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# AFM characterization of spin-coated multilayered dry lipid films prepared from aqueous vesicle suspensions

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ABSTRACT

We present a detailed AFM study on multilayered dry lipid films prepared from aqueous vesicle suspensions. Different preparation techniques were applied in order to optimize the preparation of homogeneous lipid films of various film thicknesses. Suspensions of preformed DOPC/DPPC vesicles were adsorbed onto indium tin oxide-coated glass coverslips, a substrate also commonly employed for the formation of giant liposomes. We found that the homogeneity of the lipid films could substantially be improved when applying a spin-coating step during the film preparation. These films were much more homogeneous than those prepared by conventional drop-casting and in addition the film thickness could be controlled. When using a combination of vesicle adsorption and spin-coating the quality and thickness of the films depended crucially on the lipid concentration of the vesicle suspension, the adsorption temperature and the adsorption time. For lipid films prepared by direct spin-coating the lipid concentration and the applied spin-coating sequence were critical parameters for the quality and thickness of the deposited lipid films.



## 1. Introduction

Model membrane systems such as solid-supported lipid bilayers or giant liposomes have drawn considerable interest over the past years. Solid-supported bilayers are especially suited for studying domain formation and lateral lipid organization and are often investigated by high-resolution techniques like atomic force microscopy [1–14]. Much effort was devoted to the preparation of the supported lipid bilayers and various preparation techniques were proposed. Especially the Langmuir–Blodgett-technique and the vesicle fusion method are often applied [9,15–18]. Recently, also the preparation of supported lipid bilayers by spin-coating was suggested and this technique was successfully applied for various lipid systems [19,20].

Giant liposomes on the other hand are often used as models for cellular systems [21–26]. Due to their size (>10 μm diameter) they can be observed by optical techniques such as fluorescence wide-field microscopy and are often employed, e.g., for studying domain formation, membrane curvature and lipid or protein diffusion [27–33]. Preparation of giant liposomes is in most cases achieved by the method of electroformation [34–36]. This technique relies on the deposition of a lipid film onto a substrate coated with indium tin oxide (ITO) or onto an electrode and the subsequent application of *ac* electric fields to the hydrated lipid film, which yields predominantly giant unilamellar liposomes.

One crucial parameter for the quality of the resulting giant liposomes seems to be the quality of the deposited lipid film [37,38]. Unlike solid-supported lipid bilayers, which consist of a single lipid bilayer, lipid films for the electroformation of giant liposomes are required to be built up of at least several layers. Estimates of the appropriate film thickness vary from 5 to 50 bilayers, corresponding to heights between approximately 25–250 nm [34,37,39]. The most common method for the preparation of these films on a substrate is the direct spreading of lipids dissolved in organic solvents despite the fact that this preparation method can result in inhomogeneous lipid films [20,37,38]. The homogeneity of the films prepared from lipids dissolved in organic solvents could, however, substantially be improved by using the spin-coating technique [19,20,37,40–42]. In Ref. [37] it was shown that the preparation of lipid films for the electroformation of giant liposomes by spin-coating solutions of lipids results in more homogeneous films with a controllable film thickness. Moreover, the authors could show that spin-coated lipid films are more suited for the electroformation of giant liposomes than lipid films, which were prepared by the deposition of lipid

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droplets on the substrate. Giant liposomes prepared from spin-coated films were found to be larger in size and more abundant than those derived from non-spin-coated films [37].

In most studies on giant liposomes the preparation of the liposomes was based on lipid films deposited from lipids dissolved in organic solvents. However, there are also several studies on giant liposomes with membrane-reconstituted proteins [25,26,43]. Usually, the membrane proteins are first reconstituted in preformed large unilamellar vesicles resulting in proteoliposomes and in a second step the aqueous proteoliposome suspension is deposited onto an ITO-coated substrate and left to dry on the substrate [25,26].

Unlike for lipids dissolved in an organic solvent, no systematic study has been performed to our knowledge on parameters that affect the structure of multilayered lipid films prepared from aqueous vesicle suspensions. Furthermore, no method for the preparation of solid-supported lipid films from aqueous vesicle suspension has been proposed that both ensures a homogeneous lipid film structure and controls the resulting film thickness.

To this aim we present an atomic force microscopy study on solid-supported multilayered dry lipid films prepared from aqueous vesicle suspensions with and without applying a spin-coating step in the preparation. We show how the concentration of the vesicle suspension, the adsorption time, the adsorption temperature and the applied spin-coating sequence influence the resulting lipid films and compare the morphology of these films with those prepared by the conventional drop-casting method. As a model system for our study we used dioleoyl-phospatidylcholine/dipalmitoyl-phosphatidycholine (DOPC/DPPC), which has recently be used in several studies with giant liposomes [32,33,44] and in studies elucidating, e.g., formation of solid-supported lipid bilayers and membrane domain formation [3,11,32,45–52].

## 2. Materials and methods

### 2.1. Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (dioleoyl-phospatidylcholine DOPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (dipalmitoyl-phosphatidylcholine DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and stored in chloroform at −20 °C until use. Lipids were used without further purification. All other chemicals were of reagent grade. (18 mm × 18 mm) indium tin oxide (ITO) coated microscope coverslips (ITO glasses) were prepared by the optical workshop at the University of Bayreuth by sputtering ITO onto pre-cleaned microscope coverslips (thickness no. 1; Paul Marienfeld GmbH, Germany) using the following parameters for the sputtering system (BAS450, Balzers, Liechtenstein): 400 W RF power, 7 nm/min deposition rate, ∼175 nm deposition thickness, $10^{-6}$ mbar base pressure, $2 \times 10^{-3}$ mbar argon pressure. The ITO glasses displayed a sheet resistance of 30–40 Ω as checked by the four-point-probe method (Signatone SYS-301, USA). The quality of the coating was furthermore controlled by scanning electron microscopy and atomic force microscopy.

### 2.2. Sample preparation

For the preparation of the stock solutions, DOPC and DPPC were first mixed in chloroform at a ratio of 4:1 (w/w) in a glass vial. The solvent was then evaporated under a constant flow of nitrogen. In order to remove all traces of organic solvent, the lipid films were kept under vacuum overnight. The lipid films were resuspended in 10 mM glycyl–glycine (GlyGly) buffer (pH 7.8) to a final concentration of 2 mg/ml or 10 mg/ml, respectively, and gently vortexed for a few minutes. Phospholipid vesicles were prepared by mild sonication of the suspension using an ultrasonic microtip (Labsonic P, Sartorius, Göttingen, Germany), until the suspension was almost transparent. Size and size distribution of the resulting vesicles was checked by dynamic light scattering using an ALV goniometer system with fiber optic detection and average diameters of about 180 nm were found for vesicles prepared from suspension with a lipid concentration of 2 mg/ml and 10 mg/ml, respectively.

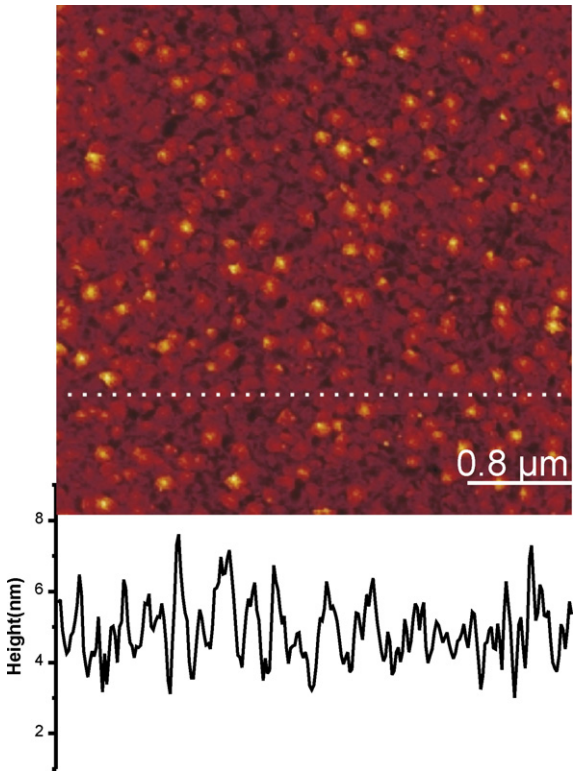


**Fig. 1.** (4 µm × 4 µm) AFM topography image (top) and height section analysis (bottom) of an ITO-coated glass substrate. The ITO-coated glass substrate had been cleaned and hydrophilized with chromosulphuric acid for 5 min, thoroughly rinsed with ultrapure water and dried under nitrogen. The dotted lines in the topography image indicate where the height profile was extracted. The scale bar in the topography image is 0.8 µm. The image was recorded in MAC mode with a scan speed of 1 line/s.

In order to clean and hydrophilize the surface of the ITO glasses, those were cleaned with chromosulphuric acid for 5 min, rinsed with copious amount of ultrapure water and dried under nitrogen. The surface roughness of the ITO glasses was not significantly affected by this treatment as checked by atomic force microscopy (AFM) (Fig. 1). Solid-supported multilayered dry films were then prepared on the freshly cleaned ITO glasses by the following methods:

A. Adsorption of a defined vesicle suspension volume (80 µl, 120 µl, or 80 µl + 80 µl) at room temperature ($T$ = 22 °C) for 3, 6, or 10 min, respectively. At the end of the adsorption period the samples were spin-coated using a sequence of 10 s at 500 rpm followed by 50 s at 2500 rpm.
B. Adsorption of a defined vesicle suspension volume (80 µl, 120 µl, or 80 µl + 80 µl) at $T$ = 60 °C for 3, 6, or 10 min, respectively. At the end of the adsorption period the samples were spin-coated at room temperature using a sequence of 10 s at 500 rpm followed by 50 s at 2500 rpm.
C. Direct spin-coating of the vesicle suspension using various spin-coating sequences.
D. A drop of 40 µl of the vesicle suspension was left to dry on the ITO-coated glass. In order to dry the resulting films thoroughly, the samples were left under vacuum overnight.