Adsorption (2010) 16: 305–312
DOI 10.1007/s10450-010-9242-1

Changes of zeta potential and particles size of silica caused by DPPC adsorption and enzyme phospholipase A_2 presence

E. Chibowski · A. Szcześ · L. Hołysz

Received: 16 March 2010 / Accepted: 20 April 2010 / Published online: 1 July 2010

Abstract The changes in electrokinetic properties of silica suspensions in the presence of 1,2-dipalmitoyl-*sn*-glycero-3-phospshocholine (DPPC) were investigated via zeta potential, mean diameter and transmittance determinations. Silica particles were precovered with monolayer (ML) or bilayer (BL) of the phospholopid from chloroform solution (SiO_2/DPPC) or covered by DPPC adsorption from aqueous solution (SiO_2 + DPPC). The zeta potential and mean diameter of SiO_2/DPPC suspension were measured as a function of NaCl concentration and due to the phospholipase A_2(PLA_2) action in 10^{-3} M NaCl solution and buffer Tris at pH = 8 and 9. It was found that the DPPC adsorption onto silica surface decreases its the zeta potential, however the suspensions were stable during the experiment time, probably because of steric stabilization. During PLA_2 enzyme action the changes in zeta potential were observed, which were caused by the hydrolysis products, especially palmitic acid molecules, which also had influence on the stability of these systems.

Keywords DPPC · Silica · Phospholipase A_2 · Zeta potential · Transmittance · Particle size · Stability

1 Introduction

Phospholipids are major components of biological membranes. In aqueous media their molecules form self-assembled spherical vesicles—liposomes, which involve one or more phospholipids bilayers. Since many years, supported phospholipid bilayers (Langmuir-Blodgett films) as well as liposomes, are commonly used models for complex biological membranes study (Jones 1995; Nielsen et al. 1999; Nagle and Tristram-Nagle 2000; Mornet et al. 2005). Model bilayers systems allow learning about biological processes taking place in living organisms. Moreover, liposomes attract great attention as drug delivery systems and carriers for the delivery of genes to cells. However, their mechanical resistance is rather poor.

Recently different lipid/particles assemblies composed of the particles core surrounded by a lipid shell have attracted attention because of their biomedical and biotechnological applications, e.g. in biological sensors and materials science (Rapuano and Carmona-Ribeiro 1997, 2000; Moura and Carmona-Ribeiro 2005; Tero et al. 2004; Castellana and Cremer 2006). The kind and strength of interactions between membrane and solid surface are very important for stabilization of the supported phospholipid layers on solid the surface, but they are still not well understood. The most frequently used particles as the lipid supports are: silica, iron oxide, polyelectrolyte capsules and polymer microgels (Troutier and Ladavière 2007). Lipid-coated silica microspheres can be applied for detection of anti-phospholipid antibodies in human body, to determine the affinity between biologically active substances and biological membranes, as well as chromatography for the separation of proteins. Because lipid shell stability on silica particles is due to hydrogen bonds and/or electrostatic interaction between lipids molecules and the hydrophilic surface, lipids affinity to silica surface varies in accordance with the sequence: anionic lipids<neutral lipids<cationic lipids (Troutier and Ladavière 2007).

The phospholipid layers on a solid support can be modified by lipolytic enzymes, whose action causes changes in

E. Chibowski (✉) · A. Szcześ · L. Hołysz
Department of Physical Chemistry-Interfacial Phenomena, Faculty of Chemistry, Maria Curie-Skłodowska University, Maria Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland
e-mail: emil.chibowski@umcs.lublin.pl

their hydrophobicity/hydrophilicity (Chibowski et al. 2008; Jurak and Chibowski 2009). For example, phospholipase A_2 (PLA_2) being water-soluble enzyme selectively catalyzes the hydrolysis of *sn*-2 fatty acyl chain in different phospholipids yielding free fatty acids and lysophospholipids as the reaction products. The activity of PLA_2 is higher in the case of the ordered lamellar structures of lipid, i.e., vesicles, multilamellar dispersions, monolayers at the air-water interface and supported bilayers in comparison to molecularly dispersed phospholipids (Dennis 1983; Grandbois et al. 1998; Mouritsen et al. 2006; Nielsen et al. 1999; Wacklin et al. 2007). Obviously, the hydrolysis products affect the layers hydrophobicity.

The aim of this study was the investigations of DPPC adsorption on the electrokinetic properties of silica suspensions in the absence and presence of PLA_2 enzyme.

2 Experimental

2.1 Materials

DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phospshocholine) (semi-synthetic, 99%) was purchased from Sigma and was used without further purification. Phospholipase A_2 from hog pancreas (200 u/mg, Fluka) was used as received to modify the phospholipid film. Buffer Tris containing 10 mM trihydroxymethylaminomethane (Sigma-Aldrich, 99.8%), 5 mM $CaCl_2$(POCH SA) and 10 mM NaCl (POCH SA) adjusted by HCl solution to pH 8 or 9 was used as the medium for an enzyme reaction. The commercial silicic acid powder ($SiO_2 \cdot xH_2O$) was purchased from Riedel-de Haen AG. The specific surface area of the SiO_2 powder determined by BET thermal desorption of nitrogen was 40 m^2/g. Water used for preparation of the suspensions was from Milli-Q system (resistivity 18.2 MΩ cm).

2.2 Methods

2.2.1 Deposition of DPPC on silica powder

The powdered samples of SiO_2 of known specific surface area were precovered with calculated statistical mono- (ML) or bi- (BL) layer of DPPC. The calculated amounts of DPPC for coverages were obtained taking 55 $Å^2$/molecule of the phospholipid cross section. Just before the deposition, DPPC was dissolved in chloroform (5.5 or 11 mg/mL for ML or BL of DPPC, respectively) (Rey Gómez-Serranillos et al. 2004). Then, the obtained solutions were mixed with 50 mg of $SiO_2 \cdot xH_2O$ placed in the beaker and then put under high vacuum in a vacuum dryer for 24 hours, in order to remove the chloroform.

2.2.2 Preparation of suspensions

In the first series of experiments the suspensions were prepared by mixing with 150 cm^3 of 10^{-3} M NaCl solution: (i) 10 mg of bare silica, (ii) 10 mg of silica powder precovered with DPPC from chloroform solution (further denoted as: SiO_2/DPPC), (iii) 10 mg of bare silica powder and powder of DPPC in the amount corresponding to 1 or 2 statistical monolayers of phospholipid (further denoted as: SiO_2 + DPPC), and (iv) DPPC alone. Before the measurements, all suspensions were sonicated using a sonicator 3000 (Misonix) for 3 min. To avoid sample heating the ultrasonic head was operating through 2 s with following 4 s breaks at the maximum power of 3.5 W.

In the second series of experiments to the suspension containing 10 mg of SiO_2/DPPC (i.e. SiO_2 pre-covered with DPPC from chloroform solution) powder dispersed in 150 mL of 10^{-3} M NaCl or buffer (Tris, pH = 8 or 9) solutions, 5 mL of the enzyme phospholipase A_2 solution was added just before sonication. Concentration of the enzyme in aqueous solution equaled to 0.1 mg/1 cm^3.

2.2.3 Zeta potential and mean diameter measurements

The zeta potentials for all the suspensions were determined as a function of the time, after 5, 15, 30 and 60 min since the moment of the suspension preparation using a Zetasizer Nano ZS, Malvern. Because in the studied systems the κa value (where κ is the Debye-Hückel parameter and a is the particle radius) was always greater than 100, therefore Helmholtz-Smoluchowski equation was applied for the zeta potential calculation from the electrophoretic mobility data. The all experiments were replicated 3–5 times at 20 ± 1 °C and arithmetic mean values were calculated.

2.2.4 Transmission light measurements

The transmission light signal of the suspensions was determined with the help of an optical analyzer Turbiscan Lab (France), which scanned every 40 μm of the sample moving along the 55 mm cell height. The scans have been taken during 1 h every 1 min. From these scans the mean light transmissions were calculated and plotted versus the time.

3 Result and discussion

3.1 Zeta potential as a function of NaCl concentration

The zeta potentials of bare silica and silica precovered with statistical mono- and bilayer of DPPC ($SiO_2/DPPC_{ML}$ or $SiO_2/DPPC_{BL}$) as a function of NaCl concentration are presented in Fig. 1. The pH of the suspension was natural and

amounted 5.95, 6.5 and 6.3 for 10^{-3}, 10^{-2} and 10^{-1} M NaCl solutions, respectively. The values of zeta potential are arithmetic means of its measured values after 5, 15, 30 and 60 min since the suspension preparation. From the marked by vertical bars ranges of the zeta potential changes during 60 min can be seen that the zeta potentials of the all suspensions change only slightly. Generally, the zeta potentials are negative and their absolute values decrease with increasing NaCl concentration. The negative zeta potential of bare silica decreases from −27.5 to −17.3 mV with increasing concentration of the indifferent electrolyte from 1 to 100 mM. This is the result of compression of the double layer thickness with the ionic strength increase (Fuerstenau and Pradip 2005).

Figure 1 shows that the negative zeta potential of silica in NaCl solutions changes after coverage its surface with a statistical mono- or bilayer of DPPC. In both cases negative zeta potential of SiO_2 is reduced. The zeta potential of $SiO_2/DPPC_{ML}$ and $SiO_2/DPPC_{BL}$ particles decreases from −20 to −7.7 mV and from −13 to −6 mV, respectively, if the concentration of electrolyte increases from 10^{-3} to 10^{-1} M, while that of bare silica decreases from −27.5 to −17.3 mV. The interactions between silica and phospholipid can occur initiated by formation of hydrogen bonds and electrostatic interactions. The charge of DPPC molecule is concentrated in the hydrophilic head group of the phospholipid. The polar part of DPPC possesses two spatially separated and oppositely charged moieties: positive choline group $–N^{(+)}(CH_3)_3$ (2 nm thickness) and negatively charged $–OPO_3^-$ (1.8 nm) (Langer and Kubica 1999). The zwitterionic head group as a whole of phosphatidylcholine is uncharged in a wide range of pH (4–10) and ionic strengths (Jones 1995; Shapovalov 1998). On the other hand, the silica particles possess many –SiOH and $–SiO^-$ groups on their surface. Two-dimensional NMR data suggested strong hydrogen bonding of silanol groups with O–P group of the polar head group of DPPC bilayer, thus changing its permeability (Chunbo et al. 1995). Moreover, because DPPC molecule is a zwitterion, its positive charge belonging to the ammonium group is favorable for electrostatic interaction with negatively charged silica surface (Shapovalov 1998) and causes relatively small decrease in the negative value of the silica particle zeta potential, because of the presence of negative $-PO_3^-$ in the zwitterion.

It should be also stressed that Na^+ ions can interact with negative part of the DPPC polar head-group. From the molecular simulation conducted by Pandit et al. (2003) it results that roughly two phospholipid molecules are bound to one Na^+ cation and it loses much of its coordinating water molecules. Simultaneously, they found that Cl^- anions much less coordinate with positive $–N^{(+)}(CH_3)_3$ of the headgroup. The greatest changes in the zeta potential of the studied systems appear in the lowest (10^{-3} M) concentration of the electrolyte. In 10^{-2} and 10^{-1} M NaCl solutions the negative zeta potentials for both $SiO_2/DPPC_{ML}$ and $SiO_2/DPPC_{BL}$ suspensions are practically the same and smaller than those of bare silica surface, but also smaller than those in 10^{-3} M (Fig. 1). These results are similar to those obtained by Sabìn et al. (2006) for the liposomes in the presence of monovalent ions (Na^+ and K^+).

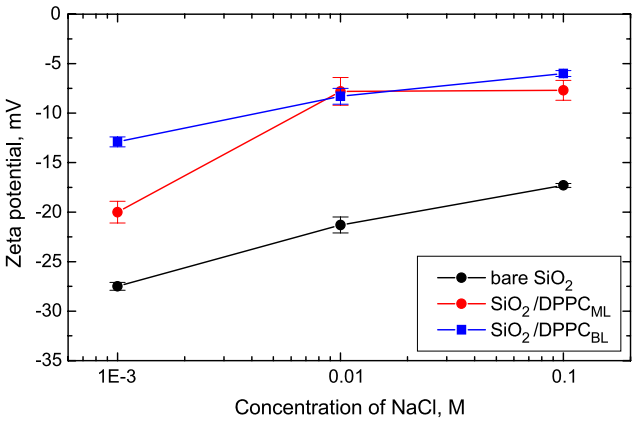

Fig. 1 Zeta potential changes of bare silica and precovered with mono- (ML) and bilayer (BL) of DPPC from chloroform solution as a function of NaCl concentration

Also from experimental studies conducted among others by Kotyńska and Figaszewski (2005) at natural pH resulted that Na^+ and Cl^- ions only slightly decreased negative surface charge density of phospholipid membrane at low concentration, however at higher concentrations the decrease was significant. Similar results were obtained also by Matsumura et al. (1995). Moreover, Kotyńska and Figaszewski (2005) observed that at pH > 6 in the presence of sodium chloride a decrease of negative charge occurred. In such conditions the degree of coverage of the membrane with Na^+ ions was evaluated by the authors to be over 0.8. Similar tendency was observed at pH < 4, when the membrane was covered by Cl^- ions. Thus the adsorption of Na^+ and Cl^- ions should be taken into account considering the changes of the zeta potential.

Moura and Carmona-Ribeiro (2005) basing on phosphatidylcholine (PC) adsorption isotherms of vesicles onto silica AEROSIL OX-50 and calculated affinity constant, stated that an increase in the solution ionic strength improved neutral lipid adsorption. However, because in aqueous systems the hydrophobic tails of DPPC molecules may tend to interact each other, it is hard to obtain uniform monolayer coverage of the supported pospholipid layer on the silica surface. Hence, probably the molecules form the bilayer patches, which nonuniformly cover the silica particles (Troutier and Ladavière 2007). Koenig et al. (1996) used the neutron reflectivity to study the structure of DPPC and DSPC bilayers adsorbed on a planar silicon surface from aqueous solution. The degree of surface coverage and the

size of the lipid domains were determined by atomic force microscopy. They found that onto hydrophilic surface of silicon, possessing thin SiO_2 layer, the lipids adsorb as a uniform layer, which was however interrupted by irregularly shaped uncovered patches of about 10–50 nm in size and the surface coverage was $70 \pm 20\%$.

It should be stressed that in our experiments the silica was first precovered with DPPC from chloroform, and after drying in vacuum it was dispersed in aqueous solution, what may caused reorientation of the phospholipid molecules on silica surface, as discussed above similar effect of DPPC ML and BL on silica zeta potential was observed in our previous paper (Jurak and Chibowski 2009), but the silica was subjected to cleaning procedure.

3.2 Zeta potential in 10^{-3} M NaCl

In Fig. 2 the zeta potential and mean diameter for two different silica particle suspensions and DPPC alone dispersion in 10^{-3} M NaCl solution are presented. The results show changes of these two parameters for silica/$DPPC_{ML}$ and silica/$DPPC_{BL}$ particles covered with phospholipid from chloroform solution and the changes for bare silica particles dispersed in 10^{-3} M NaCl where the same amount of DPPC (corresponding to 1 or 2 statistical monolayers) was added and denoted in the figure as SiO_2 + DPPC (ML) or (BL). Moreover, in Fig. 2 are plotted the potentials and particle size of the same amounts of DPPC dispersed alone in the NaCl solution without presence of SiO_2. The DPPC molecules dispersed in aqueous solution aggregate exposing the polar head groups toward water, while the hydrophobic tails are hidden (Deems 2000). It can be seen that the zeta potentials and mean diameters differ slightly if the two amounts of DPPC are dispersed in the aqueous solution. If the doubled amount is dispersed the negative zeta potential is by ca. 6 mV smaller (Fig. 2a) and the diameter is about twice greater (Fig. 2b). This can be due to formation of different DPPC aggregates possessing a bit smaller their surface charge density. But in both cases their size is significantly smaller than that of the silica particles.

As it is seen in Fig. 2 the presence of DPPC in the SiO_2 suspension affects the zeta potential and mean diameter of silica particles. If the phospholipid is added to the system simultaneously with silica, it influences the zeta potential of the SiO_2 particles which is only slightly lower than that of the particles precovered with DPPC from chloroform, especially in the case of the monolayer. Anyway in both cases of ML and BL the effect of dispersed DPPC in NaCl solution on the zeta potential of silica is clearly seen if compared to the reference system of bare silica (marked by horizontal arrow in Fig. 2a). This indicates the DPPC adsorption to the silica surface from the bulk of NaCl solution. However, no clear dependence between the particles sizes of SiO_2 in the

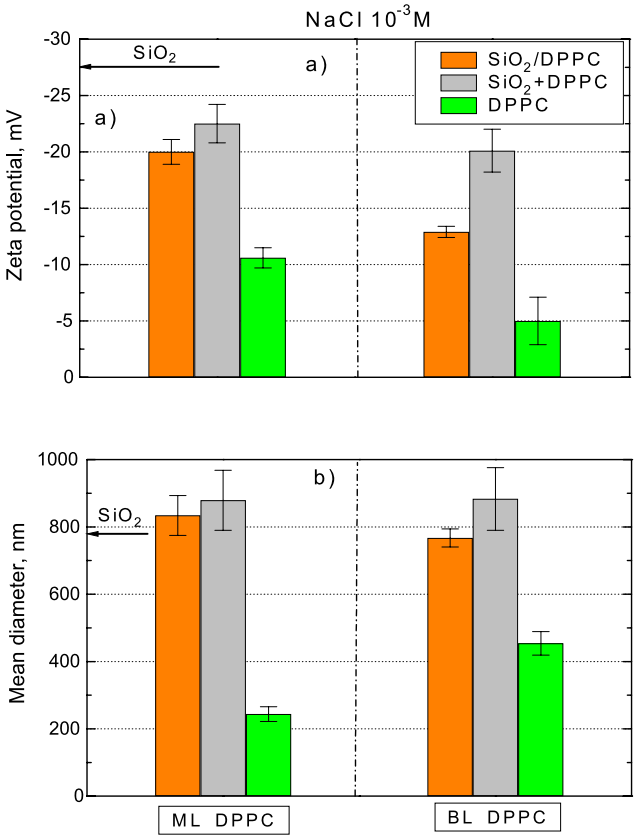

Fig. 2 Zeta potential (**a**) and mean diameter (**b**) changes of silica particles: covered with DPPC from chloroform solution (silica/DPPC) or by adsorption (SiO_2 + DPPC) and DPPC aggregates alone in 10^{-3} M NaCl

discussed three silica suspensions can be seen. Moreover, it seems, the size of silica particles (aggregates) is also independent on the amount of DPPC added. If silica was first precovered with DPPC from chloroform and then dispersed in NaCl solution, the differences in zeta potential values (Fig. 2a) indicate for indeed mono- and bilayer adsorption on the silica surface. However, after contact with NaCl solution some reorientation of the molecules could took place.

Also, electrostatic interactions in the pohospholipid bilayer play a role in the studied system (Makino et al. 1991; Satoh 1995; Matsumura et al. 1995; Imura et al. 2003). Matsumura et al. (1995) studied the electrostatic interaction between liposomes of different phospholipids in the presence of various salt solutions. The liposomes of each phospholipid had negative zeta potentials, as it also the case in Fig. 2a. Generally, the addition of electrolytes reduces the zeta potential of liposomes and the effect depends on the electrolyte concentrations and the ions valence. The presence of La^{+3} ions causes even reverse of the zeta potential sign for all phospholipid liposomes. At low concentration of NaCl, Na^{+} ions only slightly reduce the negative zeta potential of liposomes, however at higher concentrations the reduction is significant.

Satoh (1995) also measured the zeta potentials of DPPC liposomes in 5 mM Tris buffer (pH = 7.2) at different $CaCl_2$ and NaCl concentrations. In these systems the zeta potentials were positive at 10^{-4}–10^{-1} M $CaCl_2$ concentration. The presence of NaCl reduced the positive values of zeta potential by 10–20 mV, depending on its concentration. The author concluded that binding of not only Ca^{2+} ions but also Na^+ and Cl^- to the liposome surface should be taken into account, but the binding constants to the monolayer were different than for bilayer, and lower for the liquid crystalline than gel phases.

According to Makino et al. (1991) the polar head of phospholipid can reorient depending on the ionic strength, what reflects in the zeta potential, whose changes with the ionic strength can be interpreted via changes in the polar head group orientation. At a low ionic strength the choline groups are located below the phosphate group, whereas at high ionic strength the situation is reversed. According to these authors the zero zeta potential occurred if DPPC polar heads are oriented parallel to the liposome surface. However, it is hardly possibly to find a reason for the head groups orientation changes with changing the ionic strength.

In Fig. 3 are presented the mean diameters as a function of time for the mentioned earlier two different silica suspensions and DPPC aggregates alone, dispersed in 10^{-3} M NaCl solution. The mean diameter of bare SiO_2 particle aggregates slightly increases (from ca. 700–900 nm) during first 30 min since the suspension preparation (Fig. 3), which means that aggregation process occurs and then during next 30 min some sedimentation of the aggregates takes place and hence the mean diameter decreases. The zeta potential of these particles is −27.5 mV (Fig. 2a) and it is not sufficiently big to stabilize them (Heurtault et al. 2003). In the presence of $SiO_2/DPPC_{ML}$, $SiO_2/DPPC_{BL}$, and also SiO_2 + DPPC ML and BL dispersed in NaCl solution the mean diameters do not differ much from that of bare silica and the particles are relatively stable during 60 min despite decreased the zeta potentials (Fig. 2a). Comparing to bare silica aggregates, in the presence of DPPC bilayer (Fig. 3b) the suspensions are bit more stable than in the monolayer presence (Fig. 3a). Because the zeta potentials of these suspensions are smaller than of the bare one (Fig. 2a), this indicates for steric stabilization of the suspensions by DPPC adsorbed molecules. It is also worth to remark again that the dispersed DPPC in the electrolyte has to adsorb on the silica particles because the mean diameters do not differ much from those of the particle covered with DPPC from chloroform solution, but the diameters differ essentially from the DPPC aggregates diameter when SiO_2 was not present in the solution (Fig. 3).

Additional information can be obtained from the results get with the help of Turbiscan by measuring the transmitted light. As an example, in Fig. 4 is shown a print-out from the apparatus of the scans along 50 mm of the measuring cell containing $SiO_2/DPPC_{ML}$ in 10^{-3} M NaCl.

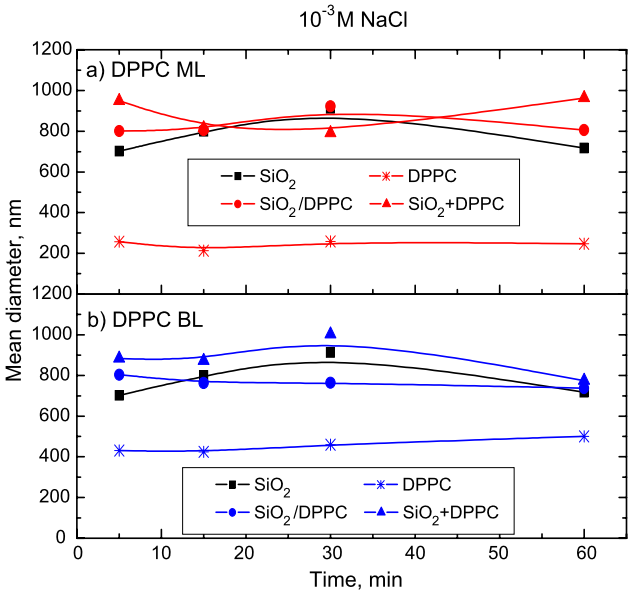

Fig. 3 Mean diameter changes vs. time of bare silica and silica particles: covered with DPPC from chloroform solution (silica/DPPC) or by adsorption (SiO_2 + DPPC) and DPPC aggregates in 10^{-3} M NaCl. The DPPC amount corresponded to: ML coverage (**a**) or BL coverage (**b**). The standard deviations of all the determination lie between 30–200 nm

The scans show that this suspension is quite stable during 60 min of the experiment, because the scans are tightly "packed" forming a thick solid line, which actually consists of 21 individual scans. In fact during 60 min 60 scans have been taken and in the figure only every other scan is printed. Such experiments were conducted for the all investigated systems. Then, from these scans the mean light transmittances were calculated and plotted versus the time. They are shown in Fig. 5, where also the transmittances for DPPC aggregates alone are also plotted.

It is clearly seen that the transmittance of the DPPC aggregates are much greater that those of silica suspensions. This is because of the smallest size of these aggregates (see Figs. 2 and 3), and they are the most stable among the systems presented. Although there are visible differences in the transmittance of the silica suspensions covered with DPPC mono- and bilayer, as well as those in the solution in which DPPC was dispersed, but there is no straight correlation with the diameters changes of these suspensions (Fig. 3). Nevertheless, relatively small changes in the transmittances also point to stability of these systems and the mean diameters changes did.

3.3 Zeta potential in the presence of PLA_2

Zeta potentials changes of silica precovered with DPPC and dispersed in NaCl solution in the presence of the enzyme phospholipase A_2 are depicted in Fig. 6 both for silica particles precovered with ML and BL of DPPC. The enzyme

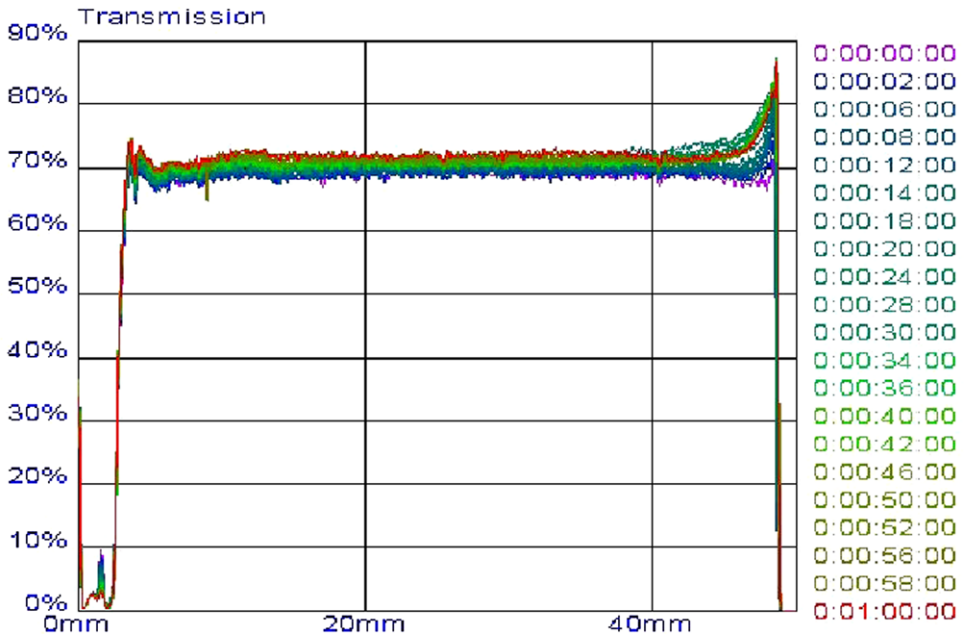

Fig. 4 The light transmittance trough $SiO_2/DPPC_{ML}$ suspension in 10^{-3} M NaCl along the suspension column in the measuring cell

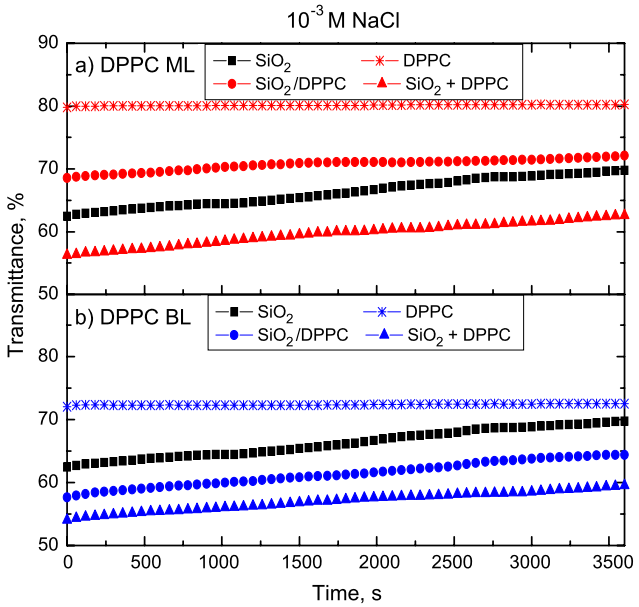

Fig. 5 The mean transmittance changes vs. time through the silica suspensions shown in Fig. 4

causes decrease of the negative zeta potential in comparison to the systems without PLA_2. Only small fluctuations of the electrokinetic potential during 60 min of the measurements indicate that most of the enzyme action takes place during the time of the suspension sonication. Moreover, after 60 min zeta potential of both measured systems, i.e. ML and BL coverage, tends to the same value, c.a. −12 mV. Phospholipase A_2 is an interfacially activated enzyme that catalyzes stereospecific hydrolysis of *sn*-2 acyl ester linkages of *sn*-3 glycerophospholipids producing fatty palmitic acid and lysophospholipid products. The smaller negative zeta potential after the enzyme treatment indicates that the resulting on the silica surface charge is less negative than it was with adsorbed DPPC. But, it should be noticed that the decrease in the negative zeta potential amounts only 5–10 mV (Fig. 6). The changes should result from the presence of the hydrolysis products as well as adsorption of PLA_2 molecules. In 10^{-3} M NaCl at natural pH = 5.95 palmitic acid appears as a product of the hydrolysis which is mostly undissociated and water insoluble. Simultaneously the lysophosphatidylcholine is also released which possesses the same choline group and only one the hydrocarbon chain. Maybe this group now causes a bit less negative zeta potential of the silica particles in comparison to that of $SiO_2/DPPC$ particles (Fig. 6). However, this is only speculation and more experiments are needed.

The zeta potentials in Tris buffer were measured at pH = 8 (Fig. 7) and pH = 9 (Fig. 8). We were interested to learn about effectiveness of PLA_2 enzyme at these two pH values, and to compare them with the results obtained in NaCl solution alone at pH = 6.3.

It appeared that in Tris at pH = 8 the zeta potential of $SiO_2/DPPC_{ML}$ particles was close to zero (∼2–3 mV) and the particles covered with DPPC (BL) possess positive zeta potential, ∼ +7 mV (Fig. 7). Moreover at pH = 9, both the particles covered with DPPC mono- and bilayer show positive zeta potential (Fig. 8). One would expect that with increasing pH the zeta potential of $SiO_2/DPPC$ should become more negative, while the changes appeared to be reversed. Such behavior can be explained taking into account possible adsorption Ca^{2+} cations (5 mM present in Tris buffer) between DPPC molecules which increases with the

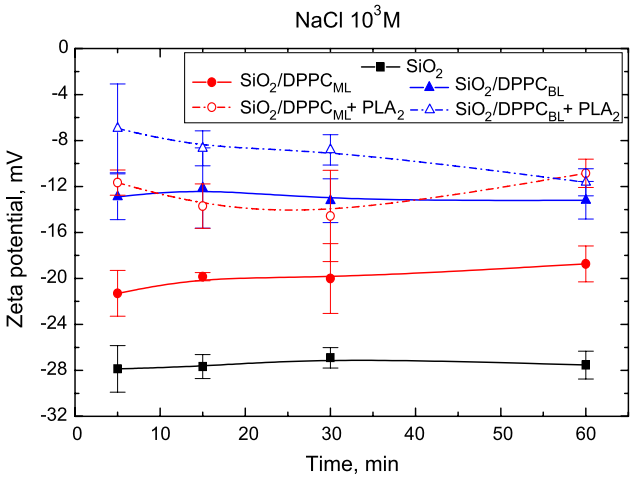

Fig. 6 Zeta potential changes vs. time of bare silica, $SiO_2/DPPC_{ML}$ and $SiO_2/DPPC_{BL}$ dispersed in 10^{-3} M NaCl due to enzyme PLA_2 action

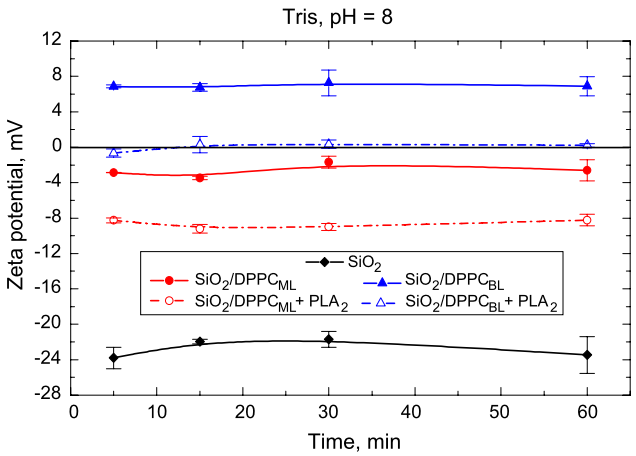

Fig. 7 Zeta potential changes vs. time of bare silica, $SiO_2/DPPC_{ML}$ and $SiO_2/DPPC_{BL}$ dispersed in Tris at pH = 8 due to enzyme PLA_2 action

pH increase, as well as to some extent adsorption of positively charged groups of trihydroxymethylaminomethane, because pK_a of the amine equals to 8.1 (Rapuano and Carmona-Ribeiro 2000). At the higher pH = 9 rather Ca^{2+} adsorption determines the observed positive zeta potential of the DPPC layers (Fig. 8).

Moreover, the effect of PLA_2 on the zeta potential of SiO_2/DPPC particles is opposite to that observed in NaCl solution (Fig. 6), where the enzyme caused the decrease of the negative zeta potential values. In the buffer at both pH values the enzyme causes shift of the values to less positive or even negative ones (Figs. 7 and 8). The same trend of the zeta potential changes was observed in our previous paper although the negative zeta potential values were greater (Jurak and Chibowski 2009). These results mean that the hydrolysis products possessing negative charge neutralize,

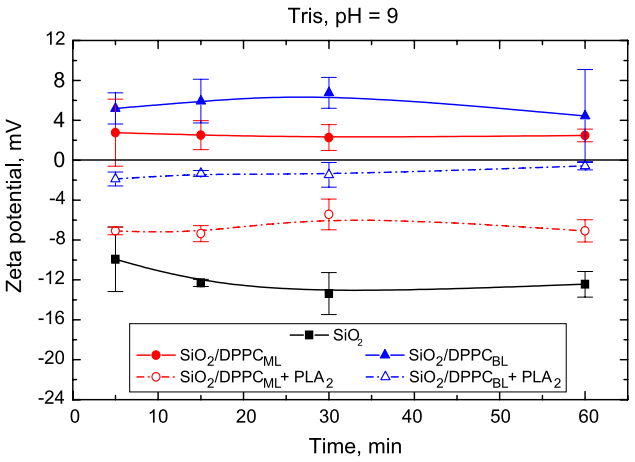

Fig. 8 Zeta potential changes vs. time of bare silica, $SiO_2/DPPC_{ML}$ and $SiO_2/DPPC_{BL}$ dispersed in Tris at pH = 9 due to enzyme PLA_2 action

at least partially, the positive charge present on the phospholipid surface. Also OH^- ions may play a role here in the zeta potential shift toward negative values. It is worth to note again that the zeta potential values of the SiO_2/DPPC particles, both without and under the enzyme action (Figs. 7 and 8), establish already during the suspension sonication.

4 Conclusions

The zeta potentials of bare silica and precovered with statistical mono- and bilayer of DPPC as a function of NaCl concentration were investigated. The negative zeta potential of silica in NaCl solution is reduced after covering its surface with phospholipid and decreases with increasing NaCl concentration as a result of compression of the double layer thickness. The greatest changes in the zeta potential of studied suspensions appear in the lowest concentration of the electrolyte. The presence of DPPC in the SiO_2 suspensions in 10^{-3} M NaCl affects the zeta potential and mean diameter of silica aggregates. If the phospholipid is added to the system simultaneously with silica, it influences the zeta potential of the SiO_2 particles which is only slightly lower than that of the particles covered with DPPC from chloroform solution. This indicates that the DPPC adsorption to the silica surface from the bulk of NaCl solution occurs. In the case when silica was precovered with DPPC from chloroform and then dispersed in NaCl solution some reorientation of the phospholipids molecules could took place. DPPC molecules deposited on SiO_2 particles as well as dispersed in the solution despite decreased zeta potentials stabilize the suspensions, probably because of steric stabilization. The mean diameter of the covered particles does not differ much from that of bare silica and the particles are relatively stable like

the transmittances vs. time. In the presence of PLA_2 the negative zeta potential of silica procovered with DPPC and dispersed in NaCl decreases. Small its variations during first 30 min indicate that most of the enzyme action takes place during the time of the suspension sonication. The observed changes should be caused by the hydrolysis products, as well as adsorption of PLA_2. The effect of enzyme action on the zeta potential of SiO_2/DPPC suspension in buffer Tris is opposite to that obtained in NaCl solution. In the buffer at both pH values the enzyme causes shift of the values to less positive or even negative ones. It may be conducted that adsorption of Tris amine on the silica particles plays here some role too.

Acknowledgements This work has been supported by polish Ministry of science and Higher Education, Project No. Project N204 128 32/3188, which we appreciate very much.

References

Castellana, E.T., Cremer, P.S.: Solid supported lipid bilayers: From biophysical studies to sensor design. Surf. Sci. Rep. **61**, 429–444 (2006)

Deems, R.A.: Interfacial enzyme kinetics at the phospholipids/water interface: practical considerations. Anal. Biochem. **287**, 1–16 (2000)

Dennis, E.A.: Phospholipases. In: Dennis, E.A. (ed.) The Enzymes, pp. 307–136. Academic Press, New York (1983)

Chibowski, E., Hołysz, L., Jurak, M.: Effect of a lypolytic enzyme on wettability and topography of phospholipid layers deposited on solid support. Colloids Surf. A **321**, 131–136 (2008)

Chunbo, Y., Daqing, Z., Aizhuo, L., Jiazuan, N.: A NMR study of the interaction of silica with dipalmitoylphosphatidylcholine liposomes. J. Colloid Interface Sci. **172**, 536–538 (1995)

Fuerstenau, D.W., Pradip: Zeta potentials in the flotation of oxide and silicate minerals. Adv. Colloid Interface Sci. **114**, 9–26 (2005)

Grandbois, M., Clause-Schaumann, H., Gaub, H.: Atomic force microscope imaging of phospholipid bilayer degradation by phospholipase A_2. Biophys. J. **74**, 2398–2404 (1998)

Heurtault, B., Saulnier, P., Pech, B., Proust, J.-E., Benoit, J.-P.: Physico-chemical stability of colloidal lipid particles. Biomaterials **24**, 4283–4300 (2003)

Imura, T., Ynagishita, H., Ikegami, T., Negishi, H., Kitamoto, D.: Drastic improvements in trapping efficiency and dispersibility for phosphatidylcholine liposomes in the presence of divalent metal ions. J. Oleo Sci. **52**, 673–679 (2003)

Jones, M.N.: The surface properties of phospholipid liposome systems and their characterization. Adv. Colloid Interface Sci. **54**, 93–128 (1995)

Jurak, M., Chibowski, E.: Zeta potential and surface free energy changes of solid + supported phospholipids (DPPC) layers caused by the enzyme phospholipase A_2 (PLA_2). Adsorption **15**, 211–219 (2009)

Koenig, B.W., Krueger, S., Orts, E.J., Majkrzak, C.F., Berk, N.F., Silverton, J.V., Gawrisch, K.: Neutron reflectivity and atomic force microscopy studies of a lipid bilayer in water adsorbed to the surface of a silicon single crystal. Langmuir **12**, 1343–1350 (1996)

Kotyńska, J., Figaszewski, Z.A.: Adsorption equilibria between liposome membrane formed of phosphatidylcholine and aqueous sodium chloride solution as a function of pH. Biochim. Biophys. Acta **1720**, 22–27 (2005)

Langer, M., Kubica, K.: The electrostatics of lipid surfaces. Chem. Phys. Lipids **101**, 3–35 (1999)

Makino, K., Yamada, T., Kimura, M., Oka, T., Oshima, H.: Temperature- and ionic strength-induced conformational changes in the lipid head group region of liposomes as suggested by zeta potential data. Biophys. Chem. **41**, 175–183 (1991)

Matsumura, H., Watanable, K., Furusawa, K.: Flocculation behaviour of egg phosphatidylcholine liposomes caused by Ca^{2+} ions. Colloids Surf. A **98**, 175–184 (1995)

Mornet, S., Lambert, O., Duguet, E., Brisson, A.: The formation of supported lipid bilayers on silica nanoparticles revealed by cryoelectron microscopy. Nano Lett. **5**, 281–285 (2005)

Moura, S.P., Carmona-Ribeiro, A.M.: Biomimetic particles: Optimization of phospholipid bilayer coverage on silica and colloid stabilization. Langmuir **21**, 10160–10164 (2005)

Mouritsen, O.G., Andersen, T.L., Halperin, A., Hansen, P.L., Jakobsen, A.F., Jensen, U.B., Jensen, M.Ø., Jørgensen, K., Kaasgaard, T., Leidy, C., Simonsen, A.C., Peters, G.H., Weiss, M.: Activation of interfacial enzymes at membranes surfaces. J. Phys., Condens. Matter **18**, 1293–1304 (2006)

Nagle, S.F., Tristram-Nagle, S.: Structure of lipid bilayers. Biochim. Biophys. Acta **1469**, 159–195 (2000)

Nielsen, L.K., Risbo, J., Callisen, T.H., Bjørnholm, T.: Lag-burst kinetics in phospholipase A_2 hydrolysis of DPPC bilayers visualized by atomic force microscopy. Biochim. Biophys. Acta **1420**, 266–271 (1999)

Pandit, S.A., Bostick, D., Berkowitz, M.L.: Molecular dynamic simulation of a dipalmitoylphosphatidylcholine bilayer with NaCl. Biophys. J. **84**, 3743–3750 (2003)

Rapuano, R., Carmona-Ribeiro, A.M.: Physical adsorption of bilayer membranes on silica. J. Colloid Interface Sci. **193**, 104–111 (1997)

Rapuano, R., Carmona-Ribeiro, A.M.: Supported bilayers on silica. J. Colloid Interface Sci. **226**, 299–307 (2000)

Rey Gómez-Serranillos, I., Miñones, J., Dynarowicz-Łątka, P., Iribarnegaray, F., Casa, M.: Study of the π-A isotherms of miltefosine monolayers spread at the air/water interface. Phys. Chem. Chem. Phys. **6**, 1580–1586 (2004)

Sabìn, J., Prieto, G., Ruso, J.M., Hidalgo-Àlvarez, R., Sarmiento, F.: Size and stability of liposomes: A possible role of hydration and osmotic forces. Eur. Phys. J. E **20**, 401–408 (2006)

Satoh, K.: Determination of binding constants Ca^{2+}, Na^{+}, and Cl^{-} ions to liposomal membranes of dipalmitoylphosphatidylcholine at gel phase by particle electrophoresis. Biochim. Biophys. Acta **1239**, 239–248 (1995)

Shapovalov, V.L.: Interaction of DPPC monolayer at air–water interface with hydrophobic ions. Thin Solid Films **327–329**, 599–602 (1998)

Tero, R., Takizawa, M., Li, Y.J., Yamazaki, M., Urisu, T.: Deposition of phospholipid layers on SiO_2 surface modified by akyl-SAM islands. Appl. Surf. Sci. **238**, 218–222 (2004)

Troutier, A.-L., Ladavière, C.: An overview of lipid membrane supported by colloidal particles. Adv. Colloid Interface Sci. **133**, 1–27 (2007)

Wacklin, H.P., Tiberg, F., Fragneto, G., Thomas, R.K.: Distribution of reaction products in phospholipase A_2 hydrolysis. Biochim. Biophys. Acta **1768**, 1036–1049 (2007)