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STABILIZATION OF LIPID BILAYERS ON SURFACES THROUGH CHARGED POLYMERS

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ABSTRACT

It is our concept to use a polymer as a hydrophilic cushion to stabilize a lipid bilayer on a solid support. This can be accomplished by using polyacrylamides with disulfides and DMPE anchors as a hydrophilic cushion. These polymers have the additional functionalities to chemisorb on gold surfaces through the disulfides and to bind a lipid bilayer on it through the insertion of the lipid anchors into the lipid bilayer. This paper shows that a polymer with the additional functionality of charged groups increases the attraction of vesicles to form a tethered supported lipid bilayer. By varying the amount of charged groups in the polymer, we are able to control the hydrophilic behavior of the polymer and therefore are able to change the wetting on a surface. This was examined by measuring the contact angles. Using the technique of the surface plasmon spectroscopy, we are able to monitor the process of vesicle fusion on the polymer support.

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INTRODUCTION

The modification of surfaces through polymer adsorption has become more important in the last years. Many scientists focus their attention on thin organic films since they provide the control of surface properties such as wetting, adhesion, and biocompatibility for future technological applications [1, 2]. Lipid bilayers have the natural behavior to form defined assemblies of nanoarchitectures. Therefore, a lot of effort is spent to prepare polymer supported lipid bilayers on solid substrates. Incorporation of specific enzymes can yield biosensors [3] (Figure 1). These biosensors would provide a very specific and sensitive detector, which can be used in all different kinds of scientific areas.

In recent years we have been following a way to build up polymer supported bilayers through vesicle fusion on an amphiphilic polymer support [4a, 4b]. Our amphiphilic polyacrylamide polymers fulfill three requirements [5]. They consist of disulfide anchor groups to chemisorb the polymer onto gold surfaces, they provide water soluble groups for a swellable polymer cushion, which decouples the lipid bilayer from the solid support, and they contain a polymer bound natural lipid (here DMPE) to induce vesicle fusion and to anchor the lipid

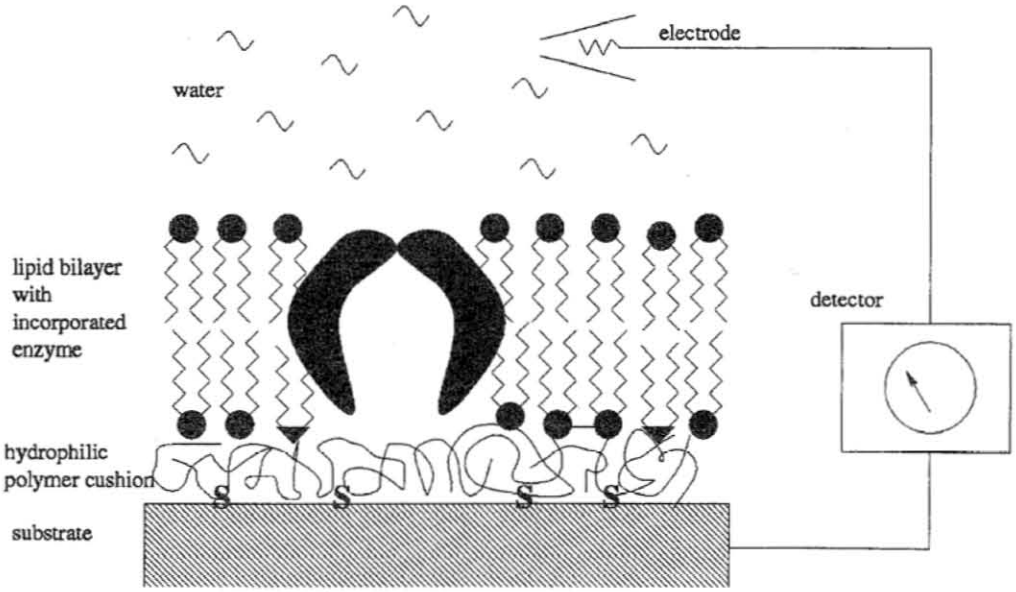

Figure 1. Schematic sketch of a lipid bilayer with an incorporated enzyme, which can be used as a biosensor.

bilayer onto the polymer support. Our synthesis strategy provides a very flexible way to receive these multifunctional polymers through a polymer analogous reaction. We use a reactive ester polymer **1** [6], which can react with a huge variety of amines (Figure 2). Alternatively, unreacted ester can be used to link the polymer to an amino functionalized surface [7].

We have studied in detail the influence of the hydrophilic part and of the lipid content on the swellability, the wetting, the properties at the air-water interface, and the fusion of vesicles onto these surfaces. We could show a dependence of the contact angle on the acrylamide used and on the amount of DMPE. The data are summarized in Table 1. In general, the more hydrophilic the amine is the

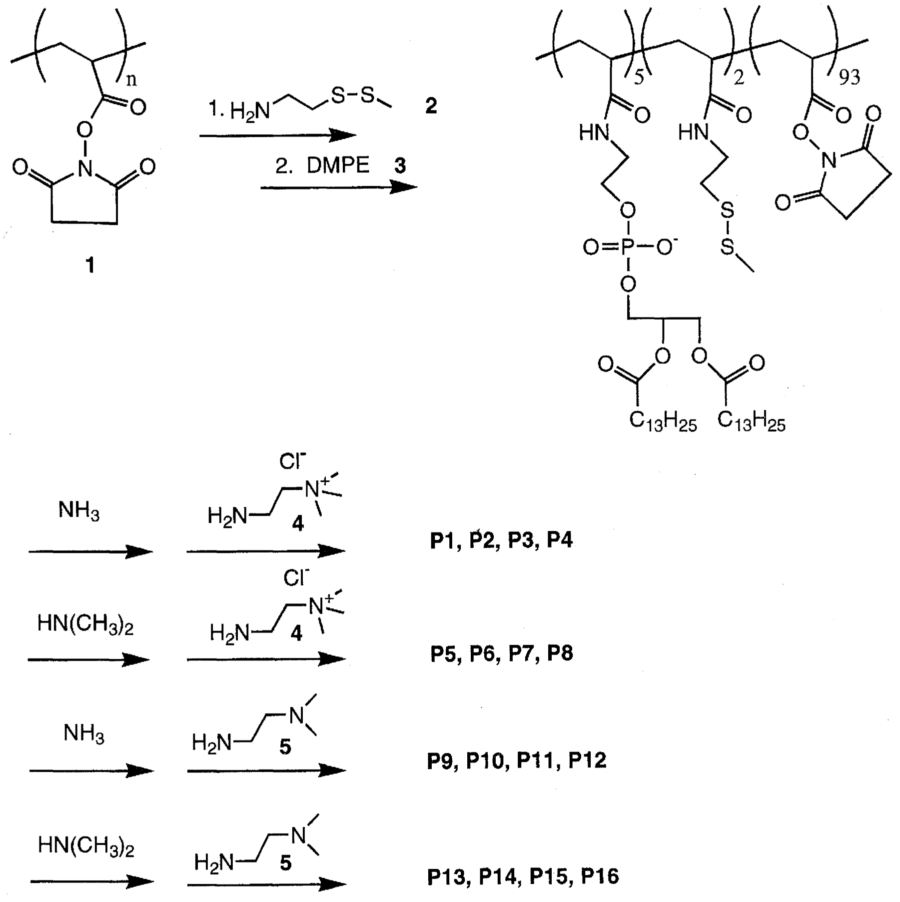

Figure 2. The polymer analogous reaction of the hydrophilic polymer spacer with a lipid anchor and a disulfide anchor.

TABLE 1. Dependence of the Contact Angle Θ_a and the Percentage of DMPE in Poly(dimethylacrylamide) and Poly-(isopropylacrylamide) [4a]

Polymer	Content of Lipid (mol%)	Θ_a (°)
poly-(isopropylacrylamide)	0	60
	1	70
	3	70
	5	72
	10	72
poly-(dimethylacrylamide)	0	35
	1	40
	3	49
	5	62

smaller the contact angle of the resulting polyacrylamide. The advancing contact angles increase also with increasing content of DMPE. With increasing lipid conanalogous reaction in four steps with different amines. First, with cysteaminemethyldisulfide **2**, which is responsible for the chemisorption of the polymer onto a gold surface. Second, with DMPE **3** (L-a-phosphatidylethanolamine, dimyristol), which acts as the lipid anchor. Third step is the reaction with (2-Aminoethyl)-trimethyl-ammoniumchloride **4**. In the last step, the polymer is treated with an excess of either ammonia or dimethylamine. The reaction scheme is summarized in Figure 2. In order to be able to compare the influence of the positive charges, polymers with 1-Amino-2-(dimethylamino)-ethane **5** instead of **4** are prepared. Table 2 shows the prepared polymers with different contents of **4** and **5**, respectively. TLC control showed the quantitative reaction

TABLE 2. Compositions of the Polymers **P1**-**P16**

Polymer	Anchor groups					
	$-NH_2$ (mol%)	$-NH(CH_3)_2$ (mol%)	Disulfid **2** (mol%)	DMPE **3** (mol%)	**4** (mol%)	**5** (mol%)
P1	88	0	2	5	5	0
P2	83	0	2	5	10	0
P3	78	0	2	5	15	0
P4	73	0	2	5	20	0
P5	0	88	2	5	5	0
P6	0	83	2	5	10	0
P7	0	78	2	5	15	0
P8	0	73	2	5	20	0
P9	88	0	2	5	0	5
P10	83	0	2	5	0	10
P11	78	0	2	5	0	15
P12	73	0	2	5	0	20
P13	0	88	2	5	0	5
P14	0	83	2	5	0	10
P15	0	78	2	5	0	15
P16	0	73	2	5	0	20

of the different amines **2-5** with the reactive polymer **1**. The polymer composition was also verified by ^{1}H-NMR, while a determination of the molecular weight by GPC was not possible.

Gold surfaces were prepared by evaporation of gold (purity > 99.99%) onto clean glass slides (BK-270) with a Blazer vapor deposition apparatus (Bae250). Before that, the glass slides were cleaned by ultrasonication in methanol, in detergent (Hellmane, Hellma, Mühlheim, Germany) and five times in Milli-Q water. Chromium and gold films (thickness 2 nm and 48 nm, respectively) were deposited through evaporation on the slides in a vacuum chamber at 5×10^{-6} mbar. Afterwards, the slides were stored under argon.

Surface plasmon spectroscopy in a standard Kretschmann configuration [9] was used to characterize the self-assembled polymer films on gold. The gold coated glass slides were refractive index matched with a 90° glass prism, used as the surface plasmon coupler. During the experiment, the resonant excitation of the surface mode, which is very sensitive to the actual interfacial architecture and, therefore, to the thickness of the adsorbed polymer, was monitored by recording the total internally reflected light from a HeNe-Laser ($\lambda = 633$ nm) as a function of the angle of incidence [10, 11]. The thickness of the thin polymer film was calculated from the SPS-curves based on a Fresnel-fitting.

Polymer adsorption was done by self-assembly of the polymers. Therefore fresh gold coated substrates were immersed for 60 minutes into a solution of 1 mg polymer in 1 mL pure water or 1 mL ethanol, respectively. The substrates were then washed thoroughly with water or ethanol and dried in an argon stream, to be used later for contact angle measurements [12]. Contact angle measurements with water were performed by using a Krüss G1 system (Krüss GmbH, Germany).

Swelling increases the thickness of the self-assembled polymers, which we performed in a self-made swelling chamber. The polymer film was attached to a chamber, in which a definite humidity was adjusted. The swelling behavior was controlled by surface plasmon spectroscopy. Three definite humidities were adjusted, 0%, 43%, and 98%. This was achieved by using solid KOH, saturated K_2CO_3 solution, and saturated KNO_3 solution [13].

Vesicle preparation was done through extrusion. DMPC was dissolved in chloroform and under an argon stream the chloroform was evaporated yielding a thin DMPC film on the glass wall. In order to get a chloroform free film, it was dried for one hour in a vacuum chamber. The DMPC film was then suspended with 0.1 mM NaCl solution at 30°C, to obtain a 1 mg/mL concentrated solution. The film was allowed to swell for one hour at 30°C. The milky solution was then pushed 30 times through a polycarbonate filter with a pore size of 50 nm (Avestin, Ottawa, Canada). The vesicles were stored at 30°C and used within five days.

Characterization

The self-assembly was traced by surface plasmon spectroscopy. A typical self-assembly kinetic curve is shown in Figure 3. All polymers produce a film of about 25 Å. So, the self-assembly is a useful method to prepare thin polymer films on gold surfaces [14, 15].

Hydrophilic behavior of all polymers was studied by measuring the advancing contact angles [16]. All charged polymers **P1-P8** self-assembled from water solutions. All uncharged polymers **P9-P16** were only soluble in ethanol and therefore, the self-assembly was done from that solution. The measured contact angles are collected in Table 3. As can be seen in the case of the polymers **P1-P5**, it is important to know from which solvent the self-assembly was performed. The contact angles of polymers self-assembled from water are all approximately 16° smaller than the ones from ethanol. This means that the solvent has a direct influence not only on the conformation of the polymer in solu-

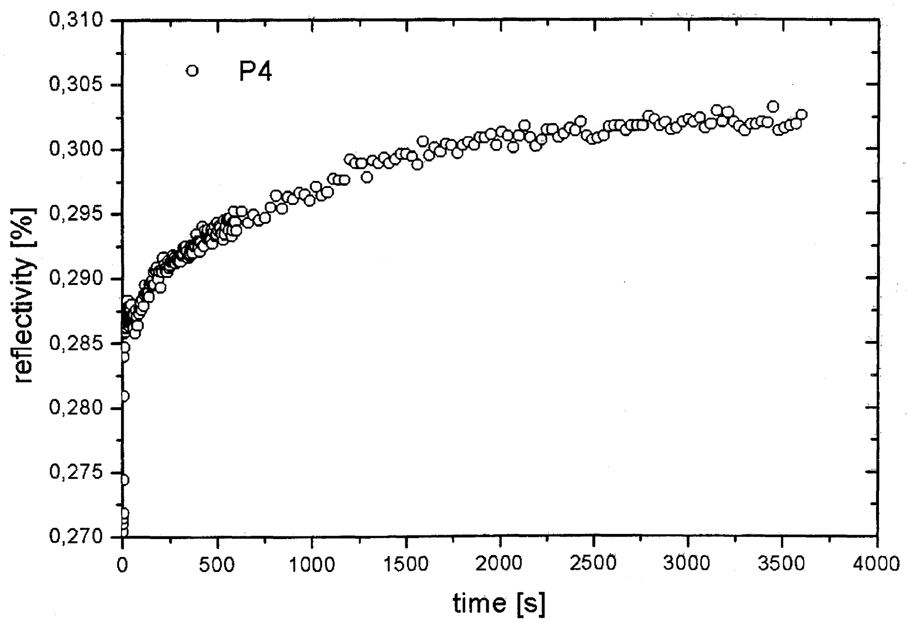

Figure 3. **P4** self-assembly from ethanol onto gold; recorded by surface plasmon spectroscopy.

TABLE 3. Contact angles Θ_a of the Polymers **P1**-**P16**

Polymer	Θ_a (°) [EtOH]	Θ_a (°) [H_2O]	Θ_r (°) [EtOH]	Θ_r (°) [H_2O]
P1	60	45	24	17
P2	58	42	20	13
P3	56	38	24	21
P4	57	-	24	-
P5	66	51	20	20
P6	-	47	-	16
P7	-	46	-	15
P8	-	42	-	16
P9	61	-	33	-
P10	63	-	27	-
P11	66	-	35	-
P12	69	-	43	-
P13	63	-	37	-
P14	69	-	40	-
P15	72	-	43	-
P16	68	-	38	-

tion, but also on an adsorbed polymer film. It seems that the hydrophobic parts of the polymer in ethanol solution approach to the surface. In contrast to the ethanol solution, the formed "micelle" in water chemisorbs directly on the surface and the polymer coil has not the possibility to rearrange to the same conformation afterwards. This is shown schematically in Figure 4.

Looking into more details at the contact angles, one can see the huge difference between Θ_a and Θ_r. Theoretically, they should have the same value if the position of hydrophilic and hydrophobic parts of the polymer is rigidly fixed. The change of the contact angle between expanding and retreating the water droplet reflects therefore, a rearrangement of the lipid chains with regard to the surface. This rearrangement is due to the change from hydrophobic air to hydrophilic water.

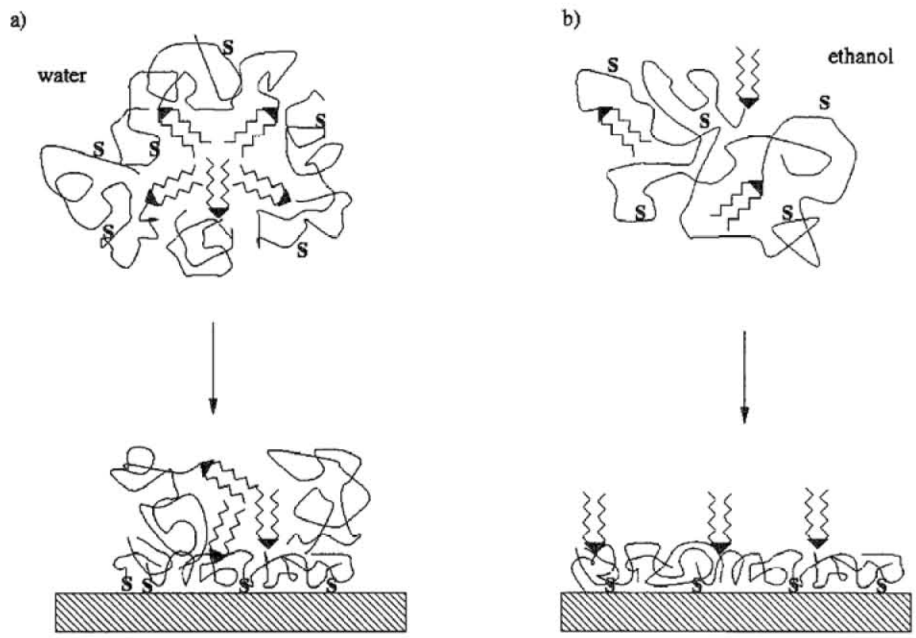

Figure 4. Schematic sketch of the conformation of amphiphilic polymers in a) water as micelles and in b) ethanol.

The dependence of the contact angle on the content of charged groups in the polymer can be better seen in a plot of Θ_a against percentage of charge. This is shown in Figures 5a and 5b for the amines **4** and **5**, respectively. There is a linear dependence between the contact angle Θ_a and the amount of charge on the polymer. The more charged the polymer is the smaller the contact angle. It is interesting to note, that there also exists a linear relation between the contact angle Θ_a and the amount of amine **5** bound to the polymer (see Figure 5b). In this case, the amine **5** acts, however, as a hydrophobic part increasing the contact angle.

Swelling of the polymers presented offers another possibility to examine the hydrophilicity of the self-assembled polymer films. Hydrophilic films should be able to take water and swell, resulting in a thicker film. A typical swelling curve is shown in Figure 6. Before measuring the swelling, the polymer film was dried for 3 hours above KOH. By changing the salt solutions one after another, the swelling was measured. It is interesting to notice that the swelling is not linear to the humidity. The polymer film swells only by one third when the humidity is changed from 0% to 43%, compared to the swelling resulting from a

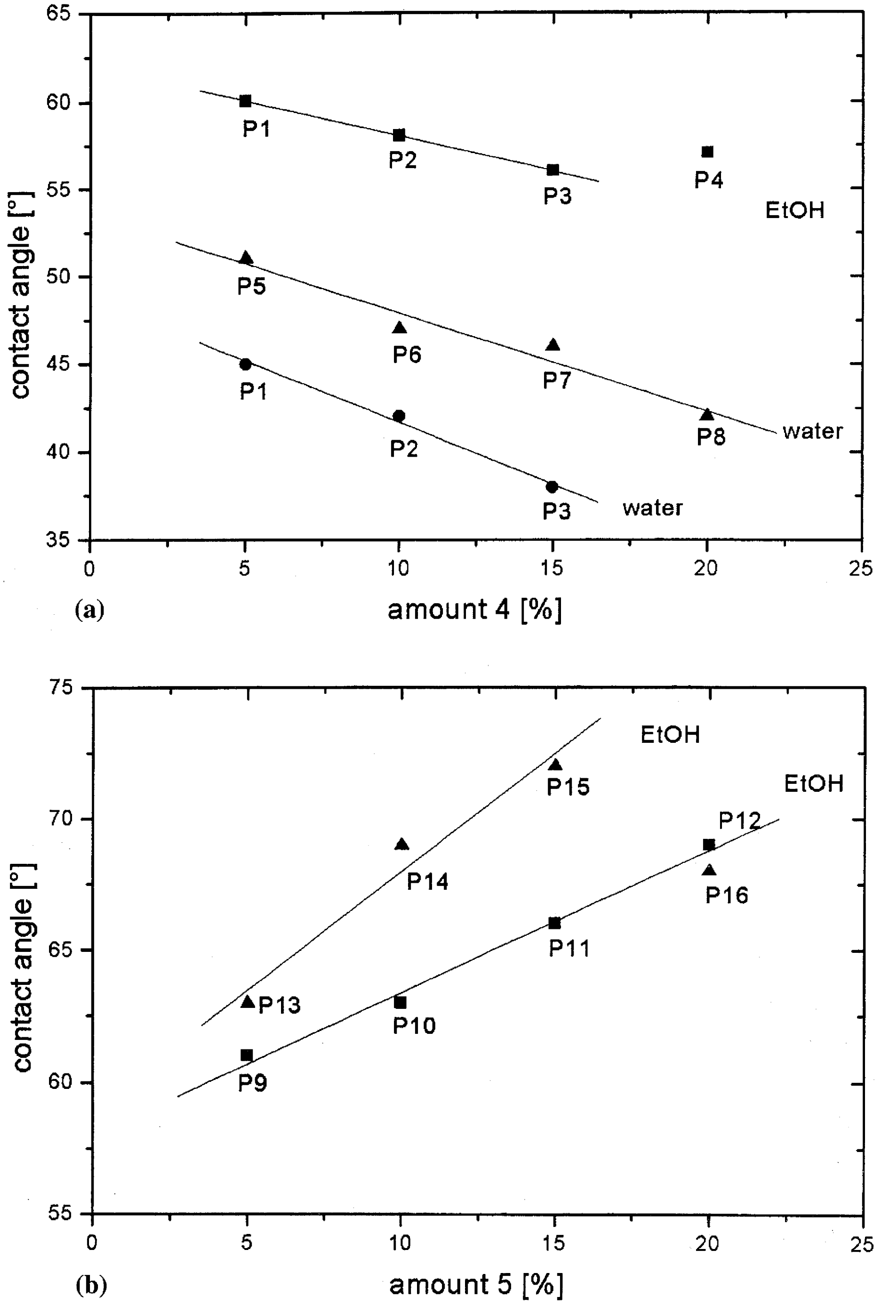

Figure 5. (a) Dependence between the contact angle Θ_a and the amount of charge on the polymer. (b) Dependence between the contact angle Θ_a and the amount of uncharged amine **5** on the polymer.

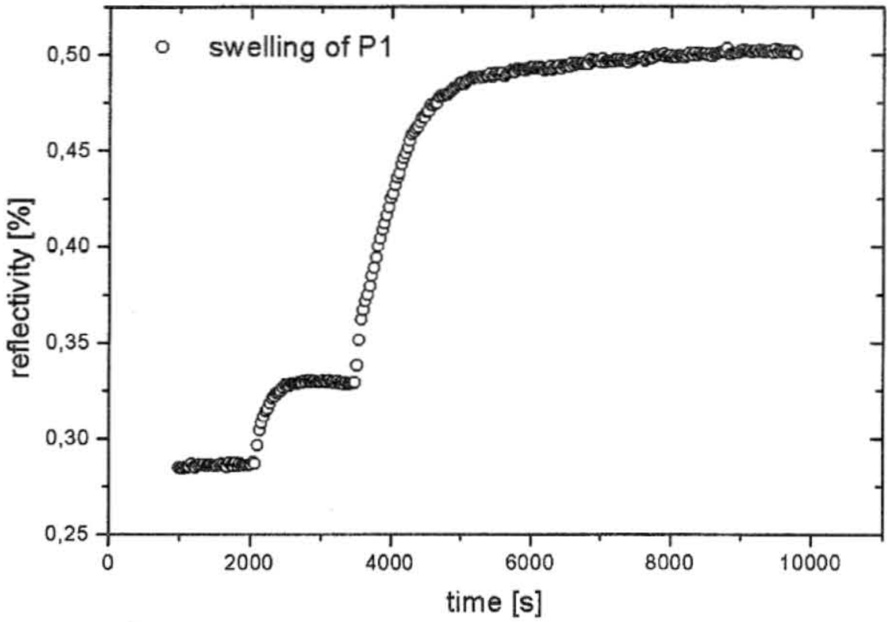

Figure 6. Swelling of **P1** starting a) at 0% humidity, b) from 0% to 43%, and c) from 43% to 98% humidity, respectively.

humidity change from 43% to 98%. This is true for all measured polymer films, charged or not. The thickening of the polymer films during swelling can be summarized as follows. The polymers **P1**-**P4** all swell approximately by 23%, **P5**-**P8** - 16%, **P9**-**P12** - 8%, and **P13**-**P16** - 6%, see Table 4. It has to be said that the accuracy is not very high, since the resolution of the surface plasmon spectroscopy is ±1Å. The reduced swelling of **P1**-**P4** as compared to **P5**-**P8** seems to be due to the less hydrophilic N,N-Dimethylacrylamide main chain spacer. But it is obvious that the charged polymers **P1**-**P8** all swell better than the

TABLE 4. Swelling of the Polymers **P1**-**P16**

Polymer	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**
Swelling (%)	22	25	21	25	12	21	11	21

Polymer	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**
Swelling (%)	0	13	13	7	8	4	5	8

uncharged polymers **P9**-**P16**. This is another result which supports the assumption that charges in the polymer result in a more hydrophilic polymer, which therefore, has a higher attraction to the hydrophilic heads of lipids.

Vesicle fusion on the self-assembled polymer films is a way to prepare tethered supported lipid bilayers [17, 18]. The idea is that vesicles adsorb from a solution onto the polymer surface. This model has two major steps. The first one is the vesicle adsorption onto the surface and the second is the "unrolling" of the vesicles to lipid bilayers, as shown schematically in Figure 7. In particular, in the first step the attraction of the polymer surface to vesicles in the solution plays

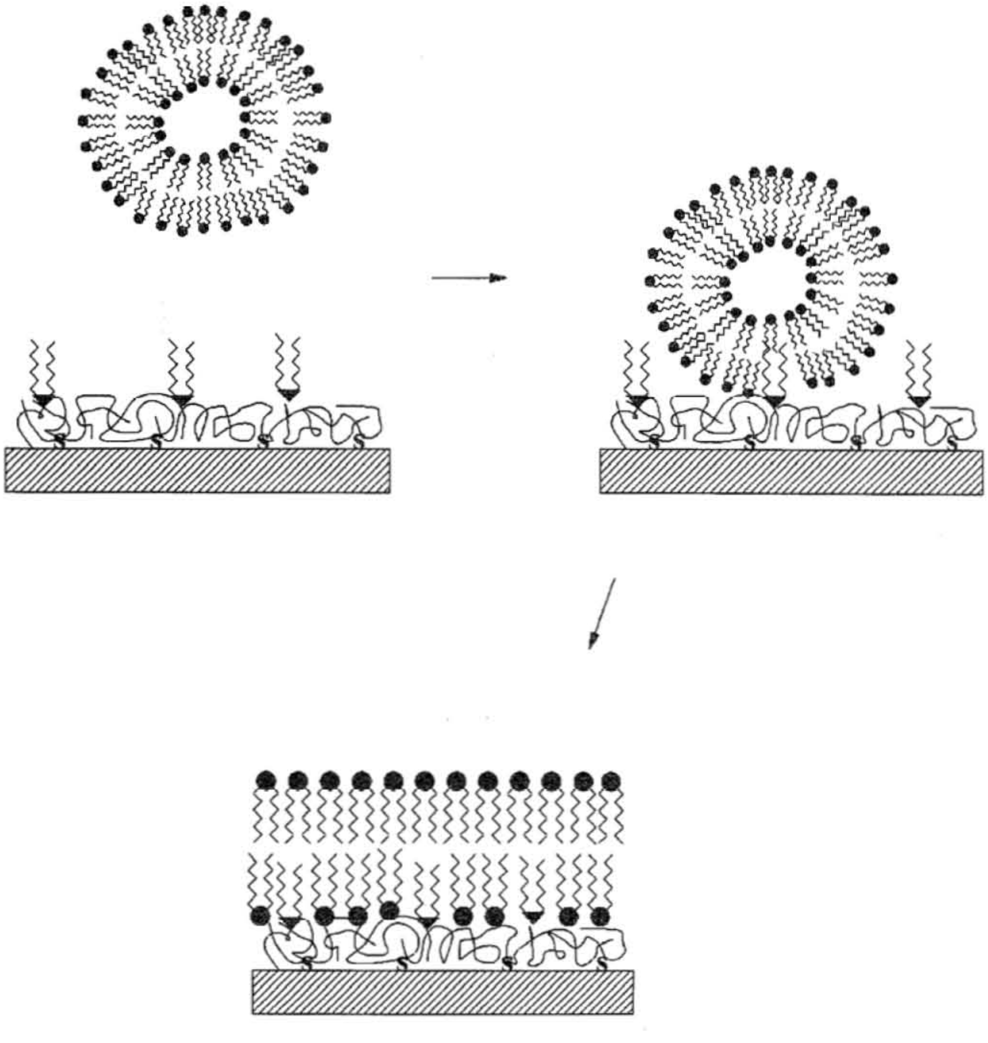

Figure 7. Schematic sketch of the vesicle fusion on polymer coated surfaces.

a major role. If our idea is true, more hydrophilic charged polymers, such as **P1-P8**, should have a higher attraction to vesicles than uncharged polymers, such as **P9-P16**. The surface plasmon measurements show that a vesicle fusion (diameter approximately 80 nm) takes place only on the charged polymer surface prepared from **P1-P8**. On the contrary, there is no increase in thickness for the uncharged polymer surface prepared from **P9-P16**. A typical kinetic mechanism of a vesicle fusion onto a polymer support is shown in Figure 8. It is evident that most of the increase takes place within the first minute. Thereafter, only a small thickness increase is observed. It is not yet clear if this is a proof for the two step model of the vesicle fusion. Further measurements to answer this question are in progress.

The resulting lipid bilayer is about 30 Å thick, which is less than the theoretical value of 50 Å for a lipid bilayer. But one has to take into account that the amount of lipid bound to the polymer has to be added to the measured value. So, the measured thickness of 30 Å seems to be reasonable and is within the range of measured thicknesses by others [15, 17].

CONCLUSION

The idea to increase the attraction of polymer surfaces to lipid vesicles in solution by charges might be seen as another step in the direction to the assem-

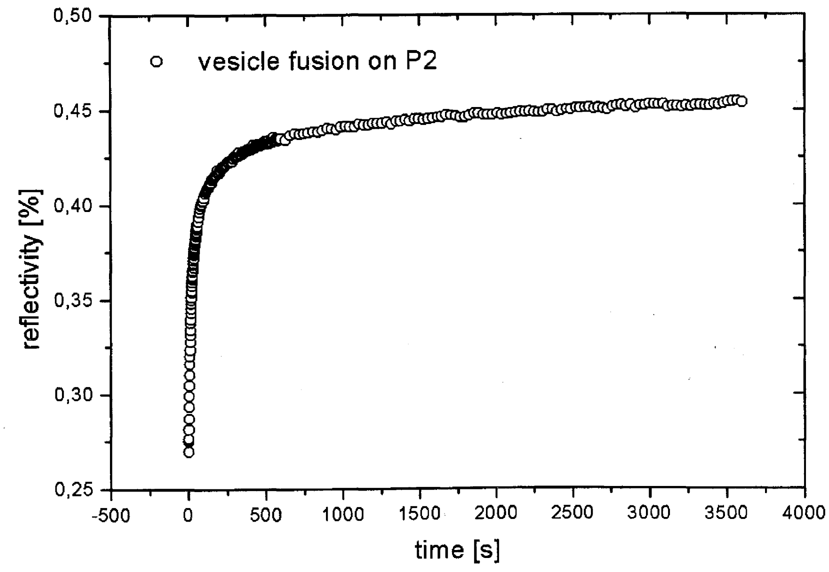

Figure 8. Monitored kinetic of the vesicle fusion on **P2**.

bly of biomaterials in nanosize dimensions. It could be shown that the hydrophilic behavior of polymer surfaces can be controlled by using charged groups. Contact angles depend linearly on the amount of charges bound to the polymers. Comparison of the charged polymers with their not charged equivalents showed a simple result. Only the charged polymer surfaces were able to initiate the fusion of vesicles on the surface. Up to now, it is not yet clear if the vesicle fusion process can be described by the two step model. Further measurements have to be done, before a detailed description can be published.

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