Selective Permeable Membrane Based on Two Dimensional Cross-linked Polysiloxane LB Films for Nitric Oxide Sensor

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A sensitive and rapid measurement method for in-situ nitric oxide (NO) monitoring is a field of great interest since NO has been recognized as a molecular messenger in biological systems[1]. Amperometric determination of NO is one of the most convenient ways. However, such systems generally suffer from electrochemical interference by other oxidizable species such as nitrite, dopamine, adrenalin and L-ascorbic acid. The use of the coating layer for blocking the other electroactive interfering substances gives all the better selectivity for NO. Moreover, a micro NO-sensing electrode equipped with a scanning electrochemical microscopic system is a useful tool for in vivo NO monitoring In order to achieve a NO-microelectrode sensing system, the development of an appropriate ultrathin permselective membrane is of importance.

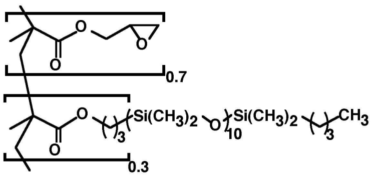
We have described homogeneous two dimensional (2D) cross-linked LB films of an amphiphilic copolymer containing the side chain of polysiloxane and epoxy group (Figure 1a)[2]. The characteristic of the film is cross-linked structure between epoxy groups on the copolymer and amino groups on polyallylamine as a cross-linker at air-water interface (Figure 1b). Such LB films with liquid crystal-like elastic structures will demonstrate superior performance as permselective membranes rather than crystalline LB films. Here, we present a method of the preparation of the NO-selective (micro)electrode based on the 2D cross-linked siloxane LB films.

Figure 1 outlines a fabricating procedure of the 2D cross-linked polysiloxane monolayers as previously reported[2]. For the amperometric measurements, the LB films modified Pt electrode, an Ag/AgCl electrode and a Pt wire were employed as the working, reference and auxiliary electrodes, respectively. The electrodes were placed in a stirred 0.1 M sodium phosphate buffer solution (pH 7.0, 20 mL) at 25.0 ± 0.2 ˚C under argon atmosphere. The magnetic stirrer and the test solution were placed underneath the Faraday cage without environmental noises. Then, either NO or interference solutions were added and the current responses at +0.85 V vs Ag/AgCl were recorded[3]. As a standard solution of NO, a NO-saturated pure water solution (NO concentration, 1.88 mM at 25 C˚) was used.

Surprisingly, the 2D cross-linked siloxane LB film shows high permselectivity for NO even in the monolayer thickness. Figure 2 shows the current response curves for the modified electrodes with the injection of NO and L-ascorbic acid solutions. In the bare electrode, the current increased immediately after the addition of samples. The modified electrode also exhibited a rapid response with the injection of NO. The current of L-ascorbic acid was drastically decreased by this modification (Figure 2b-2). This contrasted sharply with the small current decrease noted in the NO signals. The elimination of hydrophilic interferences would be dominantly originated from defect-free hydrophobic polysiloxane layers. In fact, the LB film without cross-linking revealed almost no restriction for interferences. It would indicate that the cross-linking is crucial to give a higher mechanical strength in the practical usage of ultrathin films on solid substrate.

As a NO sensor, these electrodes gave a linear current response up to 100 µM NO. The lower detection was 40 nM (signal-to-noise ratio; ca. 2). Furthermore, despite the use of ultrathin films, the modified electrodes were very stable and could be used repeatedly for more than half a year without degradation as sensors.

(a)**poly(oligosiloxane -*co* - glycidyl methacrylate)**



(b) **Polymer - polymer cross-linking at air-water interface**

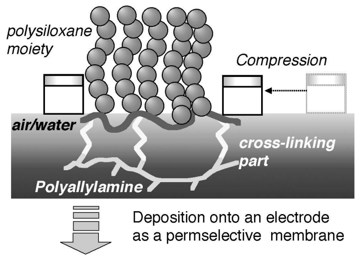


Figure 1. The chemical structure of siloxane copolymer (a) and a schematic diagram of the preparation of a 2D cross-linked LB film and modification of the film onto an electrode (b).

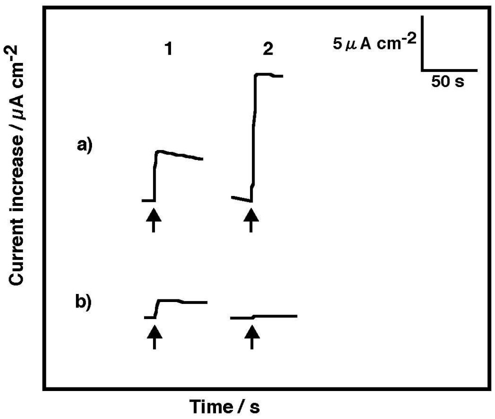


Figure 2. Current-time curves of the bare (a) and modified electrodes (1.6 mm-ø) in 20 μM NO (1) and 20 μM L-ascorbic acid (2). The arrows indicate the timing of injection.

[1] R. F. Furchgott and J. V. Zawadzki, *Nature*, **288**, 373 (1980); Li Jia, C. Bonaventura, J. Bonaventura, and J. S. Stamler, *Nature*, **380**, 221 (1996). [2] D. Kato, M. Masaike, T. Majima, Y. Hirata, F. Mizutani, M. Sakata, C. Hirayama and M. Kunitake, *Chem. Comm.*, 2616 (2002). [3] F. Mizutani, S. Yabuki, T. Sawaguchi, Y. Hirata, Y. Sato, and S. Iijima, *Sensors Actuators B*, **76**, 489 (2001).