

# Electrostatic interactions between polyelectrolyte and amphiphiles in two- and three-dimensional systems

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## Abstract

In this review we summarize some experimental investigations on the interactions between charged amphiphilic molecules and oppositely charged polyelectrolytes. We first present results on the formation, in bulk solutions, of polyelectrolyte/surfactant complexes of micrometric size. We show how the sample preparation procedure may affect the morphology of the complexes formed and how the kinetics influences the dynamics of their formation. The second and third parts are devoted to two-dimensional systems, namely a lipid bilayer attached to a polyelectrolyte multilayer cushion, on the one hand, and a lipid monolayer at the interface between air and a polyelectrolyte solution, on the other hand. We first demonstrate that, for lipids coupled to polyelectrolyte multilayers, the dynamics of the lipid is completely dominated by the electrostatic interactions with the polymer cushion. We finally investigate, by a combination of several techniques, how the interactions with a polyelectrolyte modify the properties of lipid monolayers and how ordering in an adsorption layer appears.

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## 1. Introduction

The interactions between surfactants and polymers in bulk solution have received great attention due to their numerous applications from the daily life to the various industries (e.g. pharmaceutical, biomedical application, detergency, enhanced oil recovery, paints, food and mineral processing) [1–3]. Charged amphiphilic molecules, like lipids or surfactants, and oppositely charged polyelectrolytes spontaneously form stable complexes. Such complexes are of special interest because of their great vari-

ability in structures and properties. The control of such synthetic supramolecular structures by tuning the different interactions that are responsible for the ordering opens fascinating possibilities in the manipulation of material properties on a molecular scale. This is especially important for the application of multifunctional devices. The combination of amphiphiles bearing characteristic properties in thin films and polymers with their mechanical properties is a general principle for the fabrication of new materials [4–14]. A full understanding of the mechanisms at play is however required, if we wish to take full advantage of the richness of behavior and create a large variety of new and functional materials.

This review gathers together experimental investigations on the electrostatic interactions of polyelectrolyte with mono- or bilayers of oppositely charged amphiphilic molecules, in three- and two-dimensional systems. Although very restricted compared to the wealth of experimental and theoretical studies devoted to electrostatic interactions between polyelectrolyte and charged amphiphiles, the experimental work summarized in this

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paper aims at a better characterization, and in turn a better control, of the structural and dynamical properties of composite lipid/polyelectrolyte soft materials.

In the first part of the manuscript, we present some results on the formation of polyelectrolyte/surfactant complexes in bulk solutions. We show how the sample preparation procedure may affect the morphology of the complexes formed and how the kinetics influences the dynamics for the formation of complexes. The second and third parts are devoted to two-dimensional systems, namely a lipid bilayer attached to a polyelectrolyte multilayer cushion, on the one hand, and a lipid monolayer at the interface between air and a polyelectrolyte solution, on the other hand. We first show that, for lipids coupled to polyelectrolyte multilayers, the dynamics of the lipid is completely dominated by the electrostatic interactions with the polymer cushion. We finally investigate, by a combination of several techniques, how the interactions with a polyelectrolyte modify the phase behavior of lipid monolayers.

## 2. Electrostatic interactions between multilamellar vesicles of charged surfactant and oppositely charged polyelectrolytes

The interactions between polyelectrolytes and oppositely charged surfactants can induce, in bulk solution, the formation of surfactant/polyelectrolyte complexes, which often exhibit a long-range order reminiscent of the structures classically found in surfactant/water solution (lamellar, hexagonal or cubic phases). These complexes are potentially attractive for applications in polymer and biomedical science [15–21]. However, the mechanism and kinetics underlying the process for the formation of this class of complexes is rather complicated and much remains unknown at present. We studied the formation of polyelectrolyte/surfactant complexes taking place in three-dimensional samples, between cationic uni- and multilamellar vesicles and anionic polyelectrolytes, by optical microscopy and small-angle X-ray scattering. A double-tail surfactant, didodecyldimethyl ammonium bromide, DDAB,  $(C_{12}H_{25})_2N^+(CH_3)_2Br^-$ , as a cationic surfactant, and an alternating copolymer of styrene and maleic acid in its sodium salt form,  $-CH_2CH(C_6H_5)CH(CO_2^-Na^+)CH(CO_2^-Na^+)-$  as a flexible anionic polyelectrolyte, were employed. The polyelectrolyte and surfactant were purchased from Aldrich and used as received. The molar weight of the polyelectrolyte was 120 000 g/mol, which corresponds on average to 454 monomers per molecule. The typical surfactant concentrations used ranged between 0.1 and 1% in weight. These concentrations are larger than the critical micelle concentration of DDAB and lower than the concentration above which a homogeneous lamellar phase is obtained [22]. In this range of concentration, multilamellar vesicles formed spontaneously in solution [22], that can be directly observed by light microscopy.

Our results showed that the preparation procedure and the kinetics had a significant effect on the morphology and microstructure, on the resulting surfactant/polyelectrolyte complexes, and on the dynamics of their formation.

### 2.1. Morphology and microstructure of surfactant/polyelectrolyte complexes formed with different preparation procedures

The complexes formed by the double-tail cationic surfactant and the anionic polyelectrolyte were investigated, when the charge ratio between the polyelectrolyte negative charges and the surfactant positive charges was varied. The morphology and microstructure of the complexes were studied by light microscopy and small-angle X-ray scattering for different preparation conditions [23]. In the experiments, the polyelectrolyte solution was put in a vial, and then a suitable amount of surfactant solution was gently incorporated above the polyelectrolyte solution. A sharp and white interface formed instantly between the surfactant and polyelectrolyte solutions, showing a rapid and spontaneous formation of complexes of micrometric size. In the first procedure, we mixed the above sample by shaking the vial. A turbid solution, containing micrometric surfactant/polyelectrolyte complexes, was thus obtained, which did not evolve with time. In the other procedure, the vial with the initial sharp interface was kept undisturbed. With time, the interface gradually went up. After equilibrium, the complexes were concentrated in a thin layer at the top of the vial. The microscopic structure and the morphology of complexes formed using these two procedures were investigated. Independently on the sample preparation procedure and on the charge ratio, X-ray results showed that the microscopic structure of the complexes was a condensed lamellar phase. The condensed phase results from the bridging of the surfactant bilayers by the polyelectrolyte, due to the strong electrostatic attractive interaction between the head group of the surfactant molecules and the charged backbone of the polyelectrolyte [7–13,24,25]. Surprisingly, the morphology of the complexes changed dramatically with the preparation procedure. The complexes formed by mixing a surfactant solution and a polyelectrolyte solution strongly depend on the charge ratio and are always extremely heterogeneous in size and shape (as shown in Ref. [26]). By contrast, when the two solutions were let inter-diffuse slowly, monodisperse spherical complexes of micrometric size were systematically obtained, independently of the initial relative amount of surfactant and polyelectrolyte (Fig. 1). When observed between crossed polarizers, these complexes exhibited a well-defined spherulite texture with a distinct Maltese cross, which indicated the formation of a concentric and regular lamellar structure on a micrometer scale (right hand side of Fig. 1). These complexes were very stable when diluted in pure water or in positively charged surfactant solution, while they were deformed, and eventually dissolved, in presence of negative surfactant or polyelectrolyte molecules. Our whole set of light microscopy observations and the X-ray scattering results suggested that these complexes were positively charged, and hence selected spontaneously a given local charge ratio, independently of the global charge ratio of the initial mixtures. Observation of the samples in the time course of the formation of the complexes suggested a slow and gradual growth of the soft colloids, which was presumably a key parameter for regular and uniform complexes. Very generally,

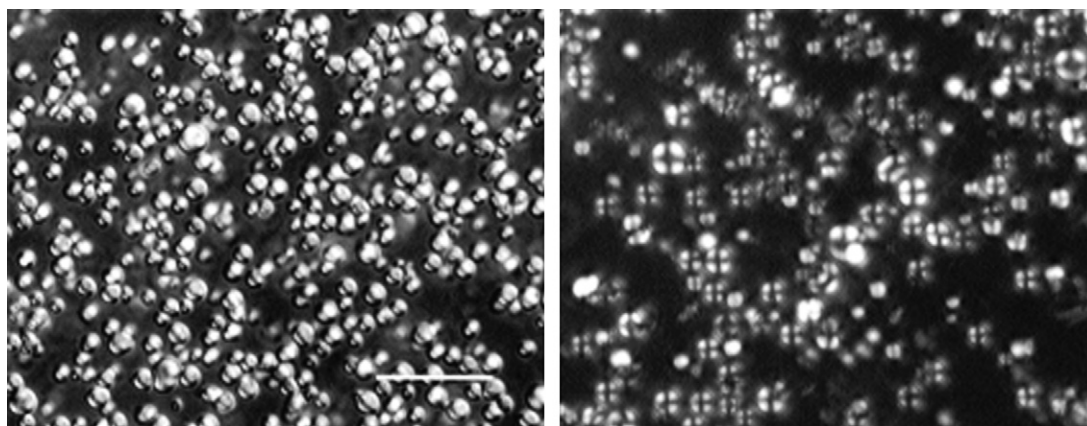


Fig. 1. Light microscopy pictures of the surfactant/polyelectrolyte complexes obtained with a “diffusion” procedure. The left (resp. right) hand side picture was taken in differential interference contrast (resp. between crossed polarizers). The scale bar represents 20  $\mu\text{m}$  and is the same for the two pictures.

we expect the crucial role of the sample preparation procedure in the microscopic structure and mesoscopic morphology of polyelectrolyte/surfactant complexes to be universal.

## 2.2. Polyelectrolyte-induced morphological transition of multilamellar vesicles

To better understand the dynamics for the formation of polyelectrolyte/surfactant complexes, we performed light microscopy experiments where we followed in real-time the effect of a polyelectrolyte solution on one multilamellar vesicle (MLV). The experimental protocol is described in detail in Ref. [26]. In brief, a surfactant solution was introduced by capillarity in an observation chamber made of a slide and coverslip, a drop of the PE solution was then put into contact with the surfactant solution, after which the apertures of the chamber were sealed with vacuum grease to avoid solvent evaporation and convective flow. In this way, a polyelectrolyte concentration gradient was created in the surfactant solution. We observed that the initial polyelectrolyte concentration,  $C_{\text{PE}}$ , and hence the kinetics of the complexation process, had dramatic effects on the intermediate structure of the vesicle as it interacted with the polyelectrolyte molecules.

We found that a dilute polyelectrolyte solution ( $C_{\text{PE}} = 0.5\%$  (w/w)) induced a discrete removal of the bilayers of the MLV one after the other, resulting in the progressive formation of complexes in the vicinity of the MLV. Along this polyelectrolyte-induced peeling of the MLV, the size of the MLV decreased continuously until its complete disappearance. Fig. 2a and b shows the initial MLV and an intermediate state, where the MLV had considerably decreased in size, and was surrounded by many small polyelectrolyte/surfactant complexes. All along the process, the interior of the MLV remained intact, as expected for a peeling mechanism, for which only the most external bilayer interacts with the polyelectrolyte molecules. A remarkable feature was that the size decrease and the formation of aggregates were associated with marked shape distortion of the initially spherical MLV. This novel phenomenon was analyzed in detail and discussed in terms of polyelectrolyte-induced tension, and pore formation and growth in a surfactant bilayer [26].

Very generally, by using multilamellar vesicles instead of unilamellar vesicles, richer behavior may occur, since cooperative effects due to the dense packing of bilayers may play an important role. This expectation was nicely illustrated by the effect of a concentrated polyelectrolyte solution ( $C_{\text{PE}} = 30\%$  (w/w)) on a MLV. When a strong gradient of polyelectrolyte concentration is imposed to the vesicle, the vesicle exhibited very spectacular morphological transitions, as shown in Fig. 2c and d. In particular, a transient state where several bilayers could be separated by distances larger than several micrometers was systematically observed (Fig. 2d). The microscopic mechanisms at the origin of this novel state, which presumably results from the progressive binding of the adjacent bilayers by the polyelectrolyte backbone, remain to be clarified [27].

## 3. Electrostatic interactions in polymer-supported lipid layers

Phospholipid bilayers and monolayers attached to various surfaces can serve as model systems for biomembranes [28,29]. In the well-defined environment of controlled nanofilms questions of membrane function and the role of different membrane components can be studied. In particular, the membrane composition can be controlled and external parameters systematically varied. Since the majority of membrane functions requires a similar flexibility of the membrane as *in vivo*, membrane model systems should preferably be built on soft rather than on rigid solid surfaces. Therefore, soft polymer cushions were established, which consist of strongly hydrated polymer and provide the flexibility of the membrane, including the mobility of distinct functional components [30–32]. In such so-called polymer-supported biomembranes the local interactions between phospholipid head groups and polymer are crucial, as they have to establish a compromise between the stability and the mobility of the system.

Several lipid diffusion studies dealt with interactions between lipids and polymers in such systems [33–36]. Electrostatic aspects of lipid–polyelectrolyte interactions between adjacent layers were studied by measuring the lateral lipid diffusion in a model membrane consisting of phospholipid monolay-



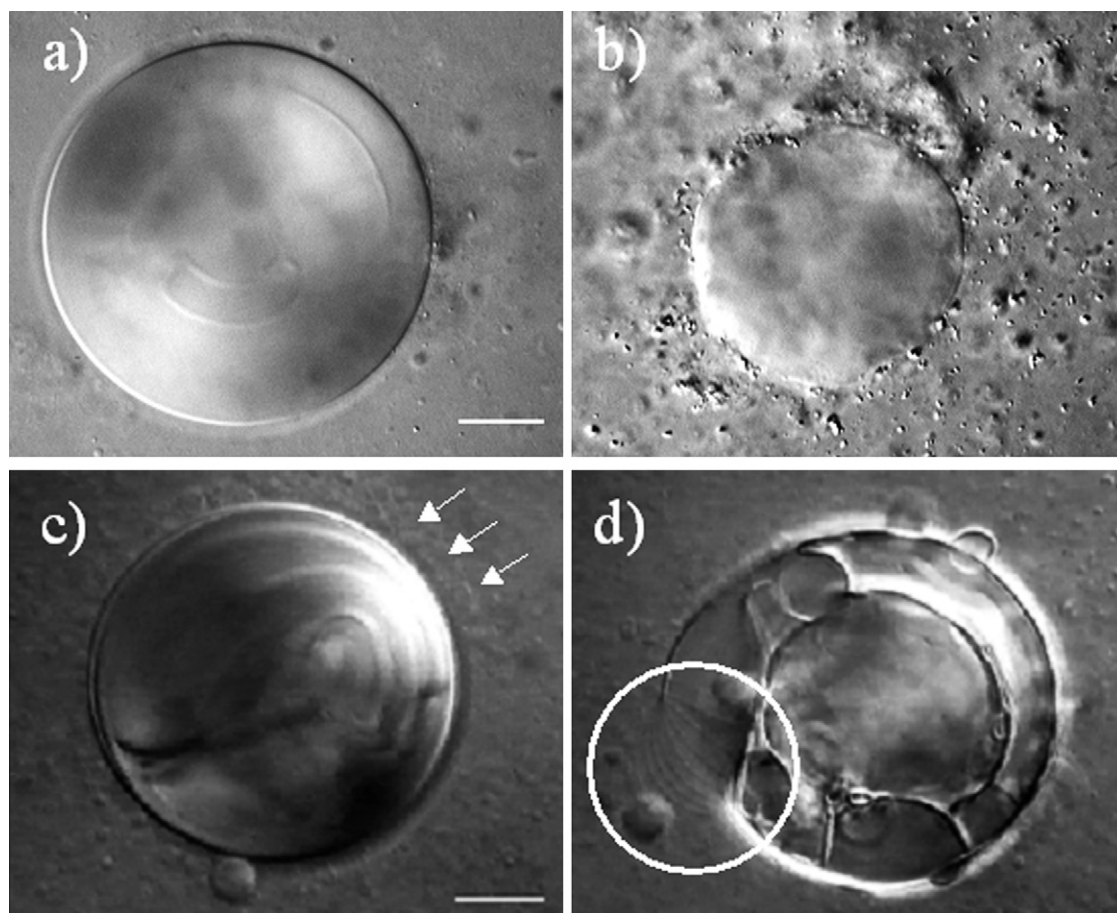


Fig. 2. Differential interference contrast micrographs showing the morphological transitions of a multilamellar vesicle (MLV) induced by a polyelectrolyte solution. In (a and b) the initial polyelectrolyte concentration was  $C_p = 0.5\%$  and in (c and d)  $C_p = 30\%$ . The time elapsed between (a) and (b) was 19 min, and between (c) and (d) is 5 min. The arrows in (c) indicates the direction for the polyelectrolyte diffusion, and the white circle in (d) show the individual and well-separated surfactant bilayers. The scale bar represents  $20\ \mu\text{m}$  and is the same for (a and b), and for (c and d).

ers attached to a polymer cushion. The polymeric support was formed by polyelectrolyte multilayers (PEM) prepared by layer-by-layer self-assembly. PEM were formed on glass substrates by alternating adsorption of a polycation poly(allylamine hydrochloride) (PAH), and a polyanion, poly(styrene sulfonate sodium salt) (PSS), from aqueous solutions [35,36]. The cushion consisted of 11 or 12 single layers, and was thus terminated either by a PSS or by a PAH layer. A lipid monolayer containing 1 mol% of fluorescent probe NBD-PC was formed and compressed on the water surface of a Langmuir trough and finally transferred to the surface of the polymer cushion.

Diffusion coefficients of fluorescence-labeled lipids were determined by means of fluorescence recovery after photobleaching (FRAP). Since lipid diffusion in dry layers turned out to be very slow ( $D$  on the order of  $10^{-12}\ \text{cm}^2\ \text{s}^{-1}$ ), a total internal reflection setup providing surface sensitivity is combined with bleaching an interference pattern by two laser beams and following its decay, which is due to lateral diffusion. Details of the setup were described previously [35].

Lipid diffusion coefficients were determined in films in contact with air for different combinations of lipids and polyelectrolyte terminating layers (see Table 1). Lipid diffusion coefficients in air are very small, on the order of  $10^{-12}\ \text{cm}^2\ \text{s}^{-1}$ , indi-

cating strong electrostatic coupling. In temperature-dependent diffusion experiments, an Arrhenius type activation behavior was found for all systems, and activation energies were determined, which are on the order of 10 kT, see Table 1 [35]. Comparing phosphatidylcholine lipids with different chain order, i.e. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with saturated chains and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) with unsaturated chains, respectively, the influence of the chain order turned out to be negligible, which is opposed to findings for free monolayers on a water/air interface. However, comparing the DOPC diffusion to that in 1,2-dioleoyl-*sn*-glycero-3-phosphate (monosodium salt) (DOPA), an influence of the head group was found in the activation energies, see Table 1 [35]. It was concluded that polymer-coupled monolayers are controlled by the head group interaction with the polymer.

The sign of charge of the terminating polyelectrolyte layer had a negligible influence on the diffusion. This was attributed to binding of the lipid head group not only to extending loops of the terminating layer, but even to segments of the second last layer, if the latter contained charges of a sign opposite to a charge in the lipid head group. From these findings the view emerged that it is the number of charges in the lipid head group, which

Table 1

Diffusion coefficients  $D$  (at 20 °C) and activation energies of diffusion  $E_a$  in different lipid monolayers

	Terminating layer			
	PSS(–)		PAH(+)	
	$D (\times 10^{-12} \text{ cm}^2/\text{s})$	$E_a \text{ (kT)}$	$D (\times 10^{-12} \text{ cm}^2/\text{s})$	$E_a \text{ (kT)}$
DMPC(+/–)	$0.54 \pm 0.05$	$10.7 \pm 0.7$	$0.60 \pm 0.06$	$12.1 \pm 1.5$
DOPC(+/–)	$0.64 \pm 0.03$	$10.6 \pm 1.6$	$0.52 \pm 0.05$	
DOPA(–)	$0.72 \pm 0.07$	$8.0 \pm 0.9$	$0.65 \pm 0.07$	$6.9 \pm 0.5$

Lipid layers were transferred to PSS/PAH multilayers, which are terminated by PAH or PSS, respectively, and were studied in air. Data according to Ref. [35].

controls the interaction strength. For zwitterionic head groups with two charges high activation energies and slightly lower diffusion coefficients were found, as compared to lipids with an ionic head group. This was irrespective of the chain order or the charge of the terminating layer. It is thus consistent with interactions of the zwitterionic head with both positive and negative polymer segments. Thus, the lipid–polyelectrolyte interaction could be described as being dominated by electrostatic forces.

The model of electrostatic control of lipid mobility was further tested by experiments of lipid–polyelectrolyte layers, which were swollen in water [36]. In water-swollen films, lipid diffusion coefficients again were independent on the sign of charge of the terminating layer and of the lipid chain order (see Table 2). The parameter controlling the interaction was again the number of charges in the lipid head group. However, in water the diffusion coefficients were larger for the zwitterionic head group than for the ionic head group. Thus, while zwitterionic lipids bind stronger than ionic ones in dry films, they showed less binding in water-swollen films.

This phenomenon was explained by a model comparing the distance dependencies of the dipole–dipole interaction and the Coulomb interaction, respectively [36]. Fig. 3 visualizes the electrostatic interactions between head group charges and polymer charges at the lipid–polyelectrolyte interface. In dry films head group–polymer distances can be assumed as small. In this case the two charges in the zwitterionic head group give rise to an activation energy between one and two times that of a single charged head group (see Table 1). This implies that the interaction strength of the zwitterionic head group corresponds to almost two Coulombic interactions of single charges. In swollen films, however, the head group–polymer distance is large and the interactions are screened by water molecules. Here, the zwitterionic head group can be considered as a dipole interacting with a dipole formed by corresponding polymer charges. Dipole–dipole interactions decay much stronger with the dis-

tance (i.e.  $r^{-3}$ ) than Coulomb interactions ( $r^{-1}$ ), therefore in the swollen system with larger distances the interaction strengths are reversed and the binding of the zwitterionic head group is less strong than that of the ionic head group.

#### 4. Coupling of polyelectrolytes to oppositely charged monolayers

A lipid monolayer at the air/water interface represents an attractive model surface to study interactions with components dissolved in the adjacent water phase:

- Its chemical composition and the density of charged groups can be varied in a broad range.
- The energetics of interactions can be measured via the surface tension.
- Structural changes of the interface and of neighboring molecules can be investigated with high precision. A broad range of new methods like X-ray diffraction and reflectivity, FT-IR spectroscopy, fluorescence and Brewster angle microscopy, nonlinear optical spectroscopy, neutron reflectivity and imaging ellipsometry has been developed [37].
- The subphase composition can be easily varied.

Therefore, studies of monolayers with polyelectrolytes in the subphase contribute substantially to our understanding of details

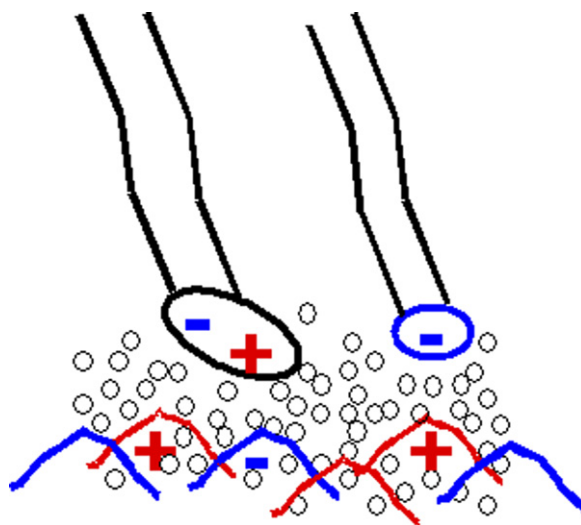


Fig. 3. Sketch of the swollen lipid–polyelectrolyte interface, representing lipid charges and polymer charges that contribute to the interfacial electrostatic interactions.

Table 2

Lipid diffusion coefficients  $D (\times 10^{-10} \text{ cm}^2/\text{s})$  (at 20 °C) in different lipid monolayers in contact with PSS/PAH multilayers swollen in water

	Terminating layer	
	PSS(–)	PAH(+)
DMPC(+/–)	$0.22 \pm 0.03$	$0.24 \pm 0.04$
DOPC(+/–)	$0.24 \pm 0.02$	
DOPA(–)	$0.10 \pm 0.02$	$0.11 \pm 0.01$

Data according to Ref. [36].

of local interactions and their manipulation. From a basic physical point of view, the most interesting question concerns the interplay between lateral forces of the lipids and entropic forces of a flexible polymer.

If a fully charged polyelectrolyte couples to a monolayer the system gets very rigid and the polymer dominates the lipid structure. Charge dilution either within the polymer or within the monolayer increases the flexibility and therefore the influence of lateral interactions in the monolayer on the structure and phase behavior [38–41].

#### 4.1. Adsorption of fully charged polyelectrolytes

The polyelectrolyte PDADMAC (poly(diallyldimethylammonium chloride)) is an un-cross-linked, linear polymer material in which the repeat unit mainly consists of a five-membered-ring species. The batch used in the described experiments had a molecular weight of  $M_n = 7.6 \times 10^4$ . The polymers were dissolved in ultra-pure water in a concentration of 1 mM (referring to the molecular weight of one monomer unit). After spreading the lipid monolayer, the polymer was given  $\sim 30$  min to adsorb. At 20 °C, DPPA (1,2-dipalmitoylphosphatidic acid) on water exhibits a fully condensed isotherm. The isotherm of DPPA coupled with PDADMAC is more expanded and exhibits less pronounced features (Fig. 4). Fluorescence microscopy reveals that the monolayer is heterogeneous with disordered and ordered regions at low and at medium pressures. The area fraction of the ordered regions increases with increasing pressure.

Grazing incidence X-ray diffraction (GIXD) measurements were performed with a Langmuir trough placed in an air-tight aluminum container with Capton windows using the liquid-surface beam-line BW1 at the synchrotron radiation facility HASYLAB at DESY (Hamburg, Germany) [42–47]. GIXD experiments show that DPPA on water exhibits the phase sequence oblique–rectangular–hexagonal on compression. Three distinct Bragg peaks (oblique chain lattice) can be seen at lower surface pressures. Increasing pressure changes the diffraction pattern into one with two Bragg peaks. One diffraction peak is located at zero  $q_z$  and the other one at  $q_z > 0$

(where  $z$  is the direction perpendicular to the interface). Such an intensity distribution is characteristic for a rectangular chain lattice with nearest-neighbor tilt ( $L_2$  phase). The phase transition from oblique to orthorhombic occurs around 15 mN/m. Above 23 mN/m, only one diffraction peak with maximum at zero  $q_z$  can be seen revealing that the aliphatic tails are hexagonally packed and not tilted. DPPA on the PDADMAC subphase behaves completely differently. At all pressures, two Bragg peaks indicative of the  $L_2$  phase can be seen. On compression, the second peak shifts towards larger  $q_{xy}$  and smaller  $q_z$  values, but this decrease of the tilt angle is much less pronounced than in the absence of the polyelectrolyte. No transitions into other phases are observed. In all cases, a cross-section per chain of  $\sim 0.2 \text{ nm}^2$  has been determined.

Summarizing, the polyelectrolyte adsorption leads to a drastic change of the monolayer structure. The oblique phase observed at low lateral pressures as well as the non-tilted hexagonal phase observed at higher surface pressures are suppressed due to interactions with PDADMAC. The coupled monolayer exhibits tilt angles which are much larger than in the pure DPPA monolayer, and there is only a slight change of the tilt angle on increasing pressure (Fig. 5). The polyelectrolyte binding decreases the lateral lipid density, and the lipid responds by an increase of the tilt angle to optimize van der Waals interactions. The oblique lattice observed on water originates from chiral interactions between the head groups. This requires orientational order of the heads and leads to certain packing constraints. The latter are obviously removed upon compression such that the density is now determined by chain packing. Also the polymer coupling leads to the removal of the oblique as well as the hexagonal lattice. This is probably due to a decoupling of the head groups and destruction of the head group lattice responsible for the chiral structure. The hexagonal lattice on the other hand requires a denser chain packing than possible in the coupled system.

As observed for many monolayer systems, the positional correlation length perpendicular to the tilt direction is much larger than in tilt direction. This has been ascribed to a one-dimensional crystallization and to weaker interactions along the tilt direction [48]. The ratio between these correlation lengths increases from

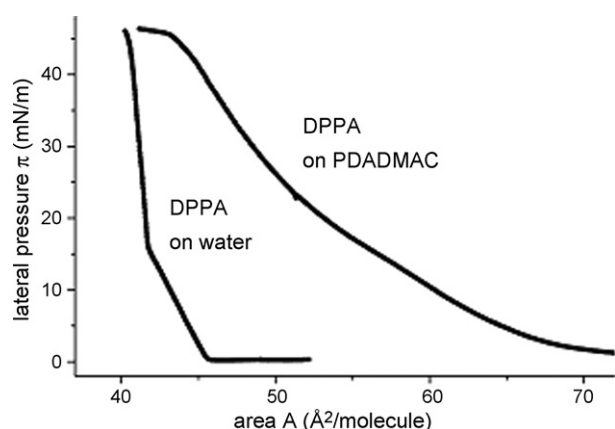


Fig. 4. Lateral pressure  $\pi$  as function of the molecular area of DPPA on water and on a PDADMAC solution at 20 °C.

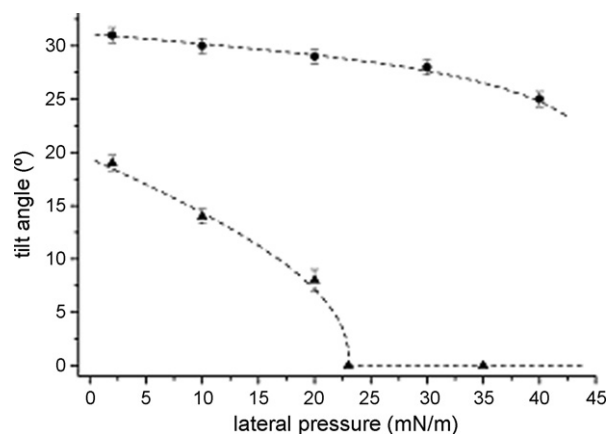


Fig. 5. Tilt angle of the aliphatic chains as function of the lateral pressure for DPPA on water (▲) and DPPA on a PDADMAC solution (●).



2:1 for DPPA on water to 6:1 after coupling with PDADMAC. This demonstrates that the polymer increases translational order perpendicular to the tilt direction and creates distortions into the tilt direction. Therefore, the PDADMAC molecule seems to be stretched underneath the phospholipid monolayer. However, no diffraction peaks corresponding to polymer/polymer correlations have been observed, which indicates that the lateral polymer order is very weak. It is not clear whether the whole polymer chain is stretched underneath the monolayer or whether it also forms loops at high lateral pressure. The well-defined structure of the polyelectrolyte-coupled monolayer is probably related to the 1:1 stoichiometry [49].

PDADMAC is basically a cylindrical molecule with a diameter of about 1.1 nm and nearly screw symmetry. The screw repeat length is 3.1 nm. The charged five-membered rings are oriented helically around the main backbone. A locally extended conformation of the molecule seems not to be favored. The distances between the charges are distributed in the range between 0.65 and 0.85 nm. At high compression, the lipid lattice parameters are  $a = 0.52$  nm and  $b = 0.85$  nm. The tilt direction is assumed to be perpendicular to the  $b$ -axis. Hence, repeat distances along the polymer backbone are commensurate with those along  $b$ . The spacing along  $a$  is about half the polymer diameter. The head groups are sufficiently flexible to enable Coulomb attraction between charged head group and polyelectrolyte. The distance along the rod is relatively fixed at all pressures.

According to this model, the thickness of the adsorption layer should basically be in the range of one polymer monolayer leading to a thickness of about 1 nm. This is in good agreement with the adsorbed polyelectrolyte amount obtained from ellipsometry measurements [41]. Charge dilution in the polyelectrolyte (fraction of charged monomers <50%) leads to an increase of the adsorbed amount as well as to much thicker adsorption layers. Summarizing these findings, adsorption to the interface leads to an alignment of PDADMAC. The polyelectrolyte couples to the DPPA monolayer as a rigid rod preferentially perpendicular to the tilt direction of the lipid chains (Fig. 6).

#### 4.2. Adsorption of charge-diluted polyelectrolytes

The copolymers (poly(diallyldimethylammonium chloride-co-acrylamide)) with different charge densities were available in three different compositions with 73% (CP-73,  $M_w = 5.8 \times 10^5$  g/mol), 47% (CP-47,  $M_w = 1.4 \times 10^6$  g/mol), and 21% (CP-21,  $M_w = 4.0 \times 10^6$  g/mol) of the charged DADMAC monomer. The structure and the phase behavior of DPPA on CP-73 are qualitatively and quantitatively the same as on PDADMAC. The coupled system exhibits the  $L_2$  phase with NN (nearest-neighbor direction) tilt at all pressures investigated. At low pressures, the tilt angles of the DPPA/CP-73 complex are nearly the same as for the DPPA/PDADMAC system. The decrease of the tilt angles on increasing pressure is more pronounced for DPPA on CP-73 than on PDADMAC, indicating a slightly higher flexibility of the DPPA/CP-73 system. Decreasing charge density in the adsorption layer leads to further reduction of the tilt angles. The chain tilt of the DPPA/CP-21 complex is decreased to values in the range of those for DPPA on

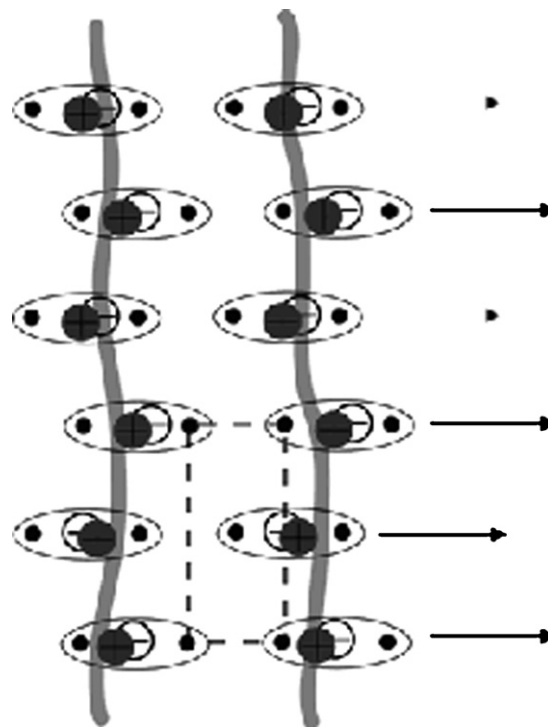


Fig. 6. Model of PDADMAC coupling to a DPPA monolayer. The polymer is stretched underneath the anionic lipid monolayer. The arrows indicate the tilt direction (nearest-neighbor tilt) of the aliphatic chains, which form a rectangular unit cell (dashed rectangle).

pure water. The flexibility of the polyelectrolyte increases continuously with decreasing charge density leading to an increased compressibility of the coupled system. However, the oblique phase observed for DPPA on water does still not appear. A consequence of increasing flexibility is the formation of tails and loops in the adsorption layer. For CP-21 an adsorption layer thickness of  $d \approx 15$  nm has been found.

Heterogeneities within the monolayer are also observed in systems with reduced charge density. In low charged systems, a penetration of polymer into the monolayer seems to be possible since the fraction of the more hydrophobic acrylamide increases.

In conclusion, competitive interactions in the hydrophilic and hydrophobic regions are important factors for the structure formation in a lipid monolayer. Additional strong forces appear if a polyelectrolyte couples to the charged lipid monolayer. PDADMAC has one positive charge per monomer unit and couples as a rigid rod in a 1:1 stoichiometry to a charged DPPA monolayer, preferentially perpendicular to the chain tilt of the lipid molecules. The electrostatic attraction between the polymer and the lipid dominates the structure formation at all pressures. The adsorption of a partly charged copolymer to a DPPA monolayer shifts this sensitive equilibrium of the different interactions. The partly charged polyelectrolyte is more flexible and forms thicker adsorption layers with many loops and tails. The 1:1 stoichiometry might be eliminated and the adsorption might not only be a result of the electrostatic coupling but also of non-electrostatic contributions to the adsorption energy. The adsorption layer is highly flexible and the dominating force for the structure formation is now the van der Waals interaction

between the hydrophobic chains. However, the coupling still suppresses the head group interactions at low lateral pressures. Therefore, no oblique phase has been observed.

This interpretation is supported by theoretical considerations showing that there should indeed be flat polyelectrolyte adsorption at low ionic strength and high charge density [50–55]. A way of viewing the adsorption behavior is that PDADMAC coupled to the charged interface is much stiffer than in solution.

#### 4.3. DNA-alignment at lipid monolayers

The understanding of DNA–lipid interactions is an important requirement to engineer artificial viruses. Non-viral gene transfer has become more and more attractive over the past decade since the application of viruses as vectors is accompanied by certain problems like immunogenicity [56–61]. Cationic lipids can easily form complexes with DNA, but they have one decisive disadvantage. They are frequently toxic for cells. Complexes composed of neutral lipids offer an alternative to cationic lipids as they are non-toxic. However, zwitterionic lipids do not interact with the DNA directly, but the interaction has to be mediated by divalent cations [62–64].

##### 4.3.1. DNA adsorption to cationic monolayers

The triple-chain methyltrioctadecylammonium bromide (TODAB) and the double-chain dimethyl-dioctadecylammonium bromide (DODAB) were used as cationic lipids. Both compounds form stable monolayers at the air/water interface. The double-stranded deoxyribonucleic acid (DNA) is a highly polymerized natural product originated from calf thymus. The DNA had not only adsorbed but also arranged parallel to the surface, even before any layer compression has been started, in contrast to the behavior of a PDADMAC adsorption layer.

Above 35 mN/m, TODAB forms a condensed phase on water. With DNA in the subphase, the film with a liquid-like compressibility is more expanded and the phase transition region disappears. However, GIXD shows an ordered lipid phase at lower lateral pressures. This indicates, that the coupling of DNA leads to a condensation of the TODAB layer. The  $L_2$  phase has been found at pressures much below the phase transition observed on water. Additionally, a second phase transition into a non-tilted LS phase occurs on compression, which was never observed on pure water [65]. The coupled film is obviously heterogeneous. This can be verified by Brewster angle microscopy (BAM). Islands corresponding to an ordered state coexist with a continuous phase (dark background in the BAM pictures). No DNA-adsorption to the water surface was observed in the absence of cationic lipids.

The most interesting finding was the observation of diffraction peaks caused by DNA ordering (Fig. 7). The Bragg peak shifts with compression to larger  $q_{xy}$  values and becomes narrow. It is remarkable that the DNA helices arrange with a one-dimensional periodicity even without compression of the monolayer. No direct geometric connection could be found between the hexagonal lipid chain packing and the arrangement of the opposite charges around the DNA helix. This geometric

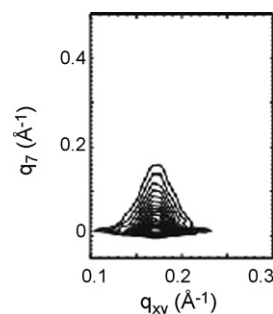


Fig. 7. Contour plots of the corrected X-ray intensities as function of the in-plane component  $q_{xy}$  and the out-of-plane component  $q_z$  of the scattering vector  $q$  of DNA coupled to a TODAB monolayer and compressed to 20 mN/m.

mismatch can possibly be compensated by counterion release, and the corresponding entropic contribution can stabilize mismatched structures.

Summarizing the coupling of DNA to both lipids, the adsorption of DNA leads to a local condensation of the lipid monolayer. The rod-like DNA forms a one-dimensional lattice (nematic alignment) under a hexagonal or distorted hexagonal (centered rectangular) lipid chain lattice although the correlation lengths are only in the order of 10 nm. A similar size of the DNA domains (near 10 neighboring chains) was reported by Safinya et al. for DNA bound in lamellar structures [7–13]. DNA and lipid density are interrelated, but not in a straightforward way like lattice commensurability.

The coupling of DNA leads to different results compared with the coupling of the synthetic (stiff) polyelectrolyte PDADMAC (see above). The PDADMAC alignment with commensurability into one direction could be deduced, although the polymer–polymer spacing could not be measured. The polyelectrolyte coupling enforced an almost pressure-independent aliphatic chain tilt, and this may be attributed to the smaller dimension of PDADMAC enabling denser lateral packing.

##### 4.3.2. DNA adsorption to zwitterionic monolayers

Systems using non-toxic zwitterionic phospholipids seem to be an interesting alternative for non-viral gene delivery. Surface pressure–area isotherms of zwitterionic DMPE (1,2-dimyristoylphosphatidylethanolamine) monolayers on water show a plateau region at  $\pi_c \sim 5.6$  mN/m indicating a first-order phase transition from the liquid expanded (LE) to a liquid condensed (LC) phase. In the LC phase, the aliphatic chains of DMPE are tilted at low surface pressures because of the large hydrated head group. At  $\sim 32$  mN/m, a second-order phase transition from a tilted to a non-tilted state can be observed characterized by a kink in the isotherm. DMPE isotherms on salt solutions or on a subphase containing only DNA without divalent cations are very similar to that on water. However, the presence of DNA and divalent cations as calcium or magnesium in the subphase shifts the isotherm to larger areas per molecule due to partial penetration of DNA into the monolayer [66,67]. The LE/LC transition is only weakly influenced. Above 30 mN/m, DNA is obviously squeezed out from the monolayer and the resulting molecular area is the same as that of DMPE on water.



Infrared reflection absorption spectroscopy (IRRAS) can be successfully used to identify a DNA adsorption layer [66,67]. The combination of IRRAS using a ‘trough shuttle technique’ and the Langmuir film balance technique enables a direct correlation of molecular structures respectively conformations with phase transitions or molecular areas in the  $\pi/A$ -isotherms [68–73]. The spectral region between 1800 and 700  $\text{cm}^{-1}$  exhibits more than 30 DNA marker bands [74]. The IRRAS experiments show clearly that DNA adsorbs to a DMPE monolayer only in the presence of divalent cations.

GIXD experiments show that the adsorption of DNA does not change the DMPE phase sequence (oblique–L<sub>2</sub>–LS). The chains are completely upright above 32 mN/m. This value agrees well with the observed kink in the isotherm. The tilt angles of DMPE on water, on salt (5 mM  $\text{MgCl}_2$ ) and on 0.1 mM DNA are very similar and lead to the same transition pressure into the non-tilted LS phase. Binding of DNA via divalent cations has a condensing effect leading to decreased tilt angles and a lower transition pressure into the LS phase [67]. Comparing GIXD and pressure/area isotherms shows that some parts of DNA must be penetrated into the DMPE monolayer but they obviously do not disturb the lipid packing.

As in the case of cationic monolayers, ordering between DNA strands is indicated by the appearance of a Bragg peak at small  $q_{xy}$  values. The inter-axial  $d$ -spacing of ordered DNA strands decreases only slightly on compression. The observed  $d$ -values depend on the type of cation used. For example, for magnesium values between 4.7 and 4.3 nm have been found whereas the distances on the subphase containing calcium ions are much smaller (3.7 nm). The decrease of only 8% is rather small compared with the above-described experiments with cationic monolayers (Fig. 8). In the case of zwitterionic monolayers, the coupled DNA layer does obviously not respond to the lipid compression. Comparing the areas per charge for DMPE and DNA shows a ratio of 1:2. This indicates that two lipids (one elementary charge per lipid head group) are correlated with one elementary charge of the DNA. The position correlation length in the LS phase is preserved even after adsorption of DNA showing that adsorbed DNA does not induce packing defects.

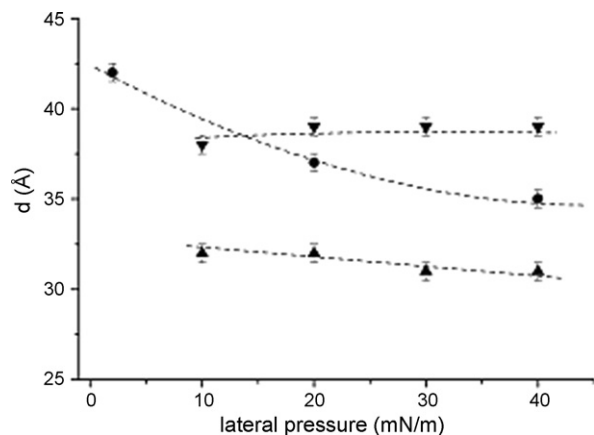


Fig. 8. Inter-strand  $d$ -spacings of ordered adsorbed DNA strands as a function of the lateral surface pressure for DNA coupled to TODAB (●) and DNA coupled to DMPE via divalent cations ( $\text{Ca}^{2+}$  (▲);  $\text{Mg}^{2+}$  (▼)).

The adsorption of DNA to a DMPE monolayer in the presence of divalent ions shows that these ions turn the zwitterionic monolayer cationic. Two modes of DNA binding to the zwitterionic lipid via divalent cations seem to be possible. Either the divalent cation bridges a phosphate of the lipid head group with a phosphate of the DNA or it connects the phosphate groups of two neighboring lipids, leaving the positively charged nitrogen pointing towards the subphase containing the DNA. Bridging between DMPE molecules seems to be more reasonable because of packing constraints.

## 5. Conclusions

We have investigated the electrostatic interactions between charged amphiphilic molecules and oppositely charged polyelectrolyte chains, for both three- and two-dimensional systems.

One remarkable results in bulk samples is that, by properly designing the sample preparation procedure, spherical surfactant/polyelectrolyte complexes, whose microstructure is that of a condensed lamellar phase, can be obtained. These complexes are uniform in size and very stable in pure water, which renders them attractive for potential application in drug controlled-release.

For lipid bilayers coupled to a polyelectrolyte multilayer cushion, one important finding is that the binding is dominated by electrostatic interactions, despite a pronounced flexibility at the polymer/lipid interface. Lipid molecules in the monolayer as well as the polymer segments provide sufficient internal flexibility to arrange into a structure dominated by interfacial electrostatic interactions. This result is of particular interest in the field of biomimetic systems as lipid bilayers coupled to a polyelectrolyte multilayer cushion constitute an important approach to control the structure and properties of model membranes.

Finally for monolayers, we have demonstrated that the subtle interplay between lateral forces of the lipids and entropic forces of the polyelectrolyte modify substantially the phase behavior of the lipid, and can induce an ordering of the polyelectrolyte molecules which interact with the monolayer.

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