ISSN 1330–0008
CODEN FIZAE4

THIN–FILM SUPPORT FOR LIPID BILAYERS

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Received 7 April 1995

Revised manuscript received 27 June 1995

UDC 538.951

PACS 68.65.+q, 73.61.-r

A strip thin-film support consisting of compact or planar interdigitated array electrodes and glass substrate is presented. A novel method of forming of s-BLM on the thin metallic film (sl-BLM) has been developed. Electromechanical characteristics of BLM were studied and the measured value of elasticity modulus perpendicular to the bilayer plane was found to change considerably with increasing dc voltage. The presented results evidence the fact that the values of $E_{\perp}$ are are strongly influenced by the degree of roughness of the metal surface. It is shown that sl-BLM modified by electron carrier can be used as a simple pH sensor.

1. Introduction

A rapid development in the miniaturization of (bio)chemical sensors has increased the significance of thin film technologies. Thin films are very important constituent of the (bio)chemical-electronic interfacing down to the micro- and nanometric scale. Their application in electrochemical microsensors can be divided into two categories: 1. Thin-fim electrodes (arrays) that serve as a base support of microsensors; 2. Sensitive anorganic-, organic- and bio–materials (like membranes, enzymes) that are used in the form of thin films. They can be utilized for miniaturization and integration of analytical electrochemical devices, e.g. miniature Clark-type oxygen thin-film sensor [1], planar thin-film differential-conductivity urea sensor [2] and integrated glucose and lactate thin-film microbiosensor [3]. Special kind of microelectrodes - the interdigitated array (IDA) of thin-film electrodes with the microband and gap widths of μm-order are very promising for electrochemical sensing. All biological membranes are based on double molecular monolayers - the bilayer lipid membrane (BLM). The possibility of formation of BLM on a solid support , the s-BLM, [4] seems to offer an attractive component of biosensors and biomolecular electronic devices.

In this paper, the thin-film support is described as a part of the integrated TF-μ S [5]. We analyzed the physical properties of supported bilayer membranes formed on smooth thin-film support (sl-BLM) and we demostrated the possibility of use of sl-BLM, modified by electron carrier TCOBQ (tetrachloro-o-benzoquinone), as a simple pH sensor.

2. *Experimental*

Two versions of thin-film support for s-BLMs have been prepared: (i) thin-film compact Pt electrodes (area of 0.36 mm^2); (ii) planar thin-film IDA Pt electrodes with the microband/gap width of 5 or 10 μm. The thin-film technology was based on the R.F. sputtering of Pt film, the patterning of microelectrodes by the “lift-off” technique and the forming/patterning of polyimide protective film [5].

A novel method of lipid bilayer preparation on thin Pt-layer (sl-BLM) was developed. Lipid solutions used were: soya-bean phosphatidylcholine (SBPC) (Calbiochem); in n-hexadecane (Merck) (40 mg/ml, w/v); or 1-monooleoyl (GMO) (Sigma) + squalene (Kodak) (20 mg/ml w/v). An atomic clean metal surface is required for the formation of BLM. Before the application of an appropriate lipid solution, the surface of thin films was bombarded by Ar-ions in order to remove adsorbed impurities on the film surface (Fig. 1). Immediately after ion treatment, a small drop of lipid solution of GMO + squalene (20 mg/ml w/v) was applied on compact or IDA electrodes through an opened window in the polyimide layer, and then thin-film was placed in 50 mM KCl solution (pH $\approx$ 6). Upon immersion of the support in the electrolyte, a spontaneous thinning process is observed, and measurement of the electrical capacitance indicates that a bilayer is formed. Schematic representation of arrangement of the lipid bilayer of convenient BLM [6], s-BLM and sl-BLM is shown on Fig. 1. Electrical contact was made directly to one side of the sl-BLM through the supporting metal surface, while a Ag/AgCl or calomel electrode was used to establish contact to the other side through the aqueous phase (see Ref. 4 for more details).

Both eleaticity modulus $E_{\perp}$ and the membrane capacitance C were measured by applying an ac voltage (amplitude U = 50 mV and frequency f = 1 kHz) to the lipid bilayer [7]. An additional valuable parameter for an analysis is the phase shift φ, defined as the difference of phase between the measured third harmonic component and that expected for an ideally elastic body [6]. Electric potentials and membrane conductance were measured by standard techniques (see e.g. Ref 8). Topology of the s-BLM and sl-BLM supporting surfaces were measured with the TALYSTEP system.

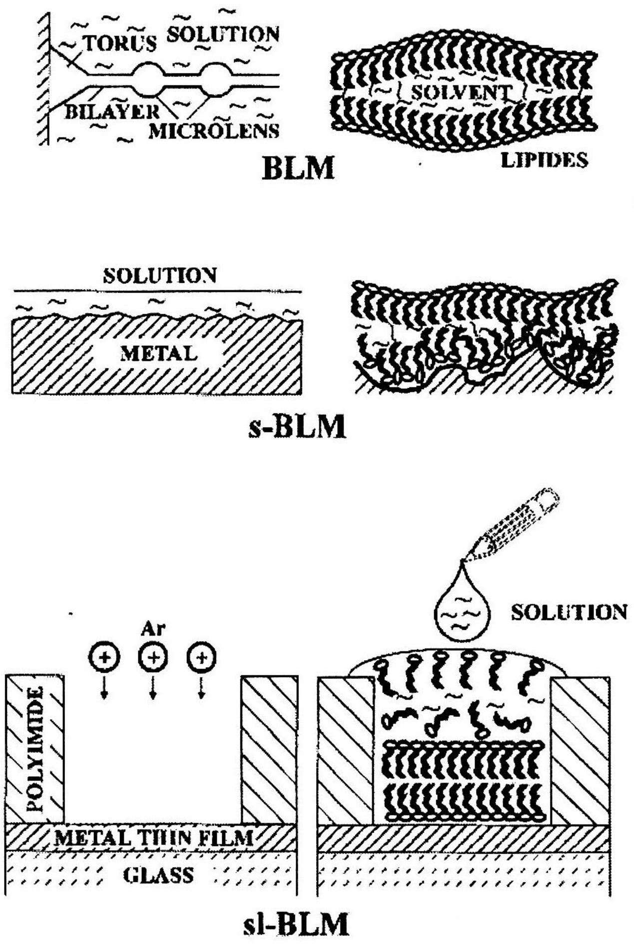

Fig. 1. (top) Schematic representation of the structure of bilayer lipid membranes formed on: (top) the wall of a cup made from hydrophobic material (BLM); (middle) a fresh cut end of a metall wire (s-BLM); (bottom) the surface of thin metal film (sl-BLM).

3. Results and discussion

The differences between s-BLM and sl-BLM may stem from the differences in the topologies (surface profile and boundary characteristics) of the metal supports. The roughness of Pt-film surface lies in the range 0.6-1.4 nm, and for the cut wire tip, the roughness reaches 100-1000 nm.

The calculated values of $E_\perp$ for sl-BLM varied greatly with the applied potential. It was up to ten times higher, $E_\perp = (5.5 \pm 0.1)\times 10^6$ Pa, than for s-BLM [5] at zero applied potential. Similar differences in $E_\perp$ were also obtained for SBPC + n-hexadecane membranes. The values of $E_\perp$ for sl-BLM are similar to those of conventional BLM [9]. We have found a complicated dependence of C, $E_\perp$ and φ on the dc voltage applied across sl-BLM with hysteresis effects (Fig. 2).

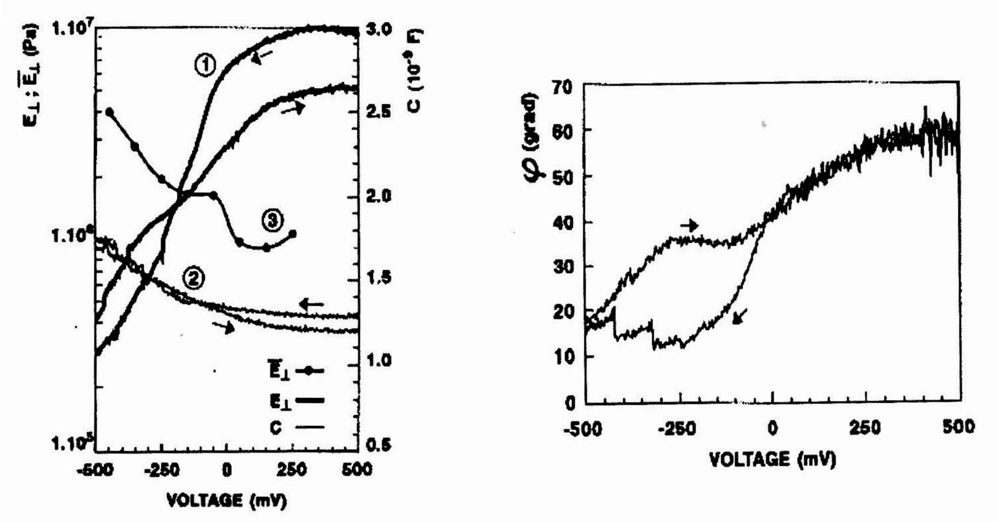

Fig. 2. Plot (a) of elasticity modulus $E_\perp$ (1), membrane capacitance $C(2)$, differential static modulus $\overline{E}_\perp$ (3) and (b) of phase shift φ vs. magnitude of dc voltage U during its cyclic application to sl-BLM with scan rate 500 mV/min *(lipid: GMO squalene). Arrows indicate the direction of dc voltage changes.*

The method of measurement of BLM elasticity by means of the third harmonic of transmembrane current is in fact a differential method for measurements on supported membrane, as small variation of voltage is applied to a membrane constricted by a high background potential. From the measurements of $C(U)$ presented in Fig. 2, which correspond roughly to "static" measurements, we should be able to calculate the differential static modulus $\overline{E}_\perp$, defined by:

$$\overline{E}_\perp(U) = -\frac{\Delta p(U)}{\Delta d/d} = \frac{\Delta p}{(C(U_1) - C(U_2))/C(\overline{U})}$$

where the change of the pressure $\Delta p = p_1 - p_2$ is the difference of the pressures constricting the membrane at two voltages U_1 and U_2 and $C(\overline{U})$ is the mean membrane capacitance at the two voltages.

For sl-BLM (Fig. 2), one can see that the differential static modulus $\overline{E}_\perp$ is smaller than the dynamic one for voltages less negative than -150 mV, whereas for more negative voltages $\overline{E}_\perp > E_\perp$. This last result is contradictory, as, barring saturation effects, a dynamic elastic modulus should always be higher than the static one. Thus one can consider that the derived value of $\overline{E}_\perp$ is an elasticity modulus for sl-BLM only at voltages $U > -150$ mV. For the voltages $U < -150$ mV, the derived value presumably reflects aberrant conditions, such as saturation of $d(U)$ or the onset of BLM breakdown.

The electrical conductance of sl-BLM were 1 - 8 nS (without and with TCOBQ); the membrane potential of the order of 10 mV has been pH dependent with significant influence of the presence of TCOBQ. Compared with the s-BLM [4], the sl-BLM on IDA electrodes displayed an electrical conductance, which was considerably higher (by a factor 20 to 40 times). Despite that, the sl-BLM displayed the same membrane potential response to pH changes (Fig. 3) as found with stainless steel wire based s-BLM [5].

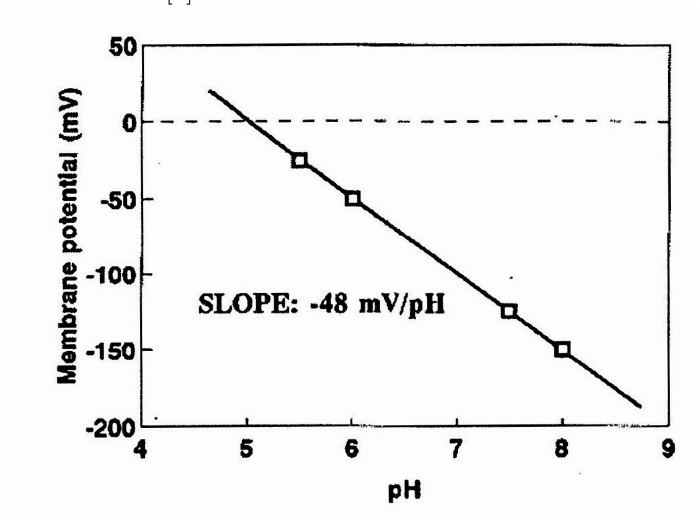

Fig. 3. The dependence of potential of sl-BLM doped by TCOBQ on pH factor.

4. Conclusion

It was proven that thin-films are suitable for the supporting of BLMs. The elastic properties of sl-BLM are consistent with the present knowledge of the membrane phenomena. Electromechanical parameters are more stable and reproducible for sl-BLM than for s-BLM. The evidence is presented that sl-BLM bilayer system doped with electron carriers TCOBQ can be used as a pH sensor.

Acknowledgement

This work was financially supported by grants: No.CIPA-CT94-0231, funded by The Commission of the European Communities; No. HRN-5600-G-2024-00, Program in Science and Technology Cooperation, Office of the Science Advisor, U.S. Agency for International Development; No.1739, Slovak Agency for Science.

References

1) M. Koudelka, Sensors and Actuators **9** (1986) 249;

2) P. Jacobs, J. Suls and W. Sansen, Sensors and Actuators **B20** (1994) 193;

3) G. Urban, G. Jobst, E. Aschauer, O Tilado, P. Svasek, M. Varahram, Ch. Ritter and J. Riegebauer, Sensors and Actuators **B18-19** (1994) 592;

4) H. Ti Tien and Z. Salamon, Bioelectrochem. Bioener. **22** (1989) 211;

5) V. Tvarozek, H. Ti Tien I. Novotny, T. Hianik, J. Dlugopolsky, W. Ziegler, A. Leitmannova-Ottova, J. Jakabovic, V. Rehacek and M. Uhlar, Sensors and Actuators **B 19** (1994) 597;

6) P. Mueller, D. O. Rudin, H. T. Tien and W.C. Wescott, Nature **194** (1962) 979;

7) V. I. Passechnik and T. Hianik, *Transversal Elasticity of Lipid Membranes*, Veda, Bratislava, 1991;

8) W. Ziegler, C.L. Slayman and C.P. Cartwright, Gen Physiol. Biophys. **12** (1993) 429;

9) T. Hianik, J. Dlugopolsky and M. Gyepessova, Bioelectrochem. Bioenerg. **31** (1993) 99.

TANKOSLOJNA PODLOGA ZA DVOSLOJEVE LIPIDA

Opisuje se podloga u obliku tanke trake koja se sastoji od nizova kompaktnih ili ravnih elektroda i staklene osnove. Razvijena je nova metoda pripreme dvoslojne lipidne membrane (s-BLM) na tankom metalnom sloju (sl-BLM). Proučavana su elektromehanička svojstva BLM i mjeren je modul elastičnosti okomito na površinu dvosloja za koji se ustanovilo da se znatno mijenja s DC naponom. Izloženi rezultati ukazuju da na vrijednosti elastičnog modula $E_\perp$ jako utječe stupanj neravnosti površine metala. Izmijenjen sl-BLM može se upotrebljavati kao jednostavna pH proba.