Visualization of Chemical Modification of Pore Internal Surfaces Using Fluorescence Microscopy

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Chemical modification of pore internal surfaces with 0.31 and 0.54 μm in diameter in poly(ethylene terephthalate) (PET) was examined using the alkylation reaction of the carboxylic acids on the surfaces. The chemical incorporation of the reagent on the surfaces was confirmed by the fluorescence microscope images of the membranes reacting with the alkylation reagent bearing a pyrene fluorophore.

Porous materials on nanometer scale such as ion track membranes and porous alumina have attracted much attention for applying to artificial organs,[1] separation membranes,[2,3] and nanoscopic electronic devices.[4–7] Ion track membranes, which are prepared by heavy ion beam radiation followed by alkaline etching, possess cylindrical pores with narrow distribution of pore diameter in a wide range of pore sizes (Figure 1).[8–12] Ion track membranes made of poly(ethylene terephthalate) (PET) can be applied to the selective separation of toxic metal ions, biomolecules, and biological cells by controlling the microscopic pore size and the properties of the internal surfaces of the pores.[2,13] Therefore, it is very important to modify the pore inner surfaces of the track membranes to adjust the chemical and physical characteristics.

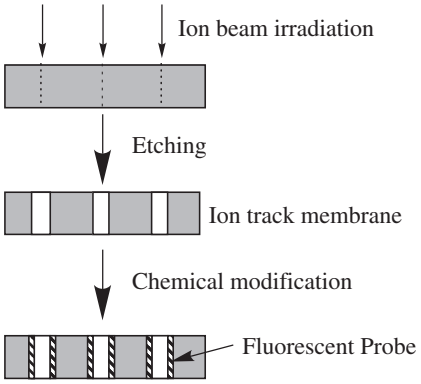
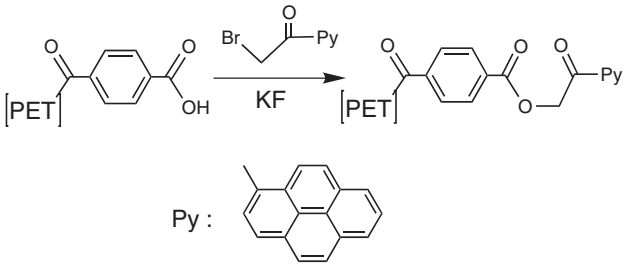


Figure 1. Schematic illustration for the preparation of ion track membranes and the modification of the pore internal surfaces.

Chemical modification is considered a suitable method for the precise control of not only surface hydrophilicity and charges but also of the quantitative introduction of functional properties, without causing surface deformations.[14,15] Recently, we reported that the hydrophilic surface of PET films was chemically modified by the selective alkylation of the carboxyl groups on the surface using acyl bromide alkylation reagents with catalytic KF, and the chemical incorporation of the electrophiles was confirmed by fluorescence spectroscopy measurements.[16] Thus, we applied the alkylation method to the chemical modification of the internal surfaces of sub-micron cylindrical pores using a fluorescence reagent and confirmed its incorporation onto the surfaces by fluorescence microscopy.

PET films with 12 μm in thickness were irradiated by $^{129}Xe^{23+}$ ion beams with energy of 3.5 MeV/n with a beam flux of 3.0×10^7 ions/cm^2. The irradiated films were illuminated by UV light at wavelengths longer than 310 nm for 6 h to enhance the dissolution rates in only ion tracks and, in consequence, etched in 0.5 M NaOH aqueous solution at 40 °C to dissolve the ion tracks.[17] The clear hole patterns with 0.31 and 0.54 μm in diameter (0.31 μm-pore membrane and 0.54 μm-pore membrane) were observed in SEM images.

We employed 1-(bromoacethyl)pyrene (**BrPy**) as a fluorescent probe because it possessed an acyl bromide moiety, which reacted with carboxyl groups of PET, and showed an excitation maximum at 360 nm, at which there was no absorption of PET films (Scheme 1).[18] According to the previous report, in which the selective alkylation of the carboxyl groups on PET surfaces using the acyl bromide had completed for 2 h judging from the change in the surface contact angles,[16] the pore internal surfaces of ion track membranes were treated with the DMF solution of 0.020 M **BrPy** and 0.050 M KF at room temperature. The DMF solution was loaded into a glass syringe, which was connected to a membrane filter holder. Then, the solution was introduced into the pores of ion track membranes with a constant pressure (5.3 kPa) to make sure the contact of the reaction solution to the internal surfaces of the pores. The flow rates of the solution through the 0.31 and 0.54 μm-pore membranes were 11 and 47 μL/min, respectively.



Scheme 1. The alkylation reaction of the carboxylic acid of PET with the reagent bearing a pyrene fluorophore.

Figure 2 shows the emission spectrum of the PET membranes reacting with **BrPy** with the excitation at 350 nm and the excitation spectrum by monitoring the emission at 430 nm; the excitation and emission spectra of PET are shown as a refer-

ence in the figure. The 0.31 μm-pore membrane showed emission maximum at 440 nm and excitation maximum at 375 nm, which were not observed in the membranes without the alkylation reaction. The 0.54 μm-pore membrane showed similar fluorescence spectra with about two time larger intensity compared to the 0.31 μm-pore membrane. The different intensities are qualitatively related to the total surface area of the pore internal surfaces of the membranes. Namely, the 0.54 μm-pore membrane possesses 1.7 (0.54/0.31) times higher total surface area than those with pores of 0.31 μm-pore membrane since these membranes have the same pore density (3.0×10^7 cm^{-2}). When the 0.54 μm-pore membrane was placed in the DMF solution of **BrPy** without KF as a control experiment, there was no change in the excitation and emission spectra of the film. The control experiment indicates that the observed fluorescence results from the chemical incorporation but not physical adsorption of the pyrene fluorophore.

The fluorescent images of the 0.54 μm-pore membrane were taken using a microscope with a standard CCD digital camera. The images were observed with the fluorescence at wavelengths longer than 420 nm (excitation: 380 nm). No fluorescence microscope image was observed on the membranes without alkylation reaction (Figure 3a). Whereas, in the same measurement condition, one can clearly observe the fluorescent spots of about 0.5 μm in diameter in the image of the membrane with **BrPy**, as shown in Figure 3b. The fluorescent spots were located at the pore position, which was determined from the optical image of the membrane at the same area (Figure 3c). Since the alkaline etching of irradiated PET films provides hydrophilic carboxyl and hydroxy groups on the film surface as well as the pore inner walls, the fluorescent probe should be also attached onto the film surfaces. However, the fluorescence was observed only at the pore position with a good contrast; namely, one can observe the emission from the fluorescent probes accumulated along the pore inner surfaces perpendicular to the film surfaces. From these observations, it is obvious that the fluorescent probe attaches chemically on the internal surfaces of the cylindrical pores with 0.54 μm in diameter.

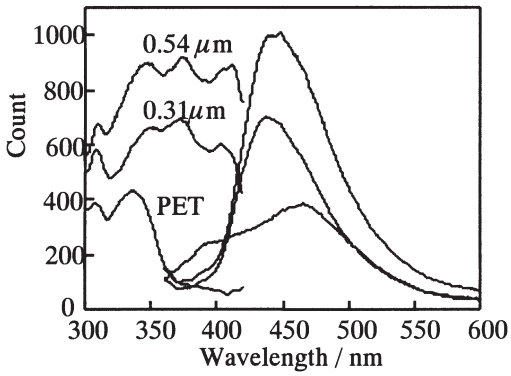


Figure 2. The emission spectra of the **BrPy**-modified PET membranes (pore diameter: 0.31 and 0.54 μm) with the excitation at 350 nm and the excitation spectrum by monitoring the emission at 430 nm; the excitation and emission spectra of PET are shown as a reference.

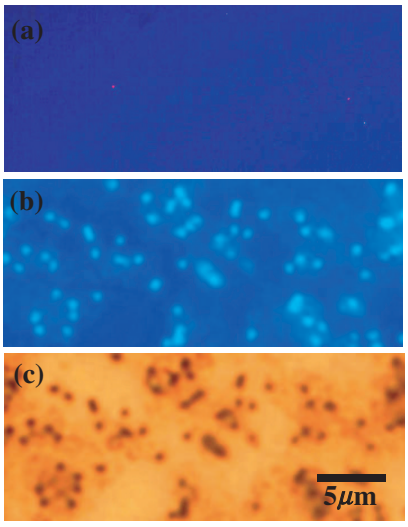


Figure 3. Fluorescence micrographs of the ion track membranes (pore diameter: 0.54 μm) (a) before and (b) after the reaction with **BrPy**. (c) The optical image at the same area of the membrane.

In conclusion, the internal surfaces of the cylindrical pores with 0.31 and 0.54 μm in diameter were modified by the reaction of the carboxylic acids on the surfaces with the alkylation reagent bearing the pyrene fluorophore. The incorporation of the fluorophore on the pore internal surfaces was confirmed by the fluorescence microscope images of the membrane. Further studies are currently underway in quantitative analysis of the surface modifications of pore inner surfaces using other chemical reagents and analytical methods.

References

1 B. D. Rantner and D. G. Castner, "Surface Modification of Polymeric Biomaterials," Plenum Press, New York and London (1997).
2 M. Yoshida, M. Asano, A. Safranj, H. Omichi, R. Spohr, J. Vetter, and R. Katakai, *Macromolecules*, **29**, 8987 (1996).
3 S. B. Lee and C. R. Martin, *Chem. Mater.*, **13**, 3236 (2001).
4 C. R. Martin, *Science*, **266**, 1961 (1994).
5 T. M. Whitney, J. S. Jiang, P. C. Searson, and C. L. Chien, *Science*, **261**, 1316 (1993).
6 M. E. T. Molares, V. Buschmann, D. Dovrev, R. Neumann, R. Sholz, I. U. Schuchert, and J. Vetter, *Adv. Mater.*, **13**, 62 (2001).
7 L. D. Pra, E. Ferain, R. Legras, and S. Demoustier-Champagne, *Nucl. Instrum. Methods*, **B196**, 81 (2002).
8 R. L. Fleisher, P. B. Price, and R. M. Walker, in "Nuclear Tracks in Solids: Principles and Applications," ed. by R. L. Fleisher, University of California Press, Berkley (1975).
9 R. Spohr, "Ion tracks and Microtechnology, Principles and Applications," Vieweg & Sohn Verlagsgesellschaft mbH, Braunschweig (1990).
10 Y. Suzuki, Y. Maekawa, M. Yoshida, K. Maeyama, and N. Yonezawa, *Chem. Mater.*, **14**, 4186 (2002).
11 Y. Maekawa, Y. Suzuki, M. Yoshida, K. Maeyama, and N. Yonezawa, *Polymer*, **44**, 2307 (2003).
12 S. Seki, K. Maeda, S. Tagawa, H. Kudoh, M. Sugimoto, Y. Morita, and H. Shibata, *Adv. Mater.*, **13**, 1663 (2001).
13 S. Sapieha, J. Cerny, J. E. Klemberg-Sapieha, and L. Martinu, *J. Adhes.*, **42**, 91 (1993).
14 A. Y. Fadeev and T. J. McCarthy, *Langmuir*, **14**, 5586 (1998).
15 W. Chen and T. J. McCarthy, *Macromolecules*, **31**, 3648 (1998).
16 J. Li, Y. Maekawa, T. Yamaki, and M. Yoshida, *Macromol. Chem. Phys.*, **203**, 2470 (2002).
17 E. Ferain and R. Legras, *Nucl. Instrum. Methods*, **B84**, 331 (1994).
18 I. Ouchi, *Polym. J.*, **15**, 225 (1983).

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