Electrical resistivity of the liquid phase of vesicular suspensions prepared by different methods

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Abstract. Giant lipid vesicles are obtained mainly by two methods of formation: (i) electroformation and (ii) gentle hydration (spontaneous swelling). Very often the electroformation is carried out in experimental cells consisting of indium-tin oxide (ITO) coated plates as electrodes and various polymer spacers. In the present work, the influence of the ITO coatings and the polymer spacers on the electrical resistivity of the liquid medium of electroformed vesicle suspensions is examined by means of electrochemical impedance spectroscopy (EIS). Our study is intended to point out possible implications of the electroformation method, especially in cases when phenomena, related to electric properties of the vesicle membranes, are investigated.

1. Introduction
The experimental research in membrane biophysics widely uses giant unilamellar lipid vesicles (GUVs) as a simple model for studying the physical properties of biological membranes [1]. Together with the classical method of spontaneous swelling, the electroformation method [2] is widely applied for GUV formation. Traditionally conceived to obtain vesicles in ion-free conditions, it has been developed recently for preparation of giant lipid vesicles in physiological conditions [3,4] i.e. upon conditions of high ionic strength of the aqueous medium. At present it is widely accepted that GUV electroformation is a method of choice to produce giant vesicles also from native cell membranes assuring the preservation of the membrane compositional asymmetry and that it can be adapted to various membrane systems including synthetic lipid mixtures, natural lipid extracts, and bilayers containing membrane proteins [3,5]. It has been shown recently in the literature that the electric fields applied during electroformation can alter the chemical structure of the lipid molecules by peroxidizing lipid acyl chains, thereby modifying the phospholipid composition and material properties of the synthesized vesicles, these effects being more pronounced for poly unsaturated acyl chains [6].

There are two general types of electroformation cells most commonly used. In the first one, the electrodes are two parallel platinum wires with diameter of several hundreds of micrometers placed

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several millimetres apart. In the second type of electroformation cells, two indium-tin dioxide-coated (ITO) plates, separated by a non-conductive spacer are used as electrodes. A possible configuration of such kind of electroformation cell is shown in figure 1. An inert material has to be used as a spacer between the two ITO-plates in order to assure stable quality of the aqueous phase during electroformation. The silicon-based two-component organic polymer polydimethylsiloxane (PDMS) [7] has gained increasing popularity and wide application in the biophysical experimental research and especially in microfluidics (soft lithography).

Good control of the composition of the suspending medium and lack of contamination of unknown origin and concentration are of major significance for the quality of vesicular suspensions. Especially ionic impurities have to be avoided when phenomena, depending on the electrolyte concentration in the aqueous phase, are being studied.

In this respect, it is important to investigate the contribution of all materials used to construct the electroformation cell on the electrochemical properties of vesicle suspensions and this constitutes the aim of the present study. The electrical resistivity of the liquid medium of the vesicular suspension is very sensitive to the presence of ionic impurities even with extremely low concentration. This is the reason why the experimental measurement of this quantity permits to verify whether the ITO coatings and the plastic spacers of the electroformation cell, as well as the lipid itself contribute to the contamination of this medium.

2. Materials and methods
Double-distilled water was obtained in a quartz distiller without ion-exchange filter at the outlet. Sucrose (cat. num. S7903, Sigma Ultra ®) was purchased from Sigma-Aldrich Chemie (Germany) and D(+) Glucose monohydrate (cat. num. 108346) – from Merck (Germany). Polydimethylsiloxane (PDMS, Sylgard 184 silicone elastomer kit) was provided by Dow Corning GmbH (Germany), and manipulated and prepared according to the manufacturer’s prescriptions with base to curing agent ratio from 10:1 to 8:1.

2.1. Preparation of lipid coatings
Double-distilled water, equilibrated in air, was used to prepare all the experimental solutions. Methanol and chloroform (“for analysis” grade) were purchased from Fluka Inc. (Germany). In our

![Figure 1. Experimental cell for electroformation – two ITO-coated plates, separated by a silicone (polydimethylsiloxane) spacer. The metal contacts between the conductive plates and the electrical wires are situated outside the cell. The used cell had thickness ~3.5 mm and area ~ 10 cm²](image-url)
experiments the lipid 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-choline (SOPC), cat. N° 850467 (Avanti Polar Lipids Inc., AL, USA) was used.

A typical electroformation cell is shown in figure 1. It consists of two ITO-coated plates, separated by a silicone (polydimethylsiloxane) spacer. Lipid depositions were made by the careful spreading of 50 µL of SOPC solution with concentration of 1 g/L in chloroform-methanol (9:1 volume parts) organic solvent on the ITO-side of each ITO-coated glass plates. In our experiments freshly prepared organic solution of the lipid (previously lyophilized and kept under vacuum at -20°C) was used, unless explicitly indicated. The lipid was held for at least 2 hours under vacuum until completely dry.

2.2. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) measurements were performed with an FFT impedance spectrometer, as described earlier [8]. A broad-band voltage signal, with a small amplitude (10 mV), representing a sum of ca. 40 selected sine waves, was applied as perturbation. The measurements were performed in a two-electrode electrochemical cell configuration, either the described above electroformation cell, or a small glass cell with two Pt-electrodes each of 25 mm² surface area placed 1 cm apart. A fast potentiostat serves to interface the cell.

The impedance data quality was validated by comparing the power spectra of the perturbation voltage and the response current for each measurement, as described earlier [9].

An EIS measurement gives information about the spectrum of the sample impedance. For our analysis we use the value of the total (not the specific one) active resistance of the medium.

3. Results and discussion

Usually, vesicular suspensions are prepared in the manner that vesicular membranes enclose sucrose solution and the suspending medium is a glucose solution with concentration, assuring in equilibrium iso-osmolar conditions across the vesicle membrane. In order to verify if the presence of glucose (and respective ionic impurities) influences the electrical conductance of the aqueous phase we measured the resistance, $R$, of 0.2 mol/L aqueous solution of glucose and compared it with the value obtained for double-distilled water (Table 1). In the frames of the experimental precision the difference between the values of $R$ in the two cases is negligible. It can be concluded that the presence of 0.2 mol/L of sugar in the water does not influence its resistivity markedly.

In our recent study [10], a strong dependence of the electrodeformation of giant vesicles in alternating electric fields on the method the vesicles were prepared (gentle hydration, electroformation in various cells) was obtained and was ascribed as a result of an uncontrolled presence of ionic impurities in the aqueous solution. These results were confirmed by our measurements of the resistivity of vesicle suspensions (not shown) – higher resistance for suspensions, which were spontaneously swollen and lower for suspensions, electroformed in different electroformation (EF) cells using various materials as spacers. In order to reveal the sources of these differences we tried to answer the practical question if there is any contribution of the materials participating in the construction of the EF chambers, namely the ITO coating and the PDMS used to prepare the spacers.

| Liquid phase                    | R [MΩ] |
|--------------------------------|--------|
| Double distilled water          | 0.509  |
| 0.2 mol/L Glucose solution      | 0.519  |
Two types of EIS measurements were performed. The first configuration of the experimental cell consisted of a glass cell with two platinum electrodes (each of them with dimensions 5x5 mm$^2$). Small pieces of freshly prepared or soaked PDMS were added and the evolution with time of the resistance of the aqueous phase in the glass cell in presence of PDMS was measured. The results are presented in figure 2. Small fluctuations in the resistance, obtained for distilled water, are due to the temperature fluctuations during measurements. It is important to underline that before measurements double-distilled water was kept in contact with air for at least a day, i.e. carbon dioxide concentration in the water was in equilibrium with air and remained constant during measurements [11,12]. Measurable influence of sodium ions dissolved from the glass walls on the water conductivity was rejected after a control measurement of the water resistance in a quartz cell. The decrease (more than two times) of the resistance in the case of immersed freshly prepared PDMS is possibly due to emission of residual products after the polymerisation of the two-component silicone polymer PDMS. This was confirmed by EIS measurements performed after soaking the PDMS pieces for 72 hours in double-distilled water, which has been regularly changed every few hours. In this case the decrease of the water resistance in the glass cell was considerably weaker than the change measured in presence of fresh polymer, cf figure 2. This result as well as measurements with different base-to-curing agent ratios (see above) show that after the polymerisation of PDMS there are residual ionic products considerably increasing the water conductance, which can be easily washed up after long enough soaking of the fresh polymerised PDMS spacers.

Figure 2. Resistance (measured by EIS; values taken at 219 Hz) of double-distilled water (●), double-distilled water with new PDMS submerged in it (▲) and double-distilled water with PDMS previously soaked for 72 hours (■). All the measurements are performed with platinum electrodes (5x5 mm$^2$) in closed glass cell.
These observations were confirmed in the second experimental series, where resistance measurements were performed directly in the EF cell as shown in figure 3. As known from the literature deionised water equilibrated with air (equilibrium carbon dioxide concentration reached) has specific resistance in the order of 1 MΩ cm [12]. Our control measurement of the resistance of the EF cell filled with double distilled water and with PDMS spacer, which had been preliminarily soaked and washed, permitted to calculate the electrical resistivity of the water using the cell dimensions. Our estimation gave quite similar value to the value published in [12]. This result is an indication for the lack of emission of any undesirable ionic impurities from the ITO during the time of measurement. In order to verify if the ionic purity of commercially available lipids is detectable, we performed the same measurements but with lipid depositions on the ITO-side of each ITO-plate. The lipid depositions were prepared identically to the preparation of the lipid depositions according to the protocol described in the previous section. As it can be seen in figure 3, the presence of the new lipid film in the EF cell reduces the resistance less than 15%. This decrease is obtained to be much more pronounced if the lipid depositions were made from an “old” lipid solution (prepared a week before and kept at -20°C). This result indicates the presence of ionic impurities in the lipid stock solution due to its chemical degradation and leads us to the conclusion to recommend the usage of only freshly prepared solutions of lipids in chloroform. In the literature it has been already warned of the effects of micromolar concentrations of ion impurities, contained in the commercial lipids, if special efforts for their purification are not exerted [13].

The most considerable reduction (~50 %) of $R$ of the EF chamber was measured when a freshly polymerized spacer from PDMS was used (figure 3). The decrease of $R$ when the spacer has been prepared about three weeks before and meanwhile left on air was less but still important (~30 %).
Usually, the overall properties of vesicular suspensions obtained via electroformation in electroformation cells composed of ITO-plates and polymeric spacers are implicitly supposed to be equivalent to those of vesicles spontaneously swollen. Our experimental study is intended to draw attention to the fact that in the particular cases, described above, the use of the electroformation is susceptible to alter the vesicle properties due to the contamination of the suspending medium with uncontrolled ionic impurities, which could influence the conditions of electroformation and/or be adsorbed on the membranes or change the vesicle behaviour in certain conditions [10]. We believe that our results could be of use not only for the electroforming biophysicists but also for this part of the biophysical community working with microfluidic devices on the basis of polydimethylsiloxane.

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