COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Permselective monolayer membrane based on two-dimensional cross-linked polysiloxane LB films for hydrogen peroxide detecting glucose sensors

Dai Kato,[a] Mio Masaike,[a] Takahito Majima,[a] Yoshiki Hirata,[b] Fumio Mizutani,[b] Masayo Sakata,[a] Chuichi Hirayama[a] and Masashi Kunitake*[a]

[a] *Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan. E-mail: kunitake@chem.kumamoto-u.ac.jp; Fax: +81-96-342-3679; Tel: +81-96-342-3675*

[b] *National Institute of Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*

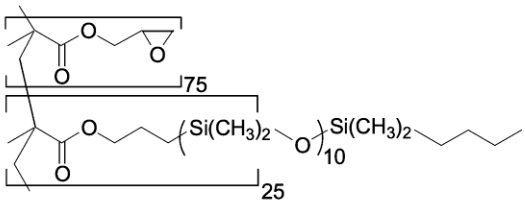
Received (in Cambridge, UK) 14th August 2002, Accepted 7th October 2002
First published as an Advance Article on the web 23rd October 2002

Novel two-dimensional (2D) cross-linked polysiloxane LB films were prepared and applied for glucose sensing as H_2O_2-permselective films in order to block other electroactive interferences, such as L-ascorbic acid, L-cysteine, uric acid and acetaminophen; the 2D cross-linked polysiloxane monolayers were remarkably effective in eliminating interfering responses and had a rapid response for glucose, even though the films were only a monolayer thick.

The Langmuir–Blodgett (LB) technique has been widely used to prepare ultrathin films[1] with layered structures for gas separation,[2] gas/ion sensors,[3] lithography[4] and so on. LB films as permselective membranes could be expected to have high selectivity because of their ordered structures and high permeation rates due to the monolayer thickness. However, the negative characteristics of the LB films, such as their fragility and structural defects, have prevented their practical application as permeable films. To overcome their fragility, polymeric LB films, particularly cross-linked polymeric LB films, are known to be effective. However, such polymeric LB films have been known to suffer from rather low quality owing to defects. Generally, almost all LB films with many defects possess polycrystalline structures, which can lead to a serious decline in permselectivity. As a new approach for the preparation of polymer LB films by the combination of soft segments and hard cross-linked structures, Kunitake *et al.* have described homogeneous 2D cross-linked LB films of oligo(dimethylsiloxane) copolymer, which were prepared by polymer–polyion complexation on an air/water interface.[5] Such LB films with non-crystal and liquid crystal-like elastic structures will give a good performance as permselective membranes.

We attempted to apply similar 2D cross-linked LB films to construct amperometric glucose sensors. The anodic detection of hydrogen peroxide (H_2O_2), which is produced by the glucose oxidase (GOx)-catalyzed oxidation of glucose, has proven to be the most convenient method of constructing amperometric glucose sensors.[6] H_2O_2 detecting electrode systems usually utilize a coating permselective layer that allows passage of the analyte (H_2O_2) but prevents interfering analytes from reaching the electrode surface.[7] The sensing system often suffers from electrochemical interferences by oxidizable species, which are found in human serum and foods, such as L-ascorbic acid, uric acid, L-cysteine and acetaminophen. Permselective films composed of siloxane-based polymer materials are often used because of their high selectivity and high permeability.[8] Very recently, Anzai and co-workers reported H_2O_2-selective membranes using polyelectrolyte 'layer by layer' thin films (typically three polymer layers).[9]

Fig. 1 shows a schematic illustration of the fabricating procedure for the glucose sensor, which includes the preparation of 2D cross-linked or non-cross-linked polysiloxane monolayers. An amphiphilic siloxane copolymer (I) was prepared by the copolymerization of a vinyl monomer with pendant oligosiloxane side-groups (SILAPLANE FM0711, M_w: 1,000; Chisso Co., Japan) and glycidyl methacrylate, which possessed the epoxy groups for cross-linking. To form a Langmuir monolayer, a chloroform solution of the copolymer was spread into a LB trough filled with pure water (Fig. 1A). The siloxane copolymers were found to form a stable monolayer at the air/water interface. Fig. 2 (dotted line) shows the surface pressure–area isotherm of the copolymer (I) on pure water. The isotherm shows a expanded phase; the molecular



Copolymer (I)

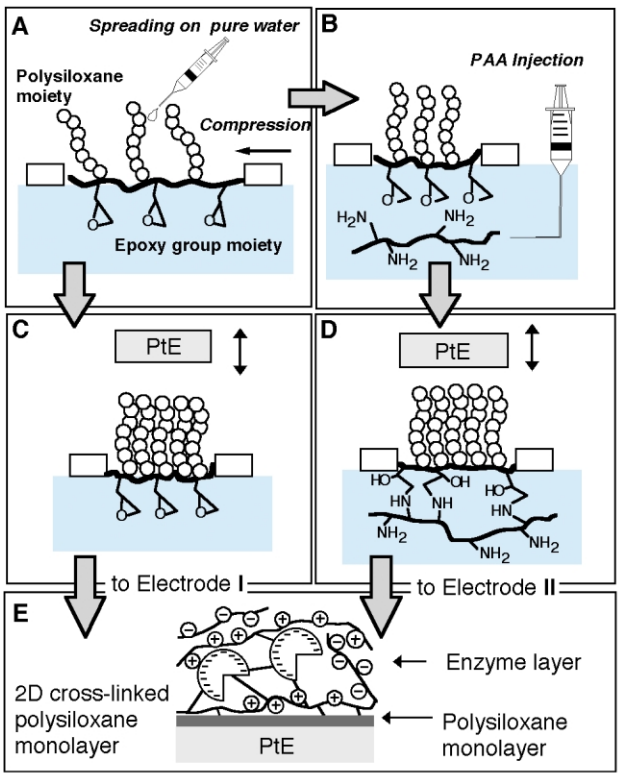


Fig. 1 Schematic illustration of the preparation of enzyme electrodes **I** (non cross-linked) and **II** (cross-linked).

DOI: 10.1039/b207957g

occupied area on pure water was approximately 1.2 nm^2/siloxane chain unit.

Epoxy groups from the copolymers can react with primary amino groups, even in aqueous solution, and the Langmuir film was cross-linked by the injection of a polyallylamine (PAA, M_w: 100,000; Nitto Boseki Co., Japan) aqueous solution in to the trough (Fig. 1B). The final concentration of PAA in the trough was *ca*. 10 unit mM. Note that this procedure, leading to cross-linking after the formation of the monolayer, is indispensable in forming homogeneous cross-linked monolayers, as described in a previous paper.[5] In the case of polyion-complexation between polymers at the air/water interface, the ordinal procedure, which is directly spreading the polymeric amphiphiles on the aqueous polyion solution, leads to the formation of a three-dimensional aggregate before the evaporation of the spreading solvent.

After the injection of the PAA solution into the subphase, the surface pressure was gradually increased and reached a constant pressure after 5 min. Fig. 2 (solid line) shows the isotherm of the cross-linked polysiloxane L film on pure water. The arrow in Fig. 2 indicates the pressure jump owing to the injection of PAA. Both non-cross-linked and 2D cross-linked monolayers were found to be microscopically homogeneous by Brewster angle microscopic observations. A single LB film was deposited onto a polished platinum disc electrode (diameter, 1.6 mm; Bioanalytical Systems, West Lafyette, IN) by a downward stroke of the horizontal deposition method (Fig. 1D, electrode **II**). The electrode without cross-linking was also prepared as electrode **I** (Fig. 1C). GOx (EC 1.1.3.4, from *Aspergillus sp*, 150 U mg^{-1}; Toyobo Co., Japan) was immobilized into polyion complex layers consisting of PAA and poly(sodium 4-styrenesulfonate) (PSS, M_w: 70,000; Aldrich, WI) according to previous reports (Fig. 1E).[10] The remaining primary amino groups on PAA in the LB film would act as electrostatic connectors to the GOx layers deposited subsequently.

The glucose-sensing system was then examined for its ability to detect glucose in the presence of interfing analytes. The potential of the enzyme electrode was set at +0.6 V *vs*. Ag/AgCl in 0.1 M sodium phosphate buffer solution (pH 7.0, 25.0 °C) saturated with air.

The total performance of the biosensor was strongly dependent on the structure of the permselective layer, especially the cross-linking. Fig. 3 shows the response-time curves of the enzyme electrodes (**I** and **II**) for glucose and/or L-ascorbic acid, which were recorded after sample solutions have been added to the buffer solution. In both electrode systems, the current increased immediately after the addition of glucose, and reached a steady state response within a few seconds. In addition, the cross-linked siloxane polymer modified electrode without GOx/polyion complex layers revealed a very rapid response (< 0.1 s) to reach a constant for H_2O_2.

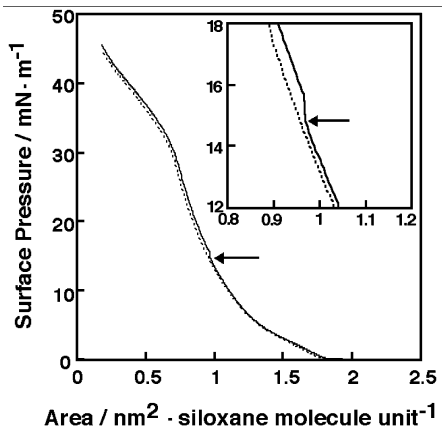


Fig. 2 π–*A* isotherms of polysiloxane L films on pure water and on *ca*. 10 unit mM PAA solution at 25 °C. The solid and dotted lines represent the 2D cross-linked and non-cross-linked monolayers, respectively.

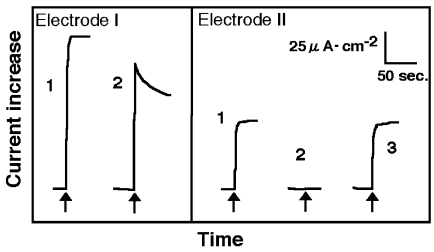


Fig. 3 Current–time curves for electrode **I** (non cross-linked) and **II** (cross-linked) to: (1) 1 mM glucose; (2) 1 mM L-ascorbic acid; and (3) a mixture of 1 mM glucose plus 1 mM L-ascorbic acid. The arrows indicate the injection time.

Electrode **I** also exhibited a similar response for interfering substances such as L-cysteine, acetaminophen, uric acid and L-ascorbic acid. In contrast, no response for these interference substances was observed using electrode **II**, in spite of the obvious response to glucose. Moreover, the current response of electrode **II** for a mixture of glucose and L-ascorbic acid was almost equal to that of a pure glucose solution. The ratios of the interference (L-cysteine, acetaminophen, uric acid and L-ascorbic acid) response to the glucose response for electrode **II** were 0.08, 0.1, 0.03 and 0.02, respectively. The ratio (ascorbic acid/glucose) for the cross-linked LB films modified electrodes without GOx layers was 0.025, suggesting that the LB film was crucial for permselectivity.

As a glucose sensor, electrode **II** gave a linear current response up to 1 mM glucose. The detection limit was 0.01 mM (signal-to-noise ratio; *ca*. 5). Although the different electrodes prepared in the same fashion showed about 10% deviation of scattering in current responses among them, each electrode exhibited consistently the same current response repeatedly, suggesting high reproducibility. The permselectivity remained constant for at least several months, with no essential change of current responses for ascorbic acid and glucose, which is promising for practical use.[10] The long-term stability dominantly depended on the deactivation of GOx but not the degradation of permselective layers. The combination of the 2D cross-linked network with the flexible side chains gave a higher mechanical strength in the practical usage of ultrathin films on solid substrates.[5]

We are very grateful to Chisso Co. for the supply of the silicone monomer. This work was supported in part by the Sagawa fundation and the CREST-JST, Japan.

Notes and references

1 G. Roberts, *Langmuir-Blodgett Films*, Plenum, New York, 1990.
2 L. H. Zhang, R. A. Hendel, P. G. Cozzi and S. L. Regen, *J. Am. Chem. Soc.*, 1999, **121**, 1621; T. Riedl, W. Nitsch and T. Michel, *Thin Solid Films*, 2000, **379**, 240.
3 S. S. Shiratori, K. Kohno and M. Yamada, *Sens. Actuators B*, 2000, **64**, 70; W. Wróblewski, M. Chudy, A. Dybko and Z. Brzózka, *Anal. Chim. Acta*, 1999, **401**, 105.
4 A. Aoki, M. Nakaya and T. Miyashita, *Macromolecules*, 1998, **31**, 7321; K. Sirkar, A. Revzin and M. V. Pishko, *Anal. Chem.*, 2000, **72**, 2930.
5 M. Kunitake, T. Nishi, H. Yamamoto, K. Nasu, O. Manabe and N. Nakashima, *Langmuir*, 1994, **10**, 3207; T. Kunitake, N. Higashi, M. Kunitake and Y. Fukushige, *Macromolecules*, 1989, **22**, 485.
6 M. F. Cardosi and S. W. Birch, *Anal. Chim. Acta*, 1993, **276**, 69; F. Mizutani, S. Yabuki and S. Iijima, *Electroanalysis*, 1995, **7**, 706.
7 R. Maiden and A. Heller, *Anal. Chem.*, 1992, **64**, 2889; A. Emr and A. M. Yacynych, *Electroanalysis*, 1995, **7**, 913.
8 T. Matsumoto, M. Furusawa, H. Fujiwara, Y. Matsumoto and N. Ito, *Sens. Actuators B*, 1998, **49**, 68; F. Mizutani, S. Yabuki and S. Iijima, *Electroanalysis*, 2001, **13**, 370.
9 T. Hoshi, H. Saiki, S. Kuwazawa, C. Tsuchiya, Q. Chen and J. Anzai, *Anal. Chem.*, 2001, **73**, 5310.
10 F. Mizutani, Y. Sato, Y. Hirata, T. Sawaguchi and S. Yabuki, *Anal. Chim. Acta*, 1998, **364**, 173.