Structures of the compounds used.

To prepare cubic phases, DOX was dissolved first in MO, ML or PT to obtain a homogeneous system, and subsequently adding appropriate buffer solution. The ratio of components was chosen on the basis of the phase diagrams for the MO/water, ML/water or PT/water systems. [8][9][10] Small angle X–ray scattering (SAXS) measurements were performed to identify the type and to structural parameters of the various liquid crystalline phases. Doping of the MO, ML and PT cubic phases with 0.6% (w/w) DOX did not affect the symmetry of the mesophases. PT-based LCPs exhibit the smallest channel size (2.3 nm) while MO-based LCPs have much larger (4.1 nm diameter) aqueous channels at both pH values.

Electrochemical measurements are useful for the studies of diffusion in the lipid mesophases because they can provide precise information on the effective diffusion coefficient, and the concentration, if unknown **[H1].** The presented in **[H1]** approach relied on performing experiments in parallel under linear and spherical diffusion regimes. Applying steady-state voltammetry on microelectrode and macroelectrode combined, allowed the determination of both diffusion coefficient (D) and concentration of the electroactive probe in the cubic phase. This approach was possible because the currents scale either with $D^{1/2}$ (chronoamperometry, chronocoulometry and voltammetry at the macroelectrode), or with D (voltammetry at the microelectrode), while both of them are directly proportional to concentration. This approach is especially useful when the concentration of the species embedded in the different compartments of the matrix cannot be precisely controlled. The representative voltammograms and chronovoltamperogrmas are presented in Figure 5.

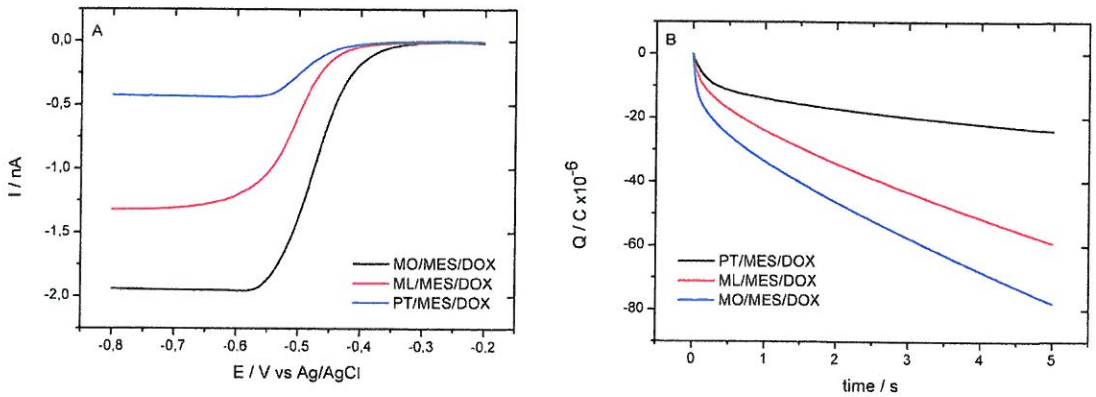


Figure 5 Representative voltammograms (A) and chronovoltamperograms (B).

By resolving the above equations, two pairs of equations are obtained for the diffusion coefficient and concentration determination.

(1) Cyclic voltammetry (CV) on macro- and microelectrode (2) CV on microelectrode and chronocoulometry

$$D = \frac{(2.69\times10^{5})^{2} n v A^{2} i_{ss}^{2}}{16F^{2}r^{2}i_{p}^{2}} \qquad D = \frac{A^{2}i_{ss}^{2}}{4r^{2}s^{2}\pi}$$

$$C_{0} = \frac{4Fri_{p}^{2}}{(2.69\times10^{5})^{2}n^{2}vA^{2}i_{ss}} \qquad C_{0} = \frac{rs^{2}\pi}{nFA^{2}i_{ss}}$$

, where i_p is the peak current (A); A: electrode area (cm^2); and v: scan rate ($V\ s^{-1}$), Q is the electrical charge (C); and t: time (s), s is the slope of the plot of the charge (Q) vs. $t^{1/2}$ in chronocoulometry, i_{ss} is the steady-state current (A); n: number of electrons exchanged; C_0: concentration of the electroactive species ($mol\ cm^{-3}$); r: radius of the microelectrode; and D: diffusion coefficient ($cm^2\ s^{-1}$).

In all systems the DOX concentration was found to be smaller than the total DOX concentration delivered to the carrier. This behavior was ascribed to the interactions of the drug with the lipids surrounding the water channels. The largest concentration was observed for the MO-based cubic phase, possessing the widest water channels, while it was much smaller for the PT-based cubic phase. Concentration of DOX depends on the size of the water channels. Additionally, the transport efficiency of DOX embedded inside the three distinct LCPs composed of monoolein (GMO), monolinolein (ML), and phytantriol (PT) was found not only to be dependent on the lipid composition but also on pH. The MD simulations showed that protonated DOX is located mainly in the aqueous channels, whereas the unprotonated form interacts with the lipid domain. **[H2]** The neutral form, present above pH 7, is embedded in the lipidic bilayer, and is less prone to being removed from the cubic phase to the surrounding water environment. Drug release depends on the drug properties, location of the drug is an important parameter influencing the the release rate. Partitioning between the lipidic and aqueous compartments, which is related to the hydrophobicity of the drug, largely determines the release kinetics. While hydrophobic drugs tend to partition into the bilayer, hydrophilic drugs reside preferentially in the aqueous channels. Kinetics of release of the anti-cancer drug doxorubicin (DOX) from the MO-based LCP was described in **[H1].** Understanding the release kinetics can provide the effective drug concentration at desired level. The known mathematic kinetic models

were used to describe the transport properties of drugs. Among the different mechanism of drug release, diffusion is dominant, however, diffusion can be significantly hindered by the interaction between drug and lipid matrix. DOX release profiles were applied to determine the kinetics and mechanism of drug release from the mesophase system. The Korsmeyer-Peppas model, used to characterize the mechanism of drug release is described by the equation:

$$\frac{M_t}{M_\infty} = K_P t^n$$

, where M_t/M_∞ is the fraction of drug released at time t, K_P is the drug release rate constant. The n value is related to the drug transport mechanism: when n=0.5 the Fickian diffusion mechanism dominates, while n value higher than 0.5 corresponds to the non-Fickian transport. The n values were found to be slightly higher than 0.5 in all cases, which suggests that in addition to diffusion also matrix effects may have an impact on the drug release. Only, on case of ML cubic phase the *n* value was equal to 0.5, which suggest that drug release is purely Fickian.

The main advantage of using lipid mesophases in DDS is the possibility of controlling the drug release. To trigger release from lipid mesophases temperature, pH, electrostatics or hydrophobic interactions can be applied. Controlled drug release can be also achieved by alterations in lipid packing that can induce phase change.

Lipid mesophase systems for controlled drug release

a) pH sensitive drug release

Between normal tissues and those affected by cancer pH gradient exists; tumours have a significantly lower pH as compared to normal tissues. Thus, pH-sensitive systems are extensively investigated as a means of increasing drug delivery to tumors. This approach offers the opportunity for triggered release of actives e.g. as a result of stimuli such as change in pH. By applying drug delivery system drugs could be accumulated at a desired site where it can release drug on demand *via* an external stimulus.

As was shown in above the positively charged DOX is located mainly in the aqueous channels, whereas the unprotonated form of the DOX is localized preferentially in the lipidic bilayer. As the pK_a of DOX is 8.2, 99.6% thereof is protonated at pH 5.5. At pH 5.5 the initial current in all formulations is significantly higher in comparison with that at pH 7.5, indicating that DOX is mainly located in the water channels where diffusion is faster. Neutral form of DOX (pH ~7), localized in the lipidic bilayer, was less prone to being removed from the cubic phase to the solution. After 10h of incubating at pH 5.5, 20 and 30 % of initial drug concentration was preserved in the ML and MO LCPs, respectively.

The release properties of the systems was found to be pH-dependent which is significant because of the potential ability to trigger the release preferentially in the environment of the cancer cells and such system can be considered as a potential drug delivery gel with respect to the affected cells. **[H1]** The pH dependence of the rate of DOX removal from the cubic phases may be exploited in the drug release into tumor cells whose pH is lower than that of the normal cells. However, in all cases the burst release of doxorubicin was observed. The burst release may lead to undesired side effect and ineffective treatment. Sustained release delivery systems are advantageous since they *i)* offer prolonged therapeutic action of drug over an extended period of time, *ii)* increase the efficiency of delivery and selectivity of action, *ii)* may significantly reduce required drug dose, *iv)* minimize its side effects. To attenuate the initial burst release for sustained release several approaches were used.

b) Electrostatic interactions

Modulation of the release properties of a positively charged drug, doxorubicin (DOX) was obtained by the incorporation of small amounts of designed charged lipids to the otherwise non-charged mesophase. **[H3]** Negatively or positively charged cubic phases were obtained by incorporating of small amounts of derivatives of monoolein. [Figure 6] By design, the charged hydrophilic head group of the dopant is exposed to the aqueous channels, whereas the hydrophobic tail is incorporated in the lipid bilayer.

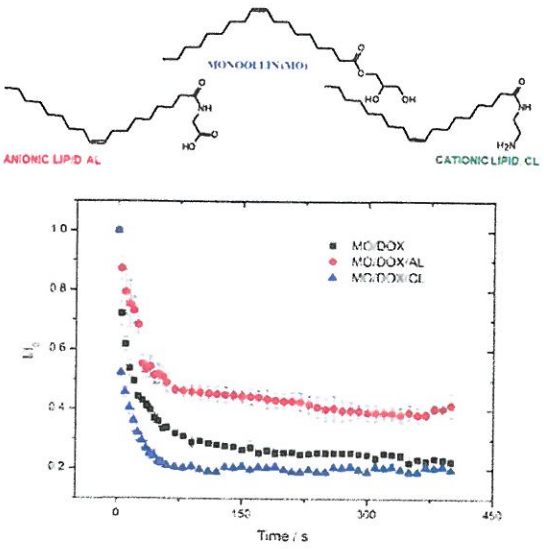


Figure 6 Release profile from mesophase systems.

In vitro DOX release profile was evaluated with the use of differential pulse voltammetry (DPV). The shift towards positive potentials observed in the presence of anionic lipid confirms that more protons are available in the

channels of cubic phase at pH 5.5. Under the pH 5.5 the negatively charged cubic phase (doped with deprotonated AL) was found to delay the release rate of an oppositely charged DOX, while introduction of a positively charged lipid (protonated CL) was found to speed up the release process slightly due to the electrostatic repulsion. Moreover, whereas in the presence of CL ca. 80% of the drug is released, only ca. 60% is released in the presence of AL. But still during the first 1h fast release of drug was noticed.

To provide independent confirmation that the observed differences in the release profiles are not due to changes in the symmetry of mesophase, SAXS measurement were performed. SAXS measurements were done on the formulations with compositions of 62.1/37.9 and 60.9/1.2/37.9 wt. % for the MO/MES buffer and MO/lipid additive/MES buffer, respectively. The X-ray patterns were best fitted to cubic phase with Pn3m symmetry for all formulations; introduction of the lipid additives at the concentration used here does not alter the internal structure of the mesophase. They do, however, affect the crystallographic unit cell size and related aqueous channel diameter. Adding DOX into the system slightly changed these parameters: in the case of the AL system were 9.6 and 4.07 nm, in the case of CL 9.3 and 3.93 nm.

The results obtained showed that *in vitro* DOX release profiles can be modulated by the electrostatic interactions between the drug molecule and the charged mesophase used. SAXS data confirm that the release behavior of DOX in the mesophase was not dependent on the mesophase symmetry, as it remains unchanged, but rather on the charges delineating the water channel compartment.

c) Phase transitions

To provide drug sustained release in the next step we moved to the hexagonal phase, structure with closed water channels. **[H4]** Amphiphiles with the small hydrophilic head (CPP $\gg$ 1) may lead to the formation of highly negatively curved hexagonal (H2) phase. According to the phase diagram for the monoolein water systems at higher temperature a transition from the cubic to the hexagonal phase occurs. At physiological conditions and at room temperature the hexagonal phase may be obtained by the addition of a third component that increases the negative curvature of the lipid layer. Additives with CPP >1 induce phase transition from cubic phase to the more negatively curved hexagonal phase. These modifications allow the formation of hexagonal mesophase.

The structure of the cubic phase consists of two continuous water channels that are believed to be open to the surrounding bulk water, while the water channels in the hexagonal phase are closed. The state of the water channels, whether open or closed, has also a great influence on the rate of drug release from the liquid crystalline phases. [11]

In publication **[H4]** we showed that a monoolein/water system additionally doped with oleic acid induced phase transition of the V2 to H2 phase stable at body temperatures.

Formulations with compositions of 62.5/37.5 and 62.5/0.6/36.9 wt. % for the GMO/MES buffer and GMO/DOX/MES buffer, respectively were prepared. SAXS was used to determine the structural parameters of all formulations.1D diffraction patterns for LCP showed reflections in the ratios of $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{9}$, which are characteristic for the cubic-Pn3m phase. For the pure MO system crystallographic unit cell size and related aqueous channel diameter were 10.5 and 4.2 nm, respectively. For DOX-doped LCP the unit cell size and the diameter of aqueous channel were 10.4 and 4.2 nm, respectively. Introduction of the DOX at the used concentration does not alter the internal structure of the LCP.

To produce hexagonal phase, formulations with compositions of 50/12.5/37.5 and 50/12.5/0.6/36.9 wt% for the GMO/OA/MES and GMO/OA/DOX/MES, respectively were prepared. To identify the phase type SAXS was used. The presence of three diffraction peaks in the ratio $1:\sqrt{3}:\sqrt{4}$, confirm the hexagonal phase formation. A similar pattern was observed for the DOX loaded hexagonal phase. At pH 5 the addition of OA to monoolein was found to increase the critical packing parameter in the system and hexagonal phase formation. Changing pH to 7 leaded to the phase transition to cubic phase: deprotonation of OA molecules in the system leads to flattering of the lipid membrane, mostly because of the charge repulsion effects between negatively charged hydrophilic head groups. This possibility of switching between the cubic and hexagonal phase by changing pH allows for designing a pH responsive system.

The release profile was determined using differential pulse voltammetry. In case of hexagonal phase the DOX release showed very slow rate. Such behavior was different from that observed in cubic phase, where initial burst release of drug was noticed. The difference in the drug release rate may be explained by the difference in the structure of both phases. The hexagonal phase, retards drug release to a greater extent than the cubic phase mainly due to the closed rod-like structure of the mesophase. Because of the slow release capabilities, hexagonal phase is a promising alternative for sustained drug delivery systems for cancer treatment. The

transport properties within hexagonal phase is affected stronger by the interaction of DOX with the carrier than it was observed in cubic phase.

d) Hydrophobic interactions

Additional control on the release properties of drug incorporated within lipid mesophases can be realized by alkylation of the small water soluble molecules. This may cause the partition of the drug into the lipid bilayer of the cubic phase. As a result drug release can be retarded form the mesophase system. In publication **[H5]** we showed that the control over release rate can be achieved by alkylation of the drug molecule. 2-methyl-1,4-naphthoquinone derivatives: menadione (VitK3), phylloquinone (VitK1) and menaquinone (VitK2) were entrapped in the lipidic cubic phase.

Effect of vitamins VitK1, VitK2 and VitK3 doping on the structure of mesophase was studied by SAXS measurement. The mesophase was a ternary system consisting of MO/VitK/H_2O at a ratio 64/1/35 wt. %. Adding vitamin VitK1 to the MO/H2O systems did not change the cubic symmetry of the phase. However, doping system with 1 wt. % of VitK2 vitamin leads to the change of the symmetry of cubic phase to Pn3m. This could be related to the less hydrophilic character of the VitK2 admixture that is incorporated mainly into lipidic bilayers.

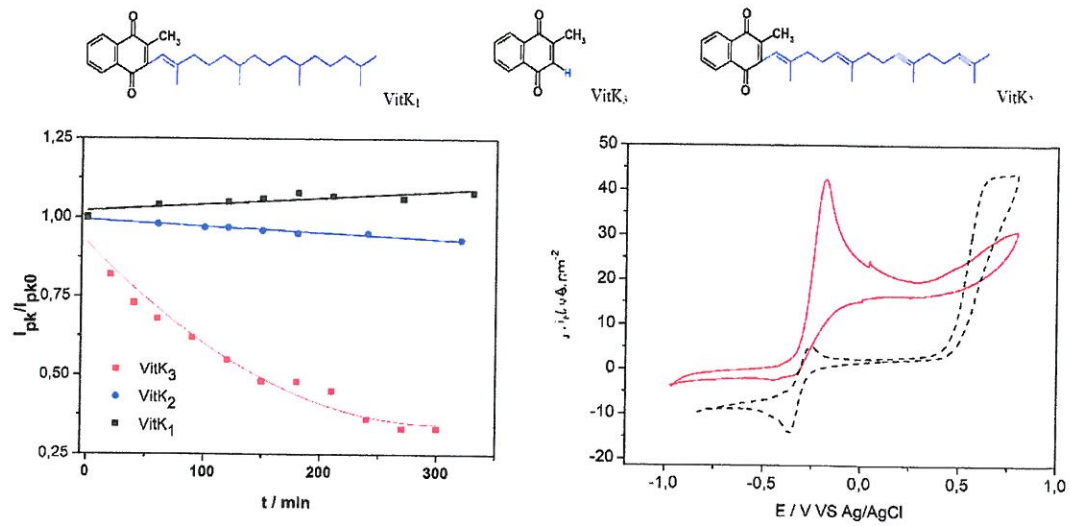


Figure 7 Structures of the molecules used. Release profile of the vitamin from the mesophase systems.

The release properties of vitamins studied using cyclic voltammetry showed that the long hydrophobic chains present in VitK1 and K2 anchor the molecules in the lipidic bilayer so that their leaking from the cubic phase film is not possible. VitK3 is less hydrophobic than VitK2 and VitK1, therefore it is removed quickly from the cubic phase to the electrolyte. In contrary to hydrophobic K1 and K2, the vitamin K3 is not anchored to the lipidic bilayer and is released with time; only residual amounts of vitamin K3 remain in the layer.

Additionally, in this paper we have demonstrated that the liquid-crystalline cubic phase may serve as the carrier and reactor that allows immobilization of enzyme cascades and mediators. Cubic phases is a convenient matrix for enzyme immobilization since it holds enzymes in their active forms close to the electrode surface and facilitates transport of substrates and products of the enzymatic process through the film and provides biocompatible environment for the reactions. Doping the cubic phase with diaphorase and glucose dehydrogenase results in further decrease of NADH oxidation overpotential and the wave appeared at -0.3 V. NADH produced in the enzymatic reaction was oxidized back to NAD+ by the second enzyme diaphorase which passes the two electrons accepted to the mediator – vitamin K. Oxidation current starts at ca. -300 mV which makes that system usable for the construction of bioanodes. [Fig. 7]

Liquid crystalline nanoparticles, cubosomes and hexosomes in drug delivery system

As was shown above using liquid crystalline phases as drug delivery systems offers opportunity for triggered release of actives e.g. as a result of stimuli such as change in pH. The bulk phase is commonly a clear, viscous, semi-solid gel. Its high viscosity makes it difficult to handle and limits its application. To overcome these drawbacks, another strategy can be applied which involve the use of dispersed bulk phase in water in the form of small particles. That dispersed cubic particles denoted as cubosomes are less viscous and they can stably exist in equilibrium with aqueous solution and concurrently retain the internal bicontinuous structure unchanged. With respect to liposome, either cubosome or hexosome possesses a larger ratio between the bilayer area and the particle volume. Cubosomes are a dispersed phase of cubic phase stabilized by a polymer coating. Similarly to the bulk systems drug release can be modulated by additives that may affect the release kinetics.

a) Interaction of cubosomes with lipidic membranes

Lipidic cubic nanoparticles (cubosomes) are known as convenient biocompatible drug delivery systems hence understanding their mode of interaction with lipidic membranes is of special interest. Better understanding of the interplay between cubosomes and lipid model membrane is essential for the improved design of drug delivery carriers for *in vivo* use. These results could have important implications for the development of cubic phase lipid nanoparticles for *in vivo* use. Therefore, we decided to characterize the interactions of cubosomes with phospholipids self-assembled at the air-water interface, which eliminates the influence of the solid support onto the model membrane and provides better control of the organization of the phospholipid monolayer **[H6].** [12][13][14] The LCNPs studied are a lipid dispersion stabilized using hydrophilic copolymer; their composition is 96.3/3.0/0.7 wt.% of water/monoolein (GMO)/block co-polymer (Pluronic F-127). Three distinct Bragg peaks with relative peaks positions of $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$ were observed. This pattern corresponds to the cubic-Im3m structure. The particle size of the cubosomes determined with DLS was 180 nm.

The interactions of monoolein (GMO) LCNP with model lipid membranes self-assembled at the air-water interface prepared by the Langmuir technique were investigated in **[H6]**. We have shown that at high surface pressures the lipid layer is densely packed and interactions can lead to the exchange of the lipid molecules between the lipid monolayer and the intact LCNP whereas at low surface pressures the monolayer is less densely packed which allows the incorporation of LCNP material into the monolayer. At low surface pressures, corresponding to less condensed phase, the monolayer is less densely packed which allows the incorporation of cubosomal material into the monolayer, while at high surface pressures the lipid layer is densely packed and interactions can lead to the exchange of the lipid molecules between the lipid monolayer and the intact cubosomes. The composition of the layers under such different conditions should be different and should reflect the mechanism of interactions of cubosomes with the lipid membranes. This work in general has revealed that:

1) cubosomes spreads at the air-water interface in the presence of less organized lipid layers,

2) changes in shape and area per molecule indicate that it is not the intact cubosome but spread components that are in the mixed layer with DPPC,

3) BAM images (not shown) reflect the changes in thickness of the layer: mixed monolayer structure is retained to high pressures,

4) less condensed layers facilitate incorporation of the cubosome material into the gaps while highly organized and packed layers keep the cubosome intact and outside the monolayer.

Despite the information gained from surface tension measurements, information about the exchange of material between the LCNPs and the monolayers, how long the cubosomes are in contact with the monolayer, and how the interfacial stoichiometry is affected by the changes in surface pressure is missing. Neutron reflectivity studies can provide direct information about the exchange of material at the interface and the quantitative information about these processes. NR studies can allow us to gain the understanding of the system.

c) pH sensitive cubosome

In work **H7** the behavior of doxorubicin in the monoolein cubic phase, where the liquid-crystalline cubic phase is considered as a potential drug delivery gel to the affected cells is described. The properties of the system where found to be pH dependent, which is interesting because of the potential ability to trigger the release of the drug only in the environment of the cancer cells. Sites of tumors have a significantly lower pH as compared to normal tissues. As such, pH-sensitive systems are extensively investigated as a means of increasing drug delivery to tumors.

To prepare cubosomes, a top-down and bottom-up approaches were adopted. The final compositions of cubosomes were: 96.3/3.0/0.7 wt. % of water/GMO/PF127 and 96.3/2.9/0.1/0.7 wt. % of water/GMO/DOX/PF127. SAXS was used to identify the type of the phase formed. Three distinct Bragg peaks with relative positions of $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, that corresponds to the cubic-Im3m, were observed for cubosome formulations. MO-based cubic phase, dispersed and stabilized with Pluronic F127, underwent a phase transition from the cubic-Pn3m type to the more swollen primitive cubic phase of Im3m symmetry.

The pH dependent release behavior of DOX was evaluated using Square Wave Voltammetry in four buffered solutions at pHs 5.5, 6.5, 7.5 and 8.5. The investigation of the release behavior confirm that the rate of DOX release from nanoparticles was pH dependent and was higher at pH 5.5 than at pH 7.5. At pH 5.5, where 99.8 % DOX is in protonated state, drug is mainly located in the aqueous channels where diffusion is faster than in the lipidic domain. The release properties from cubosomes were similar to those observed in bulk systems. pH sensitive release is crucial for a delivery system to target tumor cells.

Nevond

c) Hexosomes in sustained drug delivery system formation

Bulk hexagonal phase was shown to retard the doxorubicin release and may be considered as valuable tools for sustained drug delivery systems in cancer therapy. **[H4]** Closed water channels in hexagonal phase offer the ability to provide sustained release for certain drugs, where a prolonged delivery is preferable. The SAXS spectra was obtained for the empty and drug-loaded cubosome and hexosome formulations. 1D diffraction patterns exhibit reflections in the ratios of $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{9}$, which are characteristic for the cubic-Pn3m phase and three Bragg reflections in the ratio $1:\sqrt{3}:\sqrt{4}$, which corresponds to the hexagonal phase. The pH change was shown to trigger a change in morphology from the cubic to hexagonal phase. **[H4]** By doping monoolein with oleic acid, the cubic nanostructure transitioned to the hexagonal nanostructure. Changes in the mesophase architecture resulted in modulation of the rate of drug release from the phase. [15][11] [16]

The DOX release capabilities from the liquid crystalline nanoparticles were similar to those observed in the bulk systems. **[H4]** Change in the nanoparticle architecture resulted in different release rate of doxorubicin; DOX release rate was much more rapid from cubosomes than from the hexosomes. The drug release was sustained from hexosomes with respect to the cubosome. The ability of the hexagonal phase to slow down the rate of drug release is crucial for its further applications in sustained drug delivery.

The biological properties of the developed nanoparticles, were evaluated using confocal microscopy. Experiments were performed on the HeLa cancer cell line. HeLa cells were treated with DOX loaded cubosome or hexosome formulations. DOX localization was monitored with confocal microscopy and indicate that DOX effectively accumulated in the cell nuclei; the effect was pronounced after incubation with DOX loaded cubosomes. DOX delivery capabilities of hexosomes was clearly higher than those observed for free-DOX, but still lower than the one observed for cubosomes.

The cytotoxic effect on HeLa cells was determined using the MTT assay. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] is a tetrazolium salts, that can be reduced by viable cells to generate formazan; the absorbance measured reflects the total metabolic activity of a cells. MTS assay reveal that empty nanoparticles exhibit relatively low cytotoxicity, however when loaded with chemotherapeutic

induced a significant reduction in cell viability. Cell viability decreased after incubation of cells with DOX loaded cubosomes.

Performed in **H4** experiments confirm that hexosomes can be utilized as sustained release delivery of doxorubicin. Hexosomes can be applied when retarded release is more favorable than immense release.

a) Folate targeted lipid mesophase

The use of cytotoxic drugs in cancer treatment often leads to the side effect and the non-specific delivery of drug. The main requirement in the cancer treatment is to achieve therapeutically-relevant concentrations in target site. Active targeting that refers to the attachment of vectors which bind to specific receptors by molecular recognition may be utilized to increase accumulation and penetration into target sites. Active targeting drug to tumor tissue would reduce drug dose and side effects. The use of lipid nanoparticles conjugated to biomolecules having affinity to receptors on cancer cells are promising strategies for the development of new class of carriers for targeted therapy. **[H8]**

Liquid crystalline nanoparticles conjugated with vectors can provide selective targeting to cancer cells and avoid toxicity towards healthy tissues. To achieve accessibility for specific binding, the ligand can be attached to the stabilizer. Folic acid (FA) was used as targeting molecule and was attached to the carrier to provide selective targeting. Folic acid was conjugated with a stabilizer, Pluronic F108, thus, FA attached to the nanoparticles was exposed to the solution and increased the accessibility of the targeting group to the desired site.

Folate-targeted nanoparticles were characterized using SAXS to assess qualitative information about the internal structure of each mesophase. All formulations were prepared with a lipid content of 5 wt %, moreover the lipid to DOX ratio was maintained at a constant level. The 1D diffraction patterns for samples showed reflections in the ratios of $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{9}$, which are characteristic for the cubic-Pn3m phase. All six Bragg reflections were observed for the cubosome formulations, in the absence or presence of DOX or PF108-FA. To obtain hexosomes tetradecane was added to monoolein to induce the formation of a more negatively curved structure. SAXS profile showed three Bragg reflections in the ratio $1:\sqrt{3}:\sqrt{4}$. The X-ray patterns were best fitted to the hexagonal phase either for the non-doped or DOX-doped mesophase. SAXS data confirmed that the effect of folic acid on the structure of the cubosome or hexosome dispersions is modest.

Square wave voltammetry (SWV) was used to determine the release profile of DOX-loaded folate-conjugated nanoparticles. This technique allowed collecting scans with high accuracy in short interval times. The release properties of DOX from folate-conjugated nanoparticles was prolonged compared to free DOX. The release rate is affected by the mesophase structure, less DOX was released from the hexosomes, when compared to cubosomes (and free drug). A very slow-release profile, without an initial burst release of the drug, was observed for hexosomes. To determine the release kinetics the current values were normalized with respect to the current measured for free DOX. The drug release data were fitted into Higuchi's equation:

$$\frac{M_t}{M_\infty} = K_H t^{0.5}$$

, where M_t/M_∞ is the fraction of drug released at time t, and K_H is the drug release rate constant

The highest correlation coefficient was observed for the cubosomal formulation that suggests that DOX transport is controlled by diffusion, while in case of hexagonal phase was lower indicating that the hexagonal structure may affect the drug transport properties. The obtained results are that it will be possible to target the delivery of drugs to the site of disease and reducing unwanted side effects. Thus, the efficacy of drug delivery to cancer cells by folate-targeted lipid mesophases, cubic and hexagonal nanoparticles loaded with doxorubicin (DOX) was evaluated on three cancer-derived cell lines (KB, HeLa, T98G) exhibiting different expressional levels of folate receptor protein (FR). Both KB and HeLa cells express a high level of folate receptor protein (FR-positive), while the expression of folate receptor in T98G cells was negligible. To evaluate the DOX delivery capability of the studied folate-targeted lipid nanoparticles, confocal microscopy and fluorescence analysis of the treated cancer cells were performed. The obtained results revealed that the treatment of FR-positive HeLa and KB cells with folate-modified hexosomes results in lower intracellular DOX intake when compared to folate-targeted DOX-loaded cubosomes. In case of folate-modified cubosomes, DOX intake was significantly higher in comparison to hexosomes. In contrast, T98G cancer cells incubated with cubosomes and hexosomes exhibited similar levels of DOX intake when compared with FA-targeted structures.

To determine the number of viable cells present in a cell suspension the Trypan Blue and MTS assay were used. Trypan Blue assay is based on the principle that live cells have an intact

cell membrane, and trypan blue cannot penetrate the cell membrane of live cells. In nonviable cells trypan blue passes through the cell membrane. The viability assays showed that the administration of CUB/FA-encapsulated DOX to HeLa and KB cells rapidly decreased their survival rate and improved drug-mediated anti-tumor activity. DOX encapsulated in the folate-modified cubosomes resulted in reduction of the survival rate of KB and HeLa cells, in comparison to cubosome-treated cells (16% and 57%, respectively). Viability of T98G cells was similar in folate modified drug-loaded mesophases when compared with non-targeted carriers.

THE MAIN ACHIEVEMENTS

The most important results of my investigations are:

1. Showing that drug release form lipid liquid crystalline nanoparticles can be controlled by: pH, changing the lipid used for mesophase formation, electrostatic interactions, hydrophobic interactions, phase transitions.
2. Proving pH sensitive cubosome formation. DOX loaded cubosome exhibited pH-dependent drug release behavior and had an ability to target cancer cells, release of cargo was triggered by pH.
3. Determination of transport properties of drug incorporated within lipid mesophases. Kinetics and mechanism of drug release from the mesophase system was evaluated using electrochemical methods. Application of steady-state voltammetry on microelectrode and macroelectrode combined, allowed the determination of both diffusion coefficient (D) and concentration of the electroactive probe in the cubic phase.
4. Cubic phases may serve as the carrier and reactor that allows immobilization of enzyme cascades and mediators.
5. For the first time the Langmuir technique to study interactions of cubosomes with model membranes formed at the air-water interface. Presented data reveal that at high surface pressures the lipid layer is densely packed and interactions can lead to the exchange of the lipid molecules between the lipid monolayer.
6. It was confirmed that cubosomes likely present a more efficient and rapid drug release, while hexosomes exhibit a less prolonged drug discharge capability.

7. Biological analyses revealed that the treatment of FR-positive HeLa and KB cells with folate-modified cubosomes results in higher intracellular DOX intake when compared to folate-hexosomes.
8. Folate functionalized cubosomes provide selective targeting to cancer cells; the viability assays showed that the application of DOX loaded folate-cubosomes to FR-positive cells rapidly decreased their survival rate and improved drug-mediated anti-tumor activity.

5. Scientific achievements other than those selected for habilitation procedure

a) *Publication list - other than those selected for habilitation procedure*

1. M. Zatloukalová, E. Nazaruk, D. Novák, J. Vacek, R. Bilewicz, Lipidic liquid crystalline cubic phases for preparation of ATP-hydrolysing enzyme electrodes, Biosens. Bioelectron. 100 (2018). doi:10.1016/j.bios.2017.09.036.

$IF_{(2018)}$ = 8.173. Number of citations = 2

2. R.A. Campbell, Y. Saaka, Y. Shao, Y. Gerelli, R. Cubitt, E. Nazaruk, D. Matyszewska, M.J. Lawrence, Structure of surfactant and phospholipid monolayers at the air/water interface modeled from neutron reflectivity data, J. Colloid Interface Sci. 531 (2018). doi:10.1016/j.jcis.2018.07.022.

$IF_{(2018)}$= 5.091. Number of citations = 3

3. J. Szydłowska, A. Sitkiewicz, E. Nazaruk, D. Pociecha, P. Krzyczkowska, A. Krówczyński, E. Gorecka, Fluorescent and charge transport properties of columnar phases made of mono and bi-phenazine derivatives, Soft Matter. 14 (2018). doi:10.1039/c7sm02087b.

$IF_{(2018)}$ = 3,709. Number of citations = 0

4. Mazur, B. Rola, K. Stolarczyk, E. Nazaruk, R. Bilewicz, J. Rogalski, S. Ohga, The large scale production of Cerrena unicolor laccase on waste agricultural based media, J. Fac. Agric. Kyushu Univ. 60 (2015).

$IF_{(2015)}$ = 0,216. Number of citations = 3

5. E. Nazaruk, E.M. Landau, R. Bilewicz, Membrane Bound Enzyme Hosted in Liquid Crystalline Cubic Phase for Sensing and Fuel Cells, Electrochim. Acta. 140 (2014). doi:10.1016/j.electacta.2014.05.130.

$IF_{(2014)}$= 4,504. Number of citations = 15

6. M. Karaśkiewicz, E. Nazaruk, K. Zelechowska, J.F. Biernat, J. Rogalski, R. Bilewicz, Fully enzymatic mediatorless fuel cell with efficient naphthylated carbon nanotube-laccase composite cathodes, Electrochem. Commun. 20 (2012). doi:10.1016/j.elecom.2012.04.011.

$IF_{(2012)}$= 4,425. Number of citations = 52

7. E. Nazaruk, M. Karaskiewicz, K. Zelechowska, J.F. Biernat, J. Rogalski, R. Bilewicz, Powerful connection of laccase and carbon nanotubes: Material for mediator-free electron transport on the enzymatic cathode of the biobattery, Electrochem. Commun. 14 (2012). doi:10.1016/j.elecom.2011.11.005.

$IF_{(2012)}$ = 4,425. Number of citations = 29

8. R. Bilewicz, E. Nazaruk, K. Zelechowska, J.F. Biernat, K. Stolarczyk, K.P. Roberts, G. Ginalska, J. Rogalski, Carbon nanotubes chemically derivatized with redox systems as mediators for biofuel cell applications, Biocybern. Biomed. Eng. 31 (2011).

$IF_{(2011)}$ = 0,234. Number of citations = 6

9. E. Nazaruk, K. Sadowska, J.F. Biernat, J. Rogalski, G. Ginalska, R. Bilewicz, Enzymatic electrodes nanostructured with functionalized carbon nanotubes for biofuel cell applications, Anal. Bioanal. Chem. 398 (2010). doi:10.1007/s00216-010-4012-1.

$IF_{(2010)}$ = 3,841. Number of citations = 49

10. E. Nazaruk, K. Sadowska, K. Madrak, J.F. Biernat, J. Rogalski, R. Bilewicz, Composite bioelectrodes based on lipidic cubic phase with carbon nanotube network, Electroanalysis. 21 (2009). doi:10.1002/elan.200804435.

$IF_{(2009)}$= 2,630. Number of citations = 27

11. E. Nazaruk, S. Smoliński, M. Swatko-Ossor, G. Ginalska, J. Fiedurek, J. Rogalski, R. Bilewicz, Enzymatic biofuel cell based on electrodes modified with lipid liquid-crystalline cubic phases, J. Power Sources. 183 (2008). doi:10.1016/j.jpowsour.2008.05.061.

$IF_{(2008)}$= 3,477. Number of citations = 66

12. K. Stolarczyk, E. Nazaruk, J. Rogalski, R. Bilewicz, Nanostructured carbon electrodes for laccase-catalyzed oxygen reduction without added mediators, Electrochim. Acta. 53 (2008). doi:10.1016/j.electacta.2007.09.053.

$IF_{(2008)}$= 3,078. Number of citations = 42

13. E. Nazaruk, R. Bilewicz, G. Lindblom, B. Lindholm-Sethson, Cubic phases in biosensing systems, Anal. Bioanal. Chem. 391 (2008). doi:10.1007/s00216-008-2149-y.

$IF_{(2008)}$= 3,327. Number of citations = 40

14. E. Nazaruk, R. Bilewicz, Catalytic activity of oxidases hosted in lipidic cubic phases on electrodes, Bioelectrochemistry. 71 (2007) 8–14. doi:10.1016/j.bioelechem.2006.12.007.

$IF_{(2007)}$= 2.992. Number of citations = 23

15. E. Nazaruk, A. Michota, J. Bukowska, S. Shleev, L. Gorton, R. Bilewicz, Properties of native and hydrophobic laccases immobilized in the liquid-crystalline cubic phase on electrodes, J. Biol. Inorg. Chem. 12 (2007). doi:10.1007/s00775-006-0193-7.

$IF_{(2007)}$ = 3,325. Number of citations = 34

16. K. Stolarczyk, E. Nazaruk, J. Rogalski, R. Bilewicz, Mediatorless catalytic oxygen reduction at boron-doped diamond electrodes, Electrochem. Commun. 9 (2007). doi:10.1016/j.elecom.2006.08.044.

$IF_{(2007)}$= 2,848. Number of citations = 33

b) **Total impact factor of papers listed above according to the list from Journal Citation Reports (JCR): IFTOTAL= 83,541**

c) **Total number of citations minus self-citations to the Web of Science (WoS): 485 (without self citation)**

d) **Hirsch Index according to the Web of Science (WoS): 14**

e) **Research Projects**

Principal Investigator

- National Science Centre (Poland) OPUS13 2017/25/B/ST4/02817 "Nanostructured lipidic liquid-crystalline carriers for chemotherapeutics and corpuscular radiation emitters in targeted cancer theraphy". Start date: 2018. In progress.
- National Science Centre (Poland) SONATA5 2013/09/D/ST5/03876, "Tailored lipidic mesophases in drug delivery system development". Start date: 2014. Finish date: 2017.
- Ministry of Science and Higher Education Iuventus Plus IP „Lipidic Cubic Phase Doped with Biocatalyst and Selected Biologically Active Compound" ". Start date: 2011. Finish date: 2011.

Investigator

- National Science Centre (Poland) OPUS12 2016/23/B/ST4/03295 „Mechanism of incorporation of lipid liquid crystalline drug carriers – cubosomes and hexosomes into lipid membranes". (Principal Investigator: Prof dr hab Renata Bilewicz). Start date: 2017.
- Sinergia grant No. CRSII2 154451/1, "Design, synthesis and characterization of lipidic nanomaterials for biomedical and biosensing applications" (Principal Investigator Prof. Ehud Landau). Start date 2014; finish date 2018.
- Polish-Swiss Research Programme PSPB-079/2010 „Tailored Lipidic Mesophases as Novel Functional Nanomaterials in Bioenergetics and Biosensing". (Principal Investigator: Prof. dr hab. Renata Bilewicz). Start date 2012; finish date 2015.

- Ministry of Science and Higher Education NN204214639 „Application of chemically modified carbon nanotubes for the construction of enzymatic bioanode." (Principal Investigator: Prof. dr hab. Renata Bilewicz). Start date 2010; finish date 2013.
- The National Centre for Research and Development NR05001710 „Microbiofuel cell integrated with a biosensor". (Principal Investigator: Prof. dr hab. Renata Bilewicz). Start date 2010, finish date 2013.

Other projects

- ISIS Neutron and Muon Source Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK. 5/12/2019 to 8/12/2018; Title – "*Interactions of lipidic cubic nanoparticles with model lipid biomembranes at the air water interface*"
- Neutrons for Society, The Institut Laue-Langevin (ILL) GRENOBLE France: 27/01/2017 To 29/01/2017; Title "*Interactions of drug carriers - lipidic nanoparticles, known as cubosomes with model lipid membranes at the air-water interface*"
- Neutrons for Society, The Institut Laue-Langevin (ILL) GRENOBLE France: 15/06/2018 to 19/06/2018; Title "*Interactions of anticancer drugs with model lipid membranes - determination of the mechanisms and interfacial stoichiometry*"

f) International and polish awards for research

- 2016 - Faculty of Chemistry Scientific Council Award: Grabowski Prize for Scientific Achievements.
- 2011 - Faculty of Chemistry Scientific Council Award, II Degree for Scientific Achievements.
- 2012 - 2013 Scholarships for the best doctoral students and young PhDs from Human Capital Operational Programme, cofinanced by the European Union within the European Social Fund.

g) Summary of other scientific achievements

Of special interest of my research are the lipidic mesophases, which owning to their symmetry and internal structure have found use in diverse areas from cell biology to material science. In my PhD thesis I was focused on the application of lipid cubic phase to the development of bioelectrodes for biosensing and biofuel cell application. Because incorporation of proteins with retained activity in artificial membranes is essential for use in membrane - based sensors or biofuel cell devices, lipidic cubic phases were found to be useful matrices with potential use in biosensing or bioanode construction. When incorporated in lipidic cubic phases, the inserted redox proteins are readily recovered by mediators that shuttle electrons between the electrode surface and the protein. Such mesophases can incorporate also relatively large soluble and membrane proteins without adverse effect to activity, and allow direct spectroscopic investigation, due to their optical transparency. Thus after PhD my research I used cubic phase also to accommodation and activity study of membrane proteins. Membrane proteins in particular suffer denaturation, destabilization, and loss of activity when removed from their native membrane. These drawbacks can generally be overcome by swift reconstitution into a matrix that resembles the natural environment. The lipidic cubic phase, with its curved bilayer that spans the entire material can be regarded as convenient matrix for that purpose. I have used cubic phase to immobilize and measure activity of fructose dehydrogenase (FDH) - membrane bound enzyme. Enzyme contains flavin and heme c group and catalyses the oxidation of d-fructose to 5-keto-d-fructose. The FDH/mesophase modified electrode was used in fructose sensing. I have used also cubs phase for the reconstitution of membrane ATP-hydrolysing sodium/potassium transporter Na^+/K^+-ATPase and chloride channel (EcClC) in the monoolein cubic phase film. Activity was as determined by spectrophotometry and electrochemical methods.

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