

Selective Permeation of Nitric Oxide through Two Dimensional Cross-linked Polysiloxane LB Films

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Novel two-dimensional (2D) cross-linked polysiloxane LB films were prepared and applied to nitric oxide (NO)-permselective films in order to block other electroactive interfering substances, such as L-ascorbic acid, L-cysteine, and acetaminophen. The 2D cross-linked siloxane LB films deposited on Pt electrodes were remarkably effective in the elimination of the interfering responses and a rapid response to NO remained with modified LB films consisting of monolayers or even several layers.

Nitric oxide (NO) plays important role as a molecular messenger in biological systems. Since the epochal discovery that NO as endothelium-derived relaxing factor (EDRF) as reported by Furchtgott and co-workers,¹ a sensitive and rapid measurement method for in-situ NO monitoring has been sought. Amperometric determination of NO can be accomplished by the utilization of electrochemical oxidation on a metal² or catalyst-attached³ electrode. However, such systems generally suffer from electrochemical interference by other oxidizable species, e.g. L-ascorbic acid, L-cysteine, and acetaminophen, which results in a current response with a positive error. Other monitoring systems using microelectrodes have focused on in vivo local sensing system. In order to achieve a NO-microelectrode sensing system, the development of an appropriate ultrathin permselective membrane is required.

The Langmuir-Blodgett (LB) technique has been widely used to prepare ultrathin films with layered structures for gas separation,^{4a} gas/ion sensors,^{4b} lithography^{4c,4d} and so on. However, the negative characteristics of LB films, such as fragility and/or structural defects, have prevented their practical application as permeable films. The former is an inevitable problem in practical use and the latter will lead to a fatal decline of permselectivity. To overcome both the fragility and structural defects of LB films, a combination of soft segment and hard cross-linked structures in polymeric LB films is very effective. Nakashima and Kunitake have described homogeneous 2D cross-linked LB films of oligo(dimethyl-siloxane) copolymers, which were prepared by polymer-polyion complexation on an air-water interface.⁵ The LB films with liquid crystal-like elastic structures will demonstrate superior performance as permselective membranes rather than crystalline LB films. The present study demonstrates the selective permeation of NO through 2D cross-linked polysiloxane LB films.

Figure 1 represents a schematic illustration of the methodology used to deposit a siloxane LB film on a Pt electrode as a NO sensor. An amphiphilic siloxane copolymer (I) (Figure 1A) was prepared by the copolymerization of a vinyl monomer with

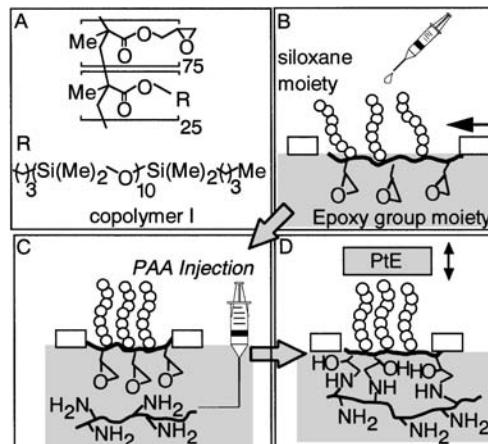


Figure 1. The chemical structure of siloxane copolymer I (A) and a schematic illustration of the preparation of a 2D cross-linked LB film (B, C) and modification of the film onto an electrode (D).

pendant oligosiloxane side-groups (SILAPLANE FM0711, Mw: 1000; Chisso Co., Japan) and glycidyl methacrylate, containing epoxy groups for covalent cross-linking. To form a Langmuir monolayer (L film), a chloroform solution of the copolymer was spread into a LB trough filled with pure water and compressed to 15 mN/m (ca. 1.2 nm²/siloxane chain unit) (Figure 1B).

The L film was cross-linked by the injection of a polyallylamine (PAA, Mw: 100000; Nitto Boseki Co., Japan) aqueous solution into the trough (final concentration of PAA, ca. 10 mM unit, Figure 1C). Epoxy groups on the copolymers react with the primary amino groups of PAA. It is noteworthy that the procedure used here, cross-linking after formation of the monolayer, is indispensable in forming a homogeneous cross-linked monolayer, as described in a previous paper.⁵ The usual procedure, which involved direct spreading of the reactive polymeric amphiphiles on aqueous polymer solution, induced a heterogeneous 3D-aggregation which was easily visualized by Brewster angle microscopy (BAM).

After injection of the PAA solution into the subphase, the surface pressure was gradually increased and reached a constant pressure after 5 min. 2D cross-linked L films were found to be microscopically homogeneous by BAM. Finally, the cross-linked L film was deposited onto a polished platinum disc electrode (diameter, 1.6 mm; BAS, West Lafayette, IN) using only the downward strokes of the horizontal deposition method (Figure 1D). After drying, the monolayer was repeatedly deposited in the

same manner.

Amperometric NO-sensing systems using LB film modified electrodes were examined for the ability to detect NO with interference from other oxidizable species. The electrodes were placed in a stirred 0.1 M sodium phosphate buffer solution (pH 7.0, 20 mL) at $25.0 \pm 0.2^\circ\text{C}$ under nitrogen atmosphere. Then, either NO or interference solutions were added and the current responses at $+0.85\text{ V}$ vs Ag/AgCl were recorded.⁶ As a standard solution of NO (99.7%, Japan Fine Products, Kanagawa), a NO-saturated pure water solution (NO concentration, 1.88 mM) was used.⁷

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 single layer modified electrode also exhibited a rapid response with the injection of NO. The currents of the interfering substances were drastically decreased by this modification (Figure 2b). This made sharp contrast to the small current decrease noted in the NO signals.

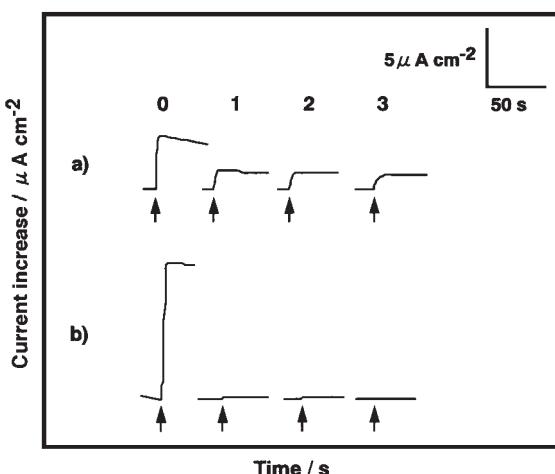


Figure 2. Current-time curves of the bare (0) and modified electrodes (the numbers 1–3 indicate the number of deposition times) in $20\text{ }\mu\text{M}$ NO (a) and $20\text{ }\mu\text{M}$ L-ascorbic acid (b). The arrows indicate the timing of injection.

Table 1. The influence of the number of deposition times of 2D cross-linked LB films on the amperometric response of NO and interfering substances

Deposited times	Response to $20\text{ }\mu\text{M}$ species/ $\mu\text{A cm}^{-2}$			
	NO	Acetaminophen	L-Cysteine	L-Ascorbic acid
Bare	3.69	7.38	5.00	11.8
1	1.51	N.D.	0.0650	0.180
2	1.32	N.D.	N.D.	0.170
3	1.11	N.D.	N.D.	0.0850

The currents were measured 50 s after the injection of samples. N.D.: Not detected.

Each current was averaged over three measurements, and was reproducible within $\pm 40\%$ in the measuring using three different electrodes.

Table 1 summarizes the current response on the modified electrodes for NO and the various interfering substances. The current response of NO decreased with as the number of deposited layers was increased. On the other hand, the responses of interfering substances were drastically decreased by the modification of siloxane LB films. The response of acetaminophen for bare electrode was 7.38, but no response was observed (less than 0.05) even for the single deposited electrode. The current responses of L-ascorbic acid and L-cysteine were decreased from 11.8 and 5.00 for a bare electrode to 0.18 and 0.065 for the single monolayer deposited electrode, respectively. In the case of the triple deposited electrode, responses of both L-ascorbic acid and L-cysteine were 0.08 and less than 0.05. The triple layer modified electrode demonstrates a relatively slow response against the bare and single layer modified electrodes as shown in Figure 2a-3. As a NO sensor, these electrodes gave a linear current response up to $20\text{ }\mu\text{M}$ NO. The lower detection was 40 nM (signal-to-noise ratio; ca. 2).

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. Despite the use of ultrathin films, the modified electrodes were very stable and could be used repeatedly for more than a week without degradation as sensors.

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