Water Sorption, Membrane Potentials, and Ion Permeability of Styrene-Grafted *Bombyx mori* Silk Fibroin Membrane

MAKOTO DEMURA, AIO KITAMURA, AKIO SHIBAMOTO, and TETSUO ASAKURA, *Faculty of Technology, Tokyo University of Agriculture and Technology, Nakamachi 2-Chome, Koganei, Tokyo 184 Japan*

Synopsis

Characterization of styrene-grafted *Bombyx mori* silk fibroin membrane was investigated. It was revealed from the water sorption and ^1H nuclear magnetic resonance (NMR) measurements that the amounts of water adsorbed on the silk fibroin membranes decreased by the styrene grafting and the states of water adsorbed on the styrene-grafted silk fibroin membranes were not homogeneous; the presence of two components of water adsorbed on the membranes at 60% relative humidity was observed. In addition, the fraction of the fast component decreased with increasing styrene grafting. The membrane potentials increased with increasing of the grafting. The KCl permeability of the membrane strongly depends on the degree of styrene grafting.

INTRODUCTION

Biotechnology with functional biopolymers has attracted the attention of many researchers and the natural biopolymers such as κ-carrageenan, collagen, and gelatin are frequently employed for the immobilization of enzymes.[1-3] Recently, silk fibroins derived from silkworms, *Bombyx mori* and *Philosamia cynthia ricini* have been used as excellent enzyme immobilization materials as well as excellent fibers.[4-6] The recognized advantages of the silk fibroins for use as biomaterials are as follows: (1) purification of the silk fibroin is relatively simple and easy compared with other biomaterials, (2) the specific activity of the enzyme entrapped in the silk fibroin and the substrate permeability of the silk fibroin membrane are controlled by the structural transition of the silk fibroin, such as occurrence of β structure induced by methanol[7-10] and/or drawing treatment of the membranes,[11] (3) there is no appreciable interaction between the silk fibroin and the enzymes such as glucose oxidase,[12,13] and (4) the functional residues in the protein can be used for chemical modification. In particular, the chemical modification of the protein provides a variety of biomaterials with unique character.

Thus far, the chemical modifications of the *silk fibers* have been carried out by graft copolymerization using vinyl monomers such as styrene, hydroxyethylmethacrylate, methylmethacrylate, and methacrylamide, etc.[14-16] However, the sample characterizations have scarcely been reported for the grafted silk fibroin *membranes*.

In this article, we report the characterization of the styrene-grafted silk fibroin membranes, which are prepared by an emulsion polymerization, from

Journal of Applied Polymer Science, Vol. 36, 535–543 (1988)
 CCC 0021-8995/88/030535-09$04.00

several points of view. The states of water adsorbed on the grafted silk fibroin membranes are analyzed by the water sorption experiments and ^{1}H-NMR spin-lattice relaxation times, T_1 of water. In addition, the membrane potential and ion permeability of the membrane were determined, because of the importance of characterizing the membrane properties in designing the functional membranes. These experiments were performed as functions of grafting degree and relative humidity. These data are considered to be useful in development of the silk fibroin membrane with high separability and high selective permeability, etc.

EXPERIMENTAL

Materials

The aqueous solution of the silk fibroin from *B. mori* cocoons (a hybrid between strains Shunrei and Shogetsu) was obtained as described elsewhere in detail.[17] The solution was cast on a polymethylmethacrylate plate and dried in air with an electric fan at room temperature. The membrane obtained was kept in the desiccator (100% relative humidity) for one day in order to prepare the insoluble membrane in water. The styrene monomer distilled in vacuum two times was grafted on the silk fibroin membrane by emulsion polymerization.[16] The styrene monomer (5 w/v %) was polymerized at 60°C with 0.02% potassium peroxydisulfate in the presence of the silk fibroin membrane and 0.02% sodium dodecyl sulfate. The solution was kept at pH 3.1 with H_2SO_4. Since small amounts of styrene homopolymer tend to attach to the membrane surface when the polymerization time is long, the homopolymer was removed from the membrane with benzene/ethanol (1 : 1) mixture by Soxhlet extractor for 30 h. Although the percentage of the homopolymer was not determined here, the amounts were clearly small, probably less than 10%. Degree of the styrene grafting was calculated according to following equation,[16]

$$\text{Degree of grafting (\%)} = (W_2 - W_1)/W_1 \times 100$$

where W_1 and W_2 were weight of the silk fibroin membrane before grafting and that of the membrane onto which styrene was chemically bound, respectively. Degree of the grafting was controlled by the reaction time.

Measurements

The amounts of water adsorbed on the styrene-grafted silk fibroin membrane were measured at 25°C with a McBain-type sorption apparatus.[18]

^{1}H NMR spectra of the water adsorbed on the styrene-grafted membrane were observed with a JEOL FX-90Q NMR spectrometer operating at 90 MHz. The spin-lattice relaxation time, T_1 of water was determined from a series of the partially relaxed ^{1}H-NMR spectra.

The membrane potential and ion permeability were determined as follows. The concentration cell of potassium chloride in which the styrene-grafted silk fibroin membrane was fixed between two half cells was prepared as follows.[19]

$$\mathrm{Hg,Hg_2Cl_2|saturated\ KCl||0.1}M\ \mathrm{KCl|membrane|0.05}M\ \mathrm{KCl}$$
$$\mathrm{||saturated\ KCl|Hg_2Cl_2,Hg}$$

The difference in the membrane potential was measured by the use of calomel electrodes which are connected to the half cells by salt bridge (saturated KCl aqueous solution containing 1% of agar) with a potentiometer (model PM-19A, TOA Electric Ltd.). pH of the solution was buffered with acetate. The permeability of KCl (initial concentration of 0.1*M*) through the styrene-grafted silk fibroin membrane was observed by electric conductivity meter as described elsewhere.[19]

RESULTS AND DISCUSSION

State of Water Adsorbed on the Styrene-Grafted Silk Fibroin Membranes

Figure 1 shows the water sorption isotherms for various styrene-grafted silk fibroin membranes at 25°C as a function of relative humidity, X. These isotherm curves were sigmoid type and the water uptake, v, decreased with increasing the degree of the styrene grafting over a wide range of the relative humidity, indicating that the swelling of the membranes is depressed by the styrene grafting. The amounts of the adsorbed water, V_m, which were presented as monolayer according to BET theory[20] and the average pore radius when the shape of the pore was assumed to be capillary tube[21] in the membranes, are summarized in Table I.

A concept of the cluster function[22] was applied to characterization of the water adsorbed on the membranes. A cluster integration, G_{11} for a two-component system, is defined by

$$G_{11} = 1/V \int \int [F_2(i, j) - 1] d(i) d(j)$$

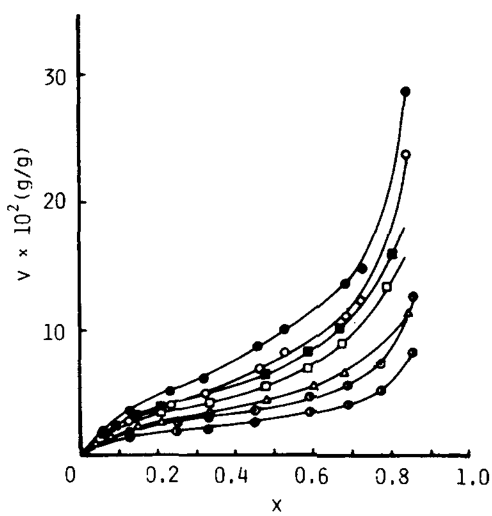

Fig. 1. Water sorption isotherm at 25°C for various styrene grafted silk fibroin membranes. v; amounts of adsorbed water, X; relative humidity. Degree of grafting: (●) 0%; (○) 10%; (■) 25%; (□) 43%; (△) 67%; (⊙) 84%; (◑) 122%.

TABLE I
Amounts of Monolayer of Adsorbed Water, V_m and Average Pore Radius, $\bar{r}$ of Styrene-Grafted Silk Fibroin Membranes

Degree of grafting (%)	V_m (g/g)	$\bar{r}$ (Å)
0	0.052	21.7
10	0.043	21.7
25	0.039	21.6
43	0.033	21.4
67	0.025	21.0
84	0.021	21.0
122	0.015	21.0

V_m and $\bar{r}$ were calculated according to BET method.

where V is volume of the swollen membrane, $F_2(i, j)$ is distribution function of a pair of water molecules, i and j. Cluster function, G_{11}/V_1 is determined by

$$G_{11}/V_1 = -v_2\left[\partial(a_1/v_1)/\partial a_1\right]_{P,T} - 1$$

where suffix 1 means the water and a_1, v_1, and V_1 stand for the activity (= relative vapor pressure), volume fraction, and partially molecular volume of the water, respectively. The cluster function is used as a measure of water clustering in the membrane. Figure 2 shows the plots of the cluster function against v_1. The value G_{11}/V_1 increases steeply with increasing v_1 for the

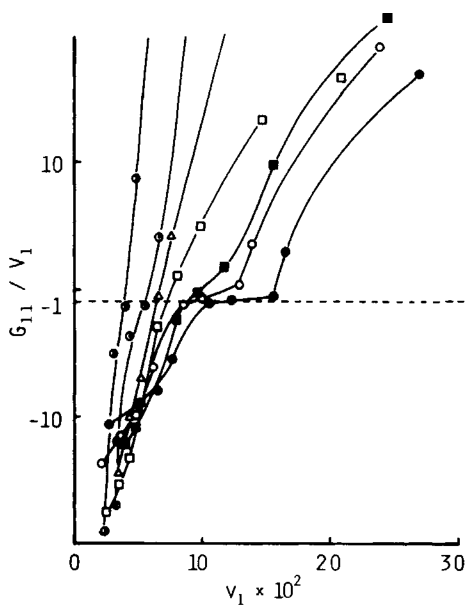

Fig. 2. Cluster function, G_{11}/V_1 vs. volume fraction of water, V_1 in the styrene-grafted silk fibroin membranes. Notations for the membranes are the same as in Fig. 1.

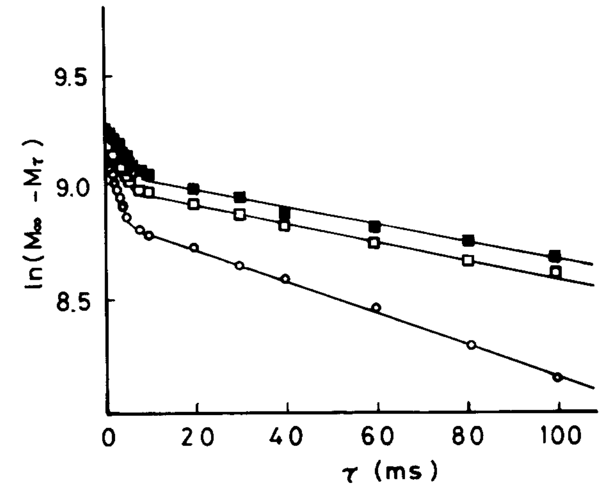

Fig. 3. Plots of $\ln(M_\infty - M_\tau)$ vs. τ for the styrene-grafted silk fibroin membranes. Degree of grafting: (○) 0%; (□) 22 %; (■) 42%. Temperature and relative humidity were 25°C and 60%, respectively.

membranes whose degrees of grafting are more than 43%. On the other hand, the curves, G_{11}/V_1 vs. v_1 for the samples of less than 25% styrene grafting tend to flatten when the value of G_{11}/V_1 is -1. These results indicate that the states of water adsorbed on the styrene-grafted silk fibroin membrane as well as the water uptakes are changed by grafting.

In order to obtain further information on the state of water adsorbed on the styrene-grafted silk fibroin membranes, the ^{1}H NMR spin-lattice relaxation time, T_1 of such a water was measured. Figure 3 shows the plots of $\ln(M_\infty - M_\tau)$ vs. τ for the ^{1}H nuclei of water adsorbed on the styrene-grafted silk fibroin membranes (the degrees of grafting were 0, 22, and 42%) at 60%

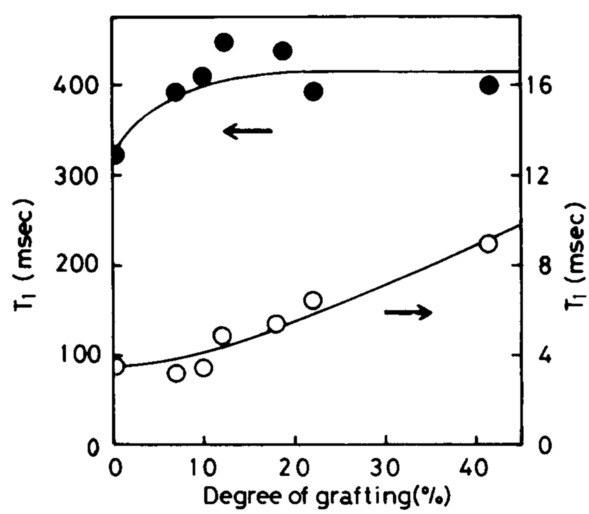

Fig. 4. Plots of T_1 of water adsorbed on the styrene-grafted silk fibroin membranes vs. degree of grafting.

TABLE II
Spin-Lattice Relaxation Time, T_1 of Two Components of Water Adsorbed on the Styrene-Grafted Silk Fibroin Membrane and the Fraction, F of Fast Component

Degree of grafting (%)	T_1 (ms) Fast	T_1 (ms) Slow	F (fast)
0	3.6	323	31.9
7	3.2	392	32.8
10	3.4	409	32.6
12	4.9	448	31.2
18	5.4	437	29.4
22	6.3	392	27.0
42	8.9	400	26.7

relative humidity, where M_∞ is the equilibrium amplitude of the fully relaxed spectrum, M_τ is the amplitude of a partially relaxed spectrum, and τ is the delay time between the 180° and 90° pulses. The plots are nonlinear and analyzed here as two components. Figure 4 and Table II represent the T_1 values of two components as a function of degree of the grafting. The T_1 values are 300–450 ms for the slow component and 3–9 ms for the fast component. The values of the slow component are smaller than that of free water. The T_1 values of both components increase with increasing the degree of styrene grafting although the line of the slow component tends to flatten. Thus, the character of water adsorbed on the membrane changed by the styrene grafting. The fraction of the fast component F decreases with increasing the degree of grafting (Table II) which corresponds to decreasing of V_m (Table I). These data indicate that the fast component may be bound water or at least, closely related to the bound water. Namely, it is presumed that the styrene grafting causes decrease of the active site of the silk fibroin membrane against the water.

Membrane Potential and Ion Permeability of Styrene-Grafted Silk Fibroin Membranes

Membrane potential and ion permeability are important factors necessary to elucidate functions of the membrane as mentioned in the Introduction. The membrane potential developed between two aqueous solutions which are separated by a membrane is reflected by the fixed charge density of the membrane.[23] Figure 5 shows the change in the membrane potential of the styrene-grafted silk fibroin membranes under the concentration gradient of KCl = 2 ($0.1M/0.05M$) as a function of degree of the styrene grafting. Sugiura et al.[19] also measured the membrane potential of the silk fibroin membrane insolubilized with ethanol and the value was approximately identical with our value of no grafted silk fibroin membrane. With increased grafting of styrene, the membrane potential increases slightly at more than ca. 30% grafting, suggesting increase of the negative fixed change density of the membrane by styrene grafting. This suggestion was supported from the decreased swelling with increased degree of styrene grafting as shown in Figure 1 and/or the shifts of the isoelectric point (IEP) of the grafted

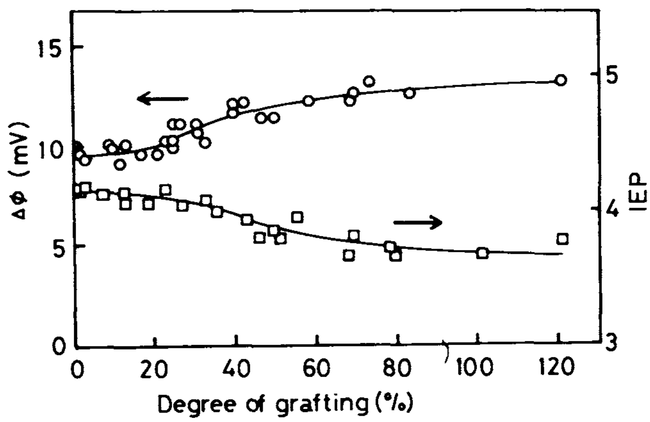

Fig. 5. Plots of membrane potential, $\Delta\phi$(mV) and IEP vs. degree of grafting. Temperature was 25°C.

membranes (Fig. 5). Specifically, the values determined from the membrane potential as a function of pH of aqueous solutions were changed from IEP = 4.2 (nongrafted) to 3.6 (100% grafted). The grafting or blocking by styrene polymer might occur at the basic sites of the membrane by taking into account the change of the IEP observed here.

The permeability coefficient, $Km \cdot d/A$ of KCl through the various styrene-grafted silk fibroin membranes was observed as shown in Figure 6, where Km, d, and A are apparent permeability coefficient, thickness of the membrane, and effective cross section, respectively. The value, $Km \cdot d/A$ decreases rapidly with increasing degree of styrene grafting in the vicinity of 30% degree of grafting. The results resemble the data of the water sorption measurements. The concept of the diffusional transport of solute in aqueous solution through the water-swollen membranes has been reported by Yasuda et al.[24-26] and Takigami et al.[27] based on the concept of the free volume theory. According to their analysis, the following equation was used to analyze our data:

$$\ln[Km \cdot d/A] = \ln\left[K_2 D_{2,1}\phi(q_2)\right] - B\left(q_2/V_{f,1}\right)(1/H - 1)$$

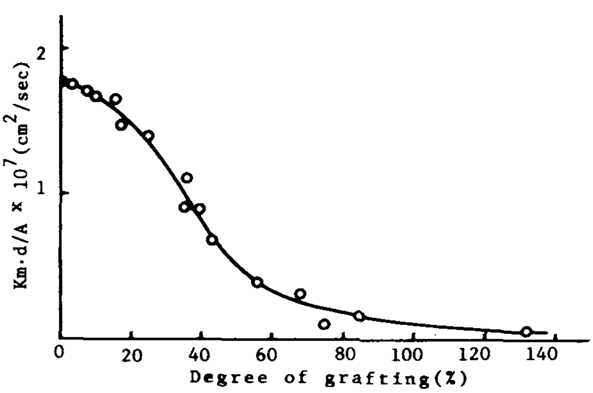

Fig. 6. Plot of permeability coefficient, $Km \cdot d/A$ of KCl vs. degree of grafting.

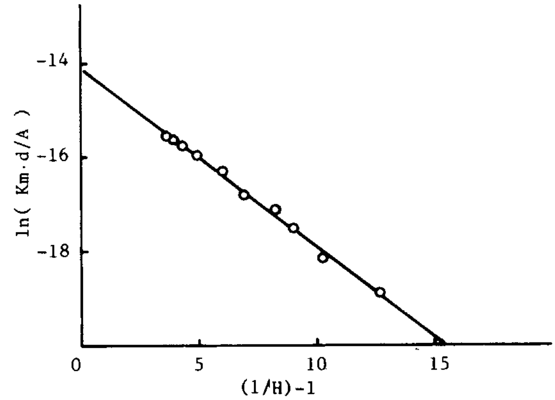

Fig. 7. Plot of ln[$Km \cdot d/A$] vs. ($1/H - 1$).

where suffixes 1 and 2 mean solvent, and solute, respectively, $D_{2,1}$ is diffusion coefficient of the solute in solution, $\phi(q_2)$ described the sieve mechanism by which small molecules are permitted to diffuse and large molecules are rejected because the macromolecular network has no hole appropriated size, $V_{f,1}$, H, B, K_2 are free volume of solvent, hydration which was calculated according to the following equation: H = [volume of the water in swollen membrane]/[volume of the swollen membrane], proportionality factor and distribution coefficient of solute, respectively. The plot of ln[$Km \cdot d/A$] against ($1/H - 1$) for the styrene-grafted silk fibroin membrane gives a straight line as shown in Figure 7. Thus the KCl permeability of the styrene-grafted silk fibroin membranes depends on the change of the hydration of the silk fibroin membrane by the grafting at the KCl concentrations of more than 0.1M. Application of these styrene-grafted silk fibroin membranes to the separation of substrates or the immobilization of biocatalysts is in progress.

References

1. O. R. Zaborsky, ed., *Immobilized Enzymes*, CRC Press, Boca Raton, FL, 1973.
2. H. H. Weetal and S. Suzuki, eds., *Immobilized Enzyme Technology*, Plenum Press, New York, 1975.
3. I. Chibata, *Immobilized Biocatalyst*, Kodansha Ltd., Tokyo, 1986.
4. L. Grasset, D. Cordier, and A. Ville, *Process Biochem.*, **14**, 2 (1979).
5. H. Kanno and N. Nakayama, *Koso Kogaku News Jpn.*, **15**, 2 (1986).
6. S. Miyairi, M. Sugiura, and S. Fukui, *Agri. Biol. Chem.*, **42**, 1661 (1978).
7. A. Kuzuhara, T. Asakura, R. Tomoda, and T. Matsunaga, *J. Biotech.*, **5**, 199 (1987).
8. H. Saito, R. Tabeta, T. Asakura, Y. Iwanaga, A. Shoji, T. Ozaki, and I. Ando, *Macromolecules*, **17**, 1405 (1984).
9. T. Asakura, A. Kuzuhara, R. Tabeta, and H. Saito, *Macromolecules*, **18**, 1841 (1985).
10. T. Asakura, H. Yoshimizu, M. Tsukada, K. Setoyama, and K. Mitsuda, *Sen-i Gakkaishi*, **43**, 335 (1987).
11. M. Demura, T. Asakura, and A. Kitamura, *Polym. Preprints Jpn.*, **36**, 645 (1987).
12. A. Kuzuhara, T. Asakura, and T. Matsunaga, *Rep. Pro. Polym. Phys. Jpn.*, **29**, 725 (1986).
13. T. Asakura, H. Yoshimizu, A. Kuzuhara, and T. Matsunaga, *J. Seric. Sci. Jpn.*, in press.
14. S. Lenka, *J. Macromol. Sci.*, **C22**, 303 (1982).
15. M. K. Mishra, *J. Appl. Polym. Sci.*, **27**, 2403 (1982).
16. A. Kitamura, *J. Seric. Sci. Jpn.*, **35**, 59 (1966).

17. T. Asakura, Y. Watanabe, A. Uchida, and H. Minagawa, *Macromolecules*, **17**, 1075 (1984).
18. A. Kitamura, *J. Seric. Sci. Jpn.*, **35**, 113 (1966).
19. M. Sugiura, *Nippon Nogei Kagaku Kaishi*, **47**, 563 (1973).
20. S. Brunauer, *Solid Surface and Gas-Solid Interface*, Adv. in Chem. Ser., Am. Chem. Soc., Washington, DC, 1961.
21. A. Kitamura, A. Shibamoto, and K. Nito, *J. Seric. Sci. Jpn.*, **48**, 477 (1979).
22. B. H. Zimm and J. L. Lundberg, *J. Phys. Chem.*, **60**, 425 (1956).
23. Y. Kobatake, K. Kurihara, and N. Kamo, *Surface Electrochemistry, Advanced Methods and Concepts*, Japan Scientific Societies Press, Tokyo, 1978.
24. H. Yashuda, C. E. Lamaze, and L. D. Ikenberry, *Makromol. Chem.*, **118**, 19 (1968).
25. H. Yasuda, L. D. Ikenberry, and C. E. Lamaze, *Makromol. Chem.*, **125**, 108 (1969).
26. H. Yasuda, A. Peterlin, C. K. Colton, K. A. Smith, and E. W. Merrill, *Makromol. Chem.*, **126**, 177 (1969).
27. S. Takigami, Y. Maeda, and A. Nakamura, *J. Appl. Polym. Sci.*, **24**, 1419 (1979).

Received July 22, 1987
Accepted October 20, 1987