Scientific paper

Interactions of Divalent Calcium Ions With Head Groups of Zwitterionic Phosphatidylcholine Liposomal Membranes

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Abstract

The interaction of the divalent calcium ions with the zwitterionic lipid membranes was studied by measuring the lipid order parameter which is inversely proportional to the membrane fluidity. Small unilamellar lipid vesicles were prepared from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and then treated with different concentrations of divalent calcium ions. An increase in the order parameter and decrease in the fluidity of the liposomal membranes were observed after treatment with the calcium ions. The presence of positively charged iron oxide nanoparticles in the suspension of liposomes negligibly changed the results. The results of experiments were discussed theoretically within modified Langevin-Poisson-Boltzmann (MLPB) model leading to the conclusion that the membrane fluidity and ordering of the membrane lipids are primarily altered by the accumulation of calcium ions in the region of negatively charged phosphate groups within the head groups of the membrane lipids.

Keywords: Anisotropy, liposomes, bilayer fluidity, iron oxide nanoparticles, electric double layer theory

1. Introduction

Liposomes encapsulating nanoparticles (NPs) with magnetic properties termed as "magnetoliposomes" have attracted a great interest in the recent past due to their potential biomedical applications especially in targeted drug delivery,[1] hyperthermia[2] and as contrast agents in magnetic resonance imaging (MRI).[3] Among the magnetic NPs, iron oxide (Fe_2O_3) NPs gains special importance in various clinical applications due to their non-toxicity and biodegradability.[4,5] As the usage of Fe_2O_3 NPs is tremendously increasing in the medical fields, it becomes impor-

tant to study the consequences of interactions of the NPs with the membrane which may alter the physical properties of the bilayer such as fluidity, phase transition temperature, morphology, mechanical stability and permeability.

Membrane fusion is an essential process which accounts for various biological events such as fertilization, embryogenesis, neuronal signalling and endocrine hormone secretion. The fusion process includes inter membrane contact, mixing of the membrane lipids and pore formation to mix the inner lipid contents to form large multilamellar vesicles.[6] Membrane fusion is generally induced *in vitro* and *in vivo* conditions[7, 8] by fusogenic agents called fusogens such as proteins and peptides. Apart from these larger molecules, divalent cations such as Ca^{2+}, Mg^{2+}, $Mn^{2+}$and Zn^{2+} have the potential to induce membrane fusion.[9] Since calcium is known to be an essential biological component and a key player in inducing membrane fusion, we have chosen divalent calcium ions to study their effect on liposomes which serve as a simple model system to understand the complex fusion process in cells.

Membrane fluidity characterizes the viscosity of the lipids in the plasma membrane. The cells maintain the optimal level of fluidity so that the mobility of the lipoproteins in the membrane is large enough to perform different biological functions.[10] Alterations in the fluidity level of the liposomal membranes can be conveniently studied by anisotropy measurements using the fluorescent probes such as 1, 6-diphenyl-1,3,5 hexatriene (DPH) (Fig. 1a).[11] Membrane fluidity can be altered by various factors such as temperature, cholesterol, lipid composition including the hydrocarbon chain length, degree of saturation and interaction of NPs.[12] Since Ca^{2+} interacts with the cell membrane, it induces alterations in the ordering of the membrane lipids and therefore affects the bilayer fluidity. Ca^{2+} may also promote the adhesion and fusion of the adjacent cells/liposomes and therefore mixing of the different lipid components.

A lot of work has been done on the study of the interaction of negatively charged membranes mediated by positively charged calcium ions but very less work was carried on with the zwitterionic liposomes. Hence in this work we intended to study the effect of Ca^{2+} on liposomes prepared with a zwitterionic lipid such as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Fig. 1b). In order to study the effect of electrostatic interactions of Ca^{2+} with the NPs in detail, we have used in this work the positively charged Fe_2O_3 NPs functionalized with amino groups. Through mathematical modeling using the modified Langevin-Poisson-Boltzmann (MLPB) model of electric double layer, we aim to discuss the theory and the obtained experimental results. The scope of the present study is to gain more knowledge for better understanding of the role of Ca^{2+} (also in the presence of Fe_2O_3 NPs) in altering the membrane lipid bilayer fluidity which is important to consider prior to their biomedical applications.

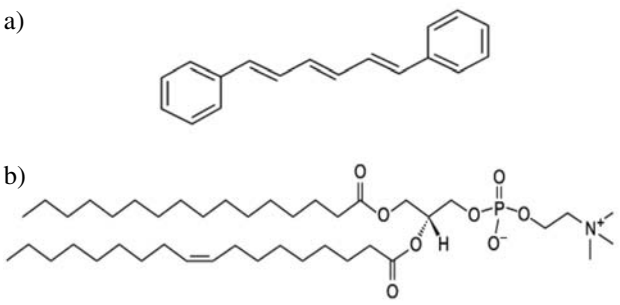

Figure 1. Structure of (a) DPH (1,6-diphenyl-1,3,5-hexatriene) and (b) POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine).

2. Materials and Methods

POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids Inc., USA. DPH (1,6-diphenyl-1,3,5-hexatriene) and anhydrous calcium chloride were obtained from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. All the chemicals obtained have high purity (> 99%) and used without any further purification. Iron oxide amine (Fe_2O_3-NH_2) NPs were obtained from J. Stefan Institute, Ljubljana, Slovenia.

2. 1. Preparation of Small Unilamellar Vesicles

Small unilamellar vesicles (SUVs) were prepared by the thin film method using the rotary evaporator. The POPC lipid (2 mg) was dissolved in 1 ml of chloroform in a round bottomed flask. The argon gas was bubbled through the lipid-chloroform mixture in the flask to avoid the oxidation of lipids. The organic solvent in the flask was slowly evaporated by the rotary evaporator under reduced pressure (1.7 kPa) to obtain a thin lipid film. The control liposomes containing pure POPC lipid were prepared by hydrating the obtained lipid film with double distilled water at pH 5.5. A thin lipid film was prepared in a similar way and hydrated with the Fe_2O_3-NH_2 NPs suspended in distilled water. The hydrodynamic radius of the Fe_2O_3-NH_2 NPs was 180 nm and their zeta potential value was 4 mV respectively.

The lipid suspensions in the flasks were vortexed vigorously with the glass beads for 10 min to obtain multilamellar vesicles (MLVs). The obtained vesicles were sonicated for 30 min total time with 10 s on-off cycles at 40% amplitude using a Vibracell Ultrasonic Disintegrator VCX 750 (Sonics and Materials, Newtown, USA) to form SUVs. In order to remove the debris formed after sonication, the sample containing SUVs were then centrifuged for 10 min at 212554 g (Eppendorf centrifuge 5415C). The SUVs were incubated with appropriate amount of calcium chloride ($CaCl_2$) solution to reach a final concen-

tration of 10 mM and 100 mM in 2.5 ml of 20 mM HEPES buffer and left for 30 min before measuring the anisotropy values.

2. 2. Fluorescence Anisotropy Measurements

DPH is one of the widely used fluorescent probes for measuring viscosity, lipid order and polarity of the membrane.[13] Being a hydrophobic probe, DPH intercalates between the tail regions of the lipid bilayer and is distributed throughout the membrane. It is almost non-fluorescent in aqueous environment but shows intense fluorescence signals after incorporation into the hydrophobic core of the membrane bilayer.[14]

Temperature dependent anisotropy values were measured following the incorporation of the fluorescent dye DPH in the POPC liposomes treated with divalent calcium ions. The measurements were carried out in a 10 mm-path-length cuvette using the Cary Eclipse fluorescence spectrophotometer (Varian, Mulgrave, Australia). In the quartz cuvette, 10 μL of DPH was added to 2.5 mL 100 μM solutions of SUVs prepared from POPC to reach a final concentration of 0.5 μM DPH. The anisotropy measurements were performed within the temperature range from 15 °C to 50 °C by gradually increasing the temperature by 5 °C for every measurement, with a time interval of 8 min with constant mixing at pH 7.0. Varian autopolarizers having the slit widths with a nominal band-pass of 5 nm was used for both the excitation and emission spectra. The fluorescent probe DPH was excited at 358 nm with the excitation polarizer oriented in the vertical position. The emitted polarized light in both the vertical and horizontal planes were recorded by a monochromator and measured at 410 nm. The anisotropy $\langle r \rangle$ values were measured using the built-in software of the instrument by applying the below formula:

$$\langle r \rangle = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + 2GI_{\perp}}, \quad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are the parallel and perpendicular emission intensities, respectively. The G-factor value (ratio of the sensitivities of the detection system for vertically [IHV] and horizontally [IHH] polarized light) was determined separately for each sample. The lipid-order parameter S was calculated from the anisotropy values using the following expression:[15]

$$S = \frac{\left[1 - 2\left(\frac{r}{r_0}\right) + 5\left(\frac{r}{r_0}\right)^2\right]^{\frac{1}{2}} - 1 + \frac{r}{r_0}}{2\left(\frac{r}{r_0}\right)}, \quad (2)$$

where r_0 is the fluorescence anisotropy of DPH in the absence of any rotational motion of the probe. The theoretical value of r_0 for DPH is 0.4, while experimental values of r_0 lie between 0.362 and 0.394.[15] In our calculation, the experimental value of r_0 was 0.370 for DPH in POPC at 5 °C.

To conclude, the anisotropy values obtained using DPH are directly proportional to lipid-order parameter (S) in the membrane, which can be calculated using the formula shown in Equation 2. Lipid-order parameter is inversely proportional to the fluidity and hence from the obtained lipid-order parameter results, bilayer fluidity can be estimated.[16]

2. 3. Theoretical Model

2. 3. 1. Pure Salt Solution in Contact With the Zwitterionic Lipid Bilayer

POPC lipid is a common representative of the zwitterionic (dipolar) lipids. Dipolar head group consists of a phosphate (negative) and amino (positive) group in the head region. Due to amphiphilic effect, the negative part of the lipid head group bounded to the lipid tails (phosphate group) is in contact with the salt solution forming negatively charged surface at $x = 0$ (see Fig. 2). The positive part of the lipid head group (amino group) penetrates into the salt solution and can be partially movable inside head group region (see Fig. 2).

In this work the contact of the zwitterionic lipid bilayer (e.g. POPC) with the salt solution containing divalent counter-ions and monovalent co-ions (e.g. $CaCl_2$) is

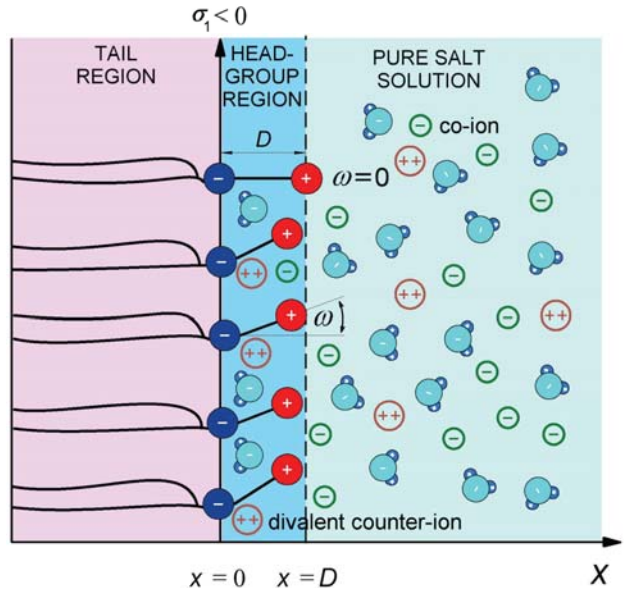

Figure 2. Schematic presentation of zwitterionic lipid bilayer in contact with the salt solution containing monovalent co-ions and divalent counter-ions (e.g. Calcium ions). Phosphate groups in the head group region are described by negatively charged surface at $x = 0$ with negative surface charge density σ_1. D is the distance between the charges in the single lipid head group, while ω describes the orientation angle of the single head group.

theoretically described by using the modified Langevin-Poisson-Boltzmann (MLPB) model.[17–19] The MLPB model takes into account the finite volumes of lipid head groups[17], the cavity field in saturation regime, and the electronic polarization of the water dipoles.[18–23] The finite volume of ions and water molecules in the solution was not taken into the account. Schematic presentation of the model system can be seen in Fig 2.

The Poisson equation describing the system presented in Fig. 2 can be written in the form:

$$\frac{d}{dx}\left[\varepsilon_0\varepsilon_r(x)\frac{d\phi(x)}{dx}\right] = -\rho_{ion}(x) - \rho_{zw}(x), \tag{3}$$

where $\phi(x)$ is the electric potential, ε_0 is permittivity of the free space, $\varepsilon_r(x)$ is the relative permittivity of the salt solution, $\rho_{ion}(x)$ is the volume charge density of ions in the salt solution and $\rho_{zw}(x)$ is the volume charge density of the positive charges of zwitterionic lipid head groups. In the model the salt solution is composed of calcium chloride ($CaCl_2$) and water. In the water solution the single $CaCl_2$ molecule dissociates into one divalent counter-ion Ca^{2+} and two monovalent co-ions Cl^-, therefore the volume charge density of ions $\rho_{ion}(x)$ can be written as:

$$\rho_{ion}(x) = -e_0 n_-(x) + 2e_0 m(x), \tag{4}$$

where $n_-(x)$ is the number density of Cl^-, $m(x)$ is the number density of Ca^{2+}.and e_0 is the elementary charge. Considering the Bolztmann distribution function for co-ions (Cl^-) and counter-ions (Ca^{2+}):

$$n_- = n_0 e^{+e_0\phi(x)\beta} \quad ; \quad m = m_0 e^{-2e_0\phi(x)\beta}, \tag{5}$$

where n_0 is bulk concentration of monovalent chloride co-ions, m_0 the bulk concentration of divalent calcium counter-ions, $\beta = 1/kT$, kT is the thermal energy. The electro neutrality condition:

$$-n_0 + 2m_0 = 0, \tag{6a}$$

yields

$$n_0 = 2m_0 \ . \tag{6b}$$

Equation 4 can be further rewritten as:

$$\rho_{ion}(x) = -e_0 n_0 e^{+e_0\phi(x)\beta} + 2e_0 m_0 e^{-2e_0\phi(x)\beta} = \\ = -2e_0 m_0\left[e^{+e_0\phi(x)\beta} - e^{-2e_0\phi(x)\beta}\right]. \tag{7}$$

The volume charge density of the positive charges of zwitterionic lipid head groups $\rho_{zw}(x)$ can be expressed as:[17]

$$\rho_{zw}(x) = \frac{e_0 P(x)}{Da_0}, \qquad 0 \le x \le D, \tag{8}$$

where P(x) is the probability density function for angle ω, D is the distance between charges in zwitterionic lipid head group (see Fig. 2) and a_0 is the area per lipid molecule. Inserting the Equation 7 and 8 into Equation 3 yields the Poisson equation in MLPB model:

$$\frac{d}{dx}\left[\varepsilon_0\varepsilon_r(x)\frac{d\phi(x)}{dx}\right] = \\ = 2e_0 m_0\left[e^{e_0\phi(x)\beta} - e^{-2e_0\phi(x)\beta}\right] - \frac{e_0 P(x)}{Da_0}, \tag{9}$$

where the last term in Equation 9 is different from zero only in the region $0 \le x \le D$ and the boundary conditions are:

$$\frac{d\phi}{dx}(x=0) = -\frac{\sigma_1}{\varepsilon_0\varepsilon_r(x=0)}\ , \tag{10}$$

$$\frac{d\phi}{dx}(x\to\infty) = 0, \tag{11}$$

$$\phi(x=D_-) = \phi(x=D_+), \tag{12}$$

$$\frac{d\phi}{dx}(x=D_-) = \frac{d\phi}{dx}(x=D_+). \tag{13}$$

The surface charge density σ_1 describes the negative surface charge density of the phosphate groups of lipid head groups at $x = 0$: $\sigma_1 = -e_0/a_0$ (see Fig. 2), where a_0 is the area per lipid molecule. Equation 9 was solved by using the standard implemented function for the multiboundary value problems (bvp4c) in Matlab2012b where the values $\varepsilon_r(x)$ and $P(x)$ were calculated in the iteration process outside of bvp4c function. The space dependent permittivity $\varepsilon_r(x)$ within MLPB model is[17,18]:

$$\varepsilon_r(x) = n^2 + \frac{n_{0w}p_0}{\varepsilon_0}\left(\frac{2+n^2}{3}\right)\frac{L(\gamma p_0 E(x)\beta)}{E(x)}, \tag{14}$$

where n is refractive index of water, n_{0w} is bulk concentration of water, p_0 is the dipole moment of water molecule, $L(u) = (\coth(u) - 1/u)$ is the Langevin function, $\gamma = (3/2)((2 + n^2)/3)$ and $E(x)$ is the magnitude (absolute value) of the electric field strength, while the probability density function $P(x)$ (taking into account the finite volumes of lipid head groups) is:[17]

$$P(x) = \Lambda\frac{\alpha e^{-e_0\phi(x)\beta}}{\alpha e^{-e_0\phi(x)\beta} - 1}, \qquad 0 \le x \le D, \tag{15}$$

where α is a model parameter (see also ref. 17) and the value of Λ is calculated in iterative procedure until the normalization condition is met:

$$\frac{1}{D}\int_0^D P(x)dx = 1. \tag{16}$$

2. 3. 2. Positively Charged Nanoparticles Added to Salt Solution

In the case when in the system are present also positively charged NPs (see Fig. 5) the boundary condition described by Equation 11 is replaced by equation:

$$\frac{d\phi}{dx}(x = D_{np}) = -\frac{\sigma_2}{\varepsilon_0 \varepsilon_r (x = D_{np})}, \tag{17}$$

where σ_2 is the surface charge density of nanoparticle located at the distance $x = D_{np}$ from the negatively charged plane in the lipid head group region (see Fig. 5).

2. 3. 3. Osmotic Pressure

Osmotic pressure between the zwitterionic lipid bilayer and nanoparticle can be derived by using the procedure described elsewhere[19] by integrating the Poisson equation (Equation 9 in our case) and subtracting the corresponding bulk osmotic pressure value between the lipid surface and nanoparticle surface located at $x = D_{np}$ (see Fig. 5). To this end, Equation 9 is first rewritten in the form:

$$-\frac{d}{dx}\left[\varepsilon_0 n^2 \frac{d\phi}{dx}\right] - n_{0w} p_0 \left(\frac{2+n^2}{3}\right)\frac{d}{dx} L(\gamma p_0 E(x)\beta) + \\ + 2e_0 m_0 \left(e^{e_0\phi(x)\beta} - e^{-2e_0\phi(x)\beta}\right) - \frac{e_0 P(x)}{Da_0} = 0, \tag{18}$$

where Equation 14 was taken into account. Multiplying equation 18 by $d\phi = \phi' dx$ followed by integration leads to:

$$-\frac{1}{2}\varepsilon_0 n^2 E(x)^2 - n_{0w} p_0 \left(\frac{2+n^2}{3}\right) L(\gamma p_0 E(x)\beta) E(x) + \\ + \left(\frac{2+n^2}{3}\right)\frac{n_{0w}}{\gamma\beta} \ln\left[\frac{\sinh(\gamma p_0 E(x)\beta)}{\gamma p_0 E(x)\beta}\right] + \\ + \frac{m_0}{\beta}\left(2e^{e_0\phi(x)\beta} + e^{-2e_0\phi(x)\beta}\right) = const.\ , \tag{19}$$

where the integration of the last term in Equation 18 (with non-zero value only in the region $0 \le x \le D$) was omitted since the osmotic pressure was always calculated outside the lipid head group region in the region $x > D$. The osmotic pressure difference $\Pi = \Pi_{inner} - \Pi_{bulk}$ can be therefore written as:

$$\Pi = -\frac{1}{2}\varepsilon_0 n^2 E(x)^2 - E(x)\left(\frac{2+n^2}{3}\right) n_{0w} p_0 L(\gamma p_0 E(x)\beta) + \\ + \left(\frac{2+n^2}{3}\right)\frac{n_{0w}}{\gamma\beta} \ln\left(\frac{\sinh(\gamma p_0 E(x)\beta)}{\gamma p_0 E(x)\beta}\right) + \\ + \frac{m_0}{\beta}(2e^{e_0\phi(x)\beta} + e^{-2e_0\phi(x)\beta} - 3). \tag{20}$$

3. Results and Discussion

The calculated number density profile of divalent calcium counter-ions (m(x)) and monovalent chloride co-ions (n_-(x)) of the salt solution as a function of the distance x from the negatively charged surface at $x = 0$ for two different bulk concentrations of divalent counter-ions can be seen in Fig. 3. Within the head group region the concentration of counter-ions decreases and the con-

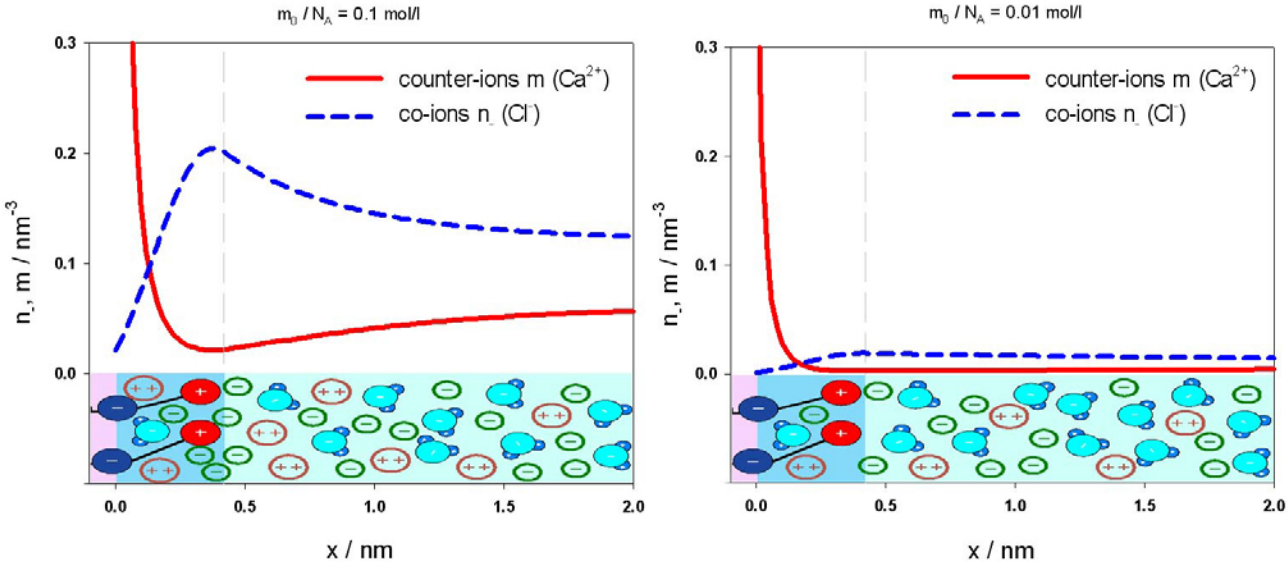

Figure 3. Number density profile of divalent counter-ions (full red line) and monovalent co-ions (dashed blue line) of the salt solution as a function of the distance x from negatively charged surface at $x = 0$ with two different bulk concentration of divalent calcium counter-ions m_0/N_A (0.1 mol/l – left panel and 0.01 mol/l – right panel). Other MLPB model parameters are: the dipole moment of water $p_0 = 3.1$ Debye, $D = 0.42$ nm, T = 298 K, $\sigma_1 = -e_0/a_0$, $a_0 = 0.60$ nm^2, $\alpha = 0.5$, bulk concentration of water $n_{0w}/N_A = 55$ mol/l, where N_A is Avogadro number.

centration of co-ions increases towards the positive charge of the lipid head groups. Near the negatively charged region of the head groups at $x = 0$ there is high accumulation of divalent counter-ions (Ca^{2+}) and the lack of monovalent co-ions (Cl^-). Far away from the zwitterionic lipid surface, concentration of co-ions is two times higher than the concentration of divalent counter-ions in accordance with the electro neutrality condition in the bulk solution (Fig.3).

The lipid-order parameter of POPC SUVs without NPs (Fig. 4A) and SUVs with adhered Fe_2O_3- NH_2 NPs (Fig. 4B) was measured for two different concentrations of Ca^{2+}. In comparison with the control, the lipid-order parameter values were highest after the treatment with $CaCl_2$ (with or without NPs). The initial order parameter values for control SUVs without NPs and SUVs with adhered positively charged Fe_2O_3-NH_2 NPs are nearly the same. Our results indicate that Ca^{2+} played a predominant role in altering the membrane fluidity. As the temperature increased, the order parameter values decreased in all the cases.

The presented experimental results can be interpreted by the calculated (relative) osmotic pressure between the zwitterionic lipid bilayer and the positively charged NPs presented in Fig. 5. At larger distances between the bilayer and the positive nanoparticle the osmotic pressure is practically equal to zero. Osmotic pressure increases with decreasing the distance between the bilayer and positive nanoparticle and practically does not depend on the bulk number density of calcium (m_0). The repulsion between the zwitterionic lipid bilayer and positively charged NPs prevent the positive NPs to interact strongly with the zwitterionic lipid bilayer. As a consequence also the membrane order parameter and fluidity are not expected to be changed by the positively charged NPs. Accordingly, the difference in the measured order parameter values is almost negligible for the control SUVs and Fe_2O_3-NH_2 NPs loaded SUVs (Fig. 4).

In SUVs treated with 10 mM and 100 mM $CaCl_2$ the order parameter is substantially increased, i.e. the fluidity is decreased. This result indicates that the accumulation of divalent calcium in the phosphate group region of the lipid head groups (Fig. 4) strongly influence the membrane order parameter and membrane fluidity. Our theoretical results presented in Fig. 3 also coincides with the results from the literature indicating that the positively charged Ca^{2+} has a strong affinity to accumulate in the region of negatively charged phosphate groups in the head region of the zwitterionic phospholipids, which may influence the fusion of the adjacent vesicles.[24–26] The accumulation of Ca^{2+} in the region of phosphate groups may reduce the repulsion between the neighbouring lipid molecules in the lipid bilayer and in this way reduce the area per lipid molecule and therefore also the membrane fluidity. Boss et al.[27] reported that addition of $CaCl_2$ to the carrot protoplast cell cultures reduced the membrane fluidity and high calcium concentrations dramatically altered the membrane structure and induced phase transition of the membrane lipids. We hypothesize that the accumulation of Ca^{2+} in the region of the negatively charged phosphate groups in the lipid head group region (Fig.3), the mobility of DPH and Fe_2O_3 NPs in the lipid bilayer is affected leading to alterations in the bilayer fluidity.

Ca^{2+} ions may play an important role also in the fusion of vesicle membranes, altering the shape of the vesicles and causes lipid mixing thereby disturbing the ordering of lipids in the membrane bilayer and the bilayer fluidity. The rotational mobility of DPH in the bila-

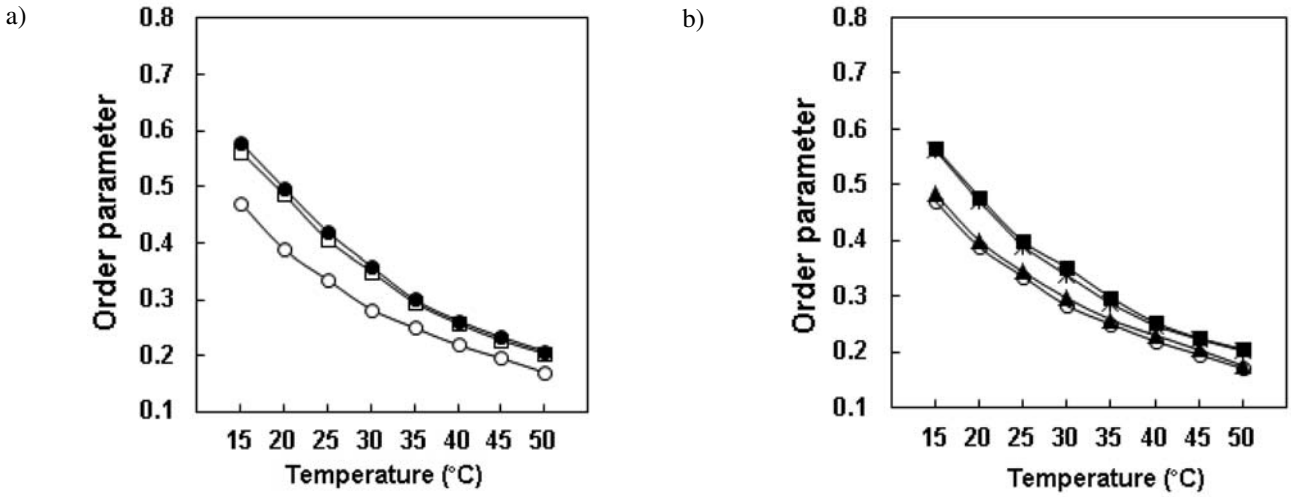

Figure 4. Effect of divalent calcium ions on the lipid-order parameter of zwitterionic POPC liposomes (A) SUVs without NPs (B) SUVs with adhered positively charged Fe_2O_3-NH_2 NPs (○ Control – SUVs without NPs; □ SUVs with 10 mM $CaCl_2$; ● SUVs with 100 mM $CaCl_2$; ▲ SUVs with adhered Fe_2O_3-NH_2 NPs; * NPs loaded SUVs treated with 10 mM $CaCl_2$; ■ NPs loaded SUVs treated with 100 mM $CaCl_2$). SUVs without NPs were incubated with 10 mM and 100 mM $CaCl_2$ for 30 min before measuring the anisotropy of DPH.

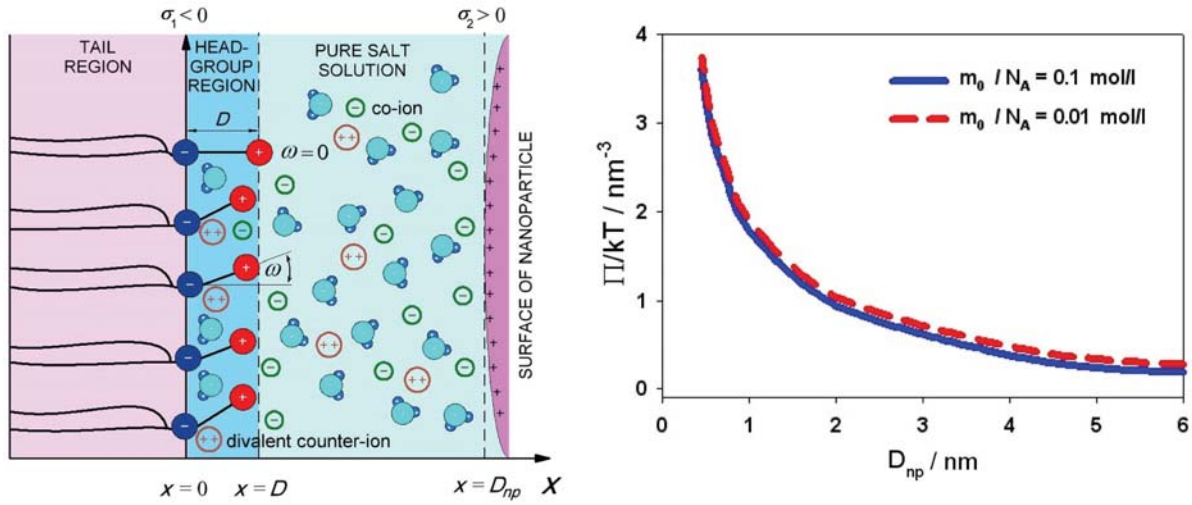

Figure 5: Left: Schematic presentation of the model for studying the interaction between zwitterionic (dipolar) lipid layer and positively charged NPs suspended in salt solution containing monovalent co-ions, and divalent counter-ions (e.g. calcium ions). D_{np} presents the distance of the nanoparticle from negatively charged surface at x = 0. **Right:** Osmotic pressure as a function of the distance (D_{np}) between negatively charged surface at $x = 0$ and positively charged NP ($\sigma_2 = -0.5\sigma_1$) calculated for two bulk concentration of divalent counter-ions (Ca^{2+}): $m_0/N_A = 0.1$ mol/l (full red line) and $m_0/N_A = 0.01$ mol/l (dashed blue line). Osmotic pressure was calculated using MLPB model. Parameter $\alpha = 1$, while all other parameters are the same as in Fig.3.

yer correlates with the ordering of lipids in the membrane. The mobility of the probe in the bilayer is affected by factors such as viscosity, temperature, size and shape of the vesicles. Taking into account of the experimental conditions, the results presented in Fig. 4 could be partially also interpreted on the basis of an increase in the size and shape of the liposomes due to fusion after incubation with Ca^{2+} which is a well-known fusogen. Mishra et al.[28] reported variation in the size and the shape of the zwitterionic phosphotidylcholine liposomal matrix due to the fusion induced by divalent cations such as Ca^{2+} and Mg^{2+}, reduced the membrane fluidity.

4. Conclusions

Our results provide information on the effect of Ca^{2+} on the bilayer fluidity of zwitterionic liposomes. The effect of Ca^{2+} on POPC SUVs adhered with and without Fe_2O_3 NPs bound with positively charged amino group on the membrane fluidity was investigated. The increase in the order parameter values of the SUVs after incubation with calcium ions can be explained by the accumulation of Ca^{2+} in the region of the negatively charged phosphate groups within the lipid head groups as shown in the presented theoretical consideration. Further studies on the influence of divalent cations on the negatively charged liposomes are needed in the future in order to gain more knowledge on the membrane electrostatic interactions, size and stability of the liposomes.

5. Acknowledgements

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Povzetek

Obravnavali smo interakcijo divalentnih kalcijevih ionov z lipidno dvojno plastjo majhnih liposomov, pripravljenih iz električno nevtralnih lipidov 1-palmitoil-2-oleoil-sn-glicero-3-fosfoholina, ki imajo zaradi notranje porazdelitve električnega naboja hidrofilne glave z od nič različnim električnim dipolnim momentom. Pokazali smo, da pri večjih koncentracijah kalcija naraste vrednost ureditvenega parametra membrane, fluidnost membrane liposomov pa se zmanjša. Prisotnost pozitivno naelektrenih nanodelcev železovega oksida v suspenziji liposomov zanemarljivo vpliva na rezultate meritev ureditvenega parametra in fluidnosti lipidne dvojne plasti membrane liposomov. Rezultate eksperimentov smo ovrednotili s pomočjo modificiranega Langevin-Poisson-Boltzmannovega modela električne dvojne plasti in prišli do zaključka, da je izmerjena variacija ureditvenega parametra in fluidnosti lipidne dvojne plasti liposomov predvsem posledica akumulacije kalcijevih ionov v področju negativno naelektrenih fosfatnih skupin v hidrofilnih glavah lipidnih molekul.