

Selective Measurement of Optical Isomer by Using Molecular Imprinting and Surface Plasmon Resonance Sensor

Satoshi NISHIMURA,^{1†} Toshifumi YOSHIDOME,² and Morihide HIGO²

^{1†} *Department of Applied Chemistry and Chemical Engineering, Graduate School of Science and Engineering, Kagoshima University, 1-21-40, Korimoto, Kagoshima 890-0065, Japan (E-mail: s-nisimu@apc.kagoshima-u.ac.jp)*

² *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, 1-21-40, Korimoto, Kagoshima 890-0065, Japan*

Polymer films that are selective toward optical isomers such as cinchonidine and cinchonine were prepared by using molecular imprinting technique, and were used as an additional part of the sensor chip of the Surface Plasmon Resonance (SPR) sensor. For the preparation of the cinchonidine-selective films, cinchonidine was used as a template; methacrylic acid, as a functional monomer; ethyleneglycol dimethacrylate, as a crosslinking monomer; and 2,2'-azobisisobutyronitrile, as an initiator. Polymerization was carried out with the use of UV light irradiation to form polymer films, which were finally washed with a mixture solution of methanol and acetic acid. With the use of the sensor chip immobilizing the cinchonidine-selective film, various concentration of cinchonidine aqueous solutions were measured to obtain the rate at which cinchonidine was caught by the cinchonidine-selective film. The rate increased linearly with increasing the concentration. The cinchonidine-selective film was selective toward cinchonidine against cinchonine; or vice versa.

(Received on August 10, 2001; Accepted on September 13, 2001)

Optical isomers become significant in the field of medical supplies, agricultural chemicals, perfume, flavoring, and electronics. This is resulted from the fact that lots of vital substances have asymmetric center, and that optical isomers play important roles in vital phenomena. Optical isomers have levo (L) forms and dextro (D) forms, and in many cases, one form is useful and the other is poisonous for life. Therefore, optical isomer should be discriminated by a simple and speedy way.¹ In general, optical isomers are discriminated by using gas chromatography, high-performance liquid chromatography (HPLC), and capillary electrophoresis. These techniques, however, are slow in response and expensive. In recent years, molecular imprinting method, a technique to produce a synthetic polymer film which selectively catches a particular substance, has been reported.² Further, separation of optical isomer has been reported by using molecular-imprinted polymers as the material for the column of HPLC.³ In this paper, the polymer film selective toward cinchonidine, which is the L form, was prepared by molecular imprinting technique, and was immobilized on the sensor chip of the SPR sensor. The SPR sensor perceives substances at the sensor chip, which is the Au film evaporated on a slide glass. The SPR sensor is convenient for measuring the molecules interacting with the sensor chip in real time.⁴ The SPR sensor with the sensor chip immobilizing the cinchonidine-selective film was investigated in applicability to the quantitative analyses of the cinchonidine aqueous solution, and in selectivity toward cinchonidine against cinchonine, which is the optical isomer of cinchonidine.

Experimental

The SPR imaging experiments were performed with the instrument (DKK Co., Ltd.) that contained a Light-emitting diode ($\lambda = 660$ nm) as a source of light and a CCD camera as a

detector. Au evaporated on a glass plate (18×18×0.15 mm) was purchased from Eliotec Corporation. A glass plate was coated with Au film (thickness 50 nm) by vacuum evaporation. To prevent Au exfoliation from the glass surface, Cr (thickness 3 nm) was evaporated prior to the evaporation of the Au film. The Au film was immersed to form self-assembled monolayer via Au-mercaptide bonding in 50 mM 2-aminoethanethiol hydrochloride aqueous solution for 20 h. Next, the pre-treated Au film was immersed to link by peptide linkage with methacrylic acid and 2-aminoethanethiol hydrochloride in an aqueous solution (50 mM N-hydroxysuccinimide and 200 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) for 1 h (Fig. 1a). For the preparation of the molecular-imprinted film (the cinchonidine-selective film), 50 mg of cinchonidine as the template was dissolved in 3 ml of chloroform. Further, 0.1 ml of methacrylic acid as the functional monomer, 1 ml of ethyleneglycol dimethacrylate as the crosslinking agent and 12 mg of 2,2'-azobisisobutyronitrile as the initiator were added into the solution. The monomer mixture was admitted into reactor (18×18×0.16 mm) and was contacted with pre-treated Au film. This reactor was placed under UV light (>290 nm) at 0 for 4 h (Fig. 1b). The cinchonidine-selective film prepared was washed exhaustively with methanol / acetic acid (4:1, v/v).

Result and discussion

At the end of the preparation of the cinchonidine-selective film, cinchonidine caught must be removed from the film before use. The film was washed with methanol-acetic acid (4:1, v/v) mixed solution. The quantities of cinchonidine further caught by the cinchonidine-selective film after washing for various times were measured for the 0.01 mol/l cinchonidine aqueous solution. By washing more than 60 s, cinchonidine was completely removed from the cinchonidine-selective film prepared.

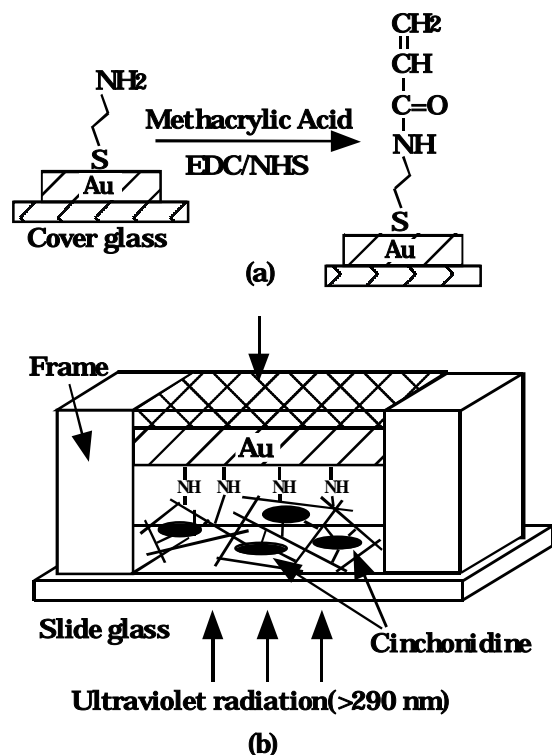


Fig. 1 Preparation procedures of the cinchonidine-selective film, which is an additional part of the sensor, chip.

The films were washed for 60 s in the subsequent experiments.

The SPR sensor responds to changes in refractive index of the medium, which exists close to the surface of the sensor chip. When the refractive index changes, the angle at which the SPR phenomenon occurs (resonance angle) changes. The resonance angle is monitored continuously over time and is registered as a sensorgram, in which the y-axis is denoted as the SPR signal indicated in degree and the x-axis is the time. The sensor chip immobilizing the cinchonidine-selective film was mounted on the SPR sensor, and applied to the cinchonidine aqueous solutions. Figure 2a shows the sensorgrams, that is, the time courses of the SPR signal for the 0.01 mol/l cinchonidine aqueous solution with the use of the cinchonidine-selective film. The SPR signal rapidly increased when the cinchonidine aqueous solution was injected after flowing water. As the SPR signal changes as a function of the refractive index of the media nearby the sensor chip, this rapid increase of the SPR signal is attributable to the presence of free cinchonidine in the aqueous solution nearby the sensor chip. For the time being, the signal slowly increased and came to an end in 3840 s. This slow increase of the signal describes cinchonidine was slowly caught by the cinchonidine-selective film, and was due to cinchonidine caught by the cinchonidine-selective film. The standard deviation was 2.2 % ($n=5$). With the use of the Au film as the sensor chip (Fig. 2b), the SPR signal rapidly increased on injection of the cinchonidine aqueous solution, and kept a constant value (69.910 degree) without its slow increase, which confirms that the slow increase observed in Fig. 2a is due to cinchonidine caught by the cinchonidine-selective film. The increase of the SPR signal observed with the use of the Au-film-sensor chip was 1.58 times larger than the overshoot observed on injection of the cinchonidine aqueous solution in Fig. 2a. This is explained as follows: the cinchonidine-selective film prevents the cinchonidine aqueous solution injected from coming close to the Au film surface of the cinchonidine-selective-film-sensor

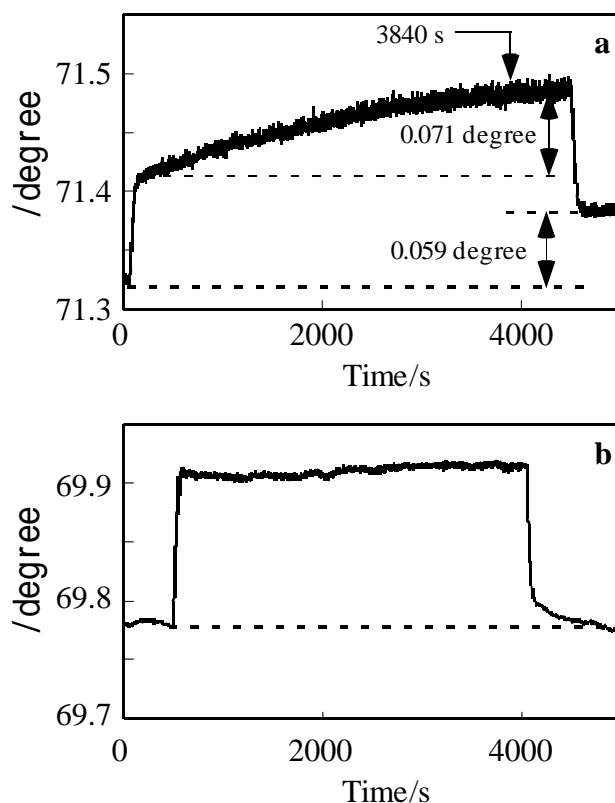


Fig. 2 Typical time course of the SPR signal for the cinchonidine aqueous solution, by using (a) the cinchonidine-selective and (b) the Au film.

chip, which leads to that the difference in refractive index nearby the Au film on injection of the cinchonidine aqueous solution was less. Thus, the cinchonidine-selective film sensor was better in sensitivity than the Au film sensor. This is based on that the interaction between cinchonidine and the cinchonidine-selective film is larger than that between cinchonidine and the Au film. On injection of water again followed by flowing the cinchonidine aqueous solution for about 4500 s in Fig. 2a, the signal rapidly decreased to reach the constant value of 71.381 degree which was larger by 0.059 degree than that observed under the previous injection of water (71.322 degree). This value of 0.059 degree should have been the same as the degree of the slow increase of 0.071 degree observed on injection of the cinchonidine aqueous solution. The difference of 0.012(0.071-0.059) degree is attributable to some cinchonidine weakly bonded to the cinchonidine-selective film. The SPR signal change by 0.1 degree corresponds to change of the density of substance on the Au film by 1 ng/mm^2 .⁵ The quantity of cinchonidine caught by the cinchonidine-selective-film-sensor chip was proved to be 0.59 ng/mm^2 by using the SPR measurement.

The cinchonidine aqueous solutions with the concentration of 10^{-5} – 10^{-2} mol/l were measured by using the SPR sensor with the cinchonidine-selective film. With increasing the cinchonidine concentration, the overshoot became larger in intensity, the slow increase became much slower in rate, and the time required for the SPR signal to reach a constant value became longer. The SPR signal shift obtained at last was of course independent of the cinchonidine concentration. The rate at which cinchonidine was caught by the cinchonidine-selective film was evaluated as the difference of the SPR signals between at 5 min and at 10 min. Figure 3 shows the relationship between the cinchonidine concentration and the rate at which cinchonidine was caught by the cinchonidine-selective film. The rate was dependent on the concentration of cinchonidine, and became larger with increasing the concentration. In accordance with this relation,

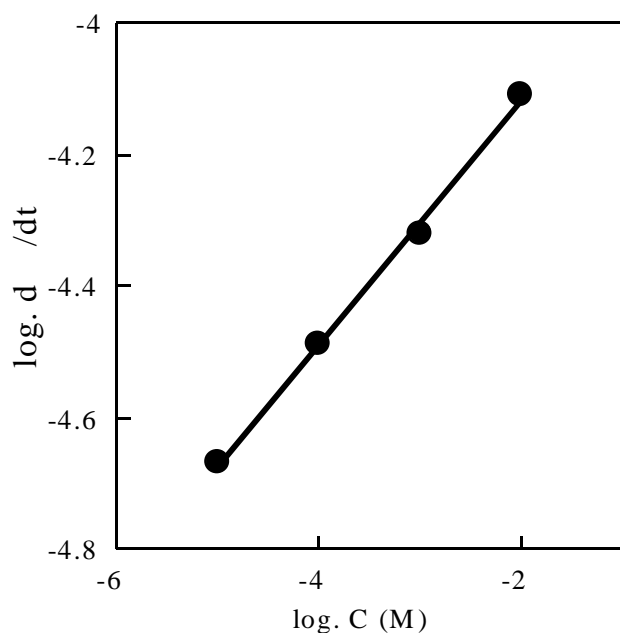


Fig.3 Relationship between the rate at which cinchonidine caught by the cinchonidine-selective film and the cinchonidine concentration.

a plot of the rate against the cinchonidine concentration was found to be linear. It was suggested that the cinchonidine concentration could be determined from the rate at which cinchonidine was caught by the cinchonidine-selective film.

The SPR sensor with the sensor chip immobilizing the cinchonidine-selective film was applied to the measurement of the aqueous solution of cinchonine, which is the optical isomer of cinchonidine. The time course of the SPR signal is shown in Fig. 4a. When the cinchonidine aqueous solution was first injected, the SPR signal increased over a period of 3840 s as stated above though the injection of the cinchonidine aqueous solution was stopped at about 450 s in the time course of Fig. 4a. In the case of the injection of the cinchonine aqueous solution, however, the slow increase of the SPR signal was not observed. This result suggests that no cinchonine was caught by the cinchonidine-selective film. Thus, the cinchonidine-selective film sensor could be used for the selective measurement of cinchonidine against cinchonine. Further, the cinchonine-selective film was prepared by using the molecular imprinting technique to form the sensor chip immobilizing the cinchonine-selective film. The time course obtained with the use of this sensor chip for the cinchonidine and the cinchonine aqueous solutions is shown in Fig. 4b. The slow increase in intensity of the SPR signal was observed for only the cinchonine aqueous solution, which means that the cinchonine-selective film caught cinchonine but not cinchonidine. Thus, selective measurement toward cinchonine against cinchonidine was possible with the use of the cinchonine-selective film sensor. Therefore, the sensor chip immobilizing the film selective toward either cinchonidine or cinchonine was constructed by the molecular imprinting technique, and had high selectivity toward each optical isomer.

Conclusions

The molecular imprinted films were prepared by using cinchonidine and cinchonine as the templates. Cinchonidine was caught by the cinchonidine-selective film; cinchonine, by the cinchonine-selective film. The rate at which cinchonidine was caught by the cinchonidine-selective film was linearly dependent on the concentration of the cinchonidine aqueous solution, which means the applicability to the quantitative analysis of the cinchonidine concentration. The cinchonidine-selective film and

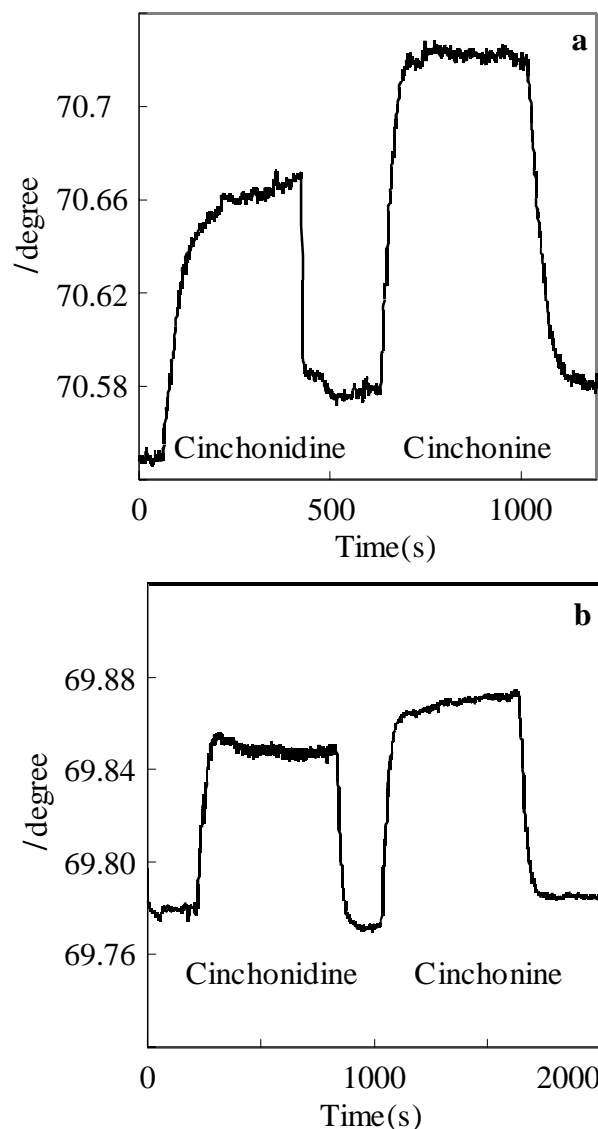


Fig.4 Time courses of the SPR signal for the cinchonidine and cinchonine aqueous solution by using (a) the cinchonidine-selective film and (b) the cinchonine-selective film.

the cinchonine-selective film could be used for discrimination between cinchonidine and cinchonine.

References

1. M. Novotny, H. Soini, and M. Stefansson, *Anal. Chem.*, **1994**, *66*, 646A.
2. J. Matsui, I. A. Nicholls, and T. Takeuchi, *Tetrahedron: Asymmetry*, **1996**, *7*, 1357.
3. S. Terabe, K. Otsuka, and H. Nishi, *J. Chromatogr. A*, **1994**, *666*, 295.
4. N. J. Deddes, A. S. Martin, F. Caruso, R. S. Urquhart, D. N. Furlong, J. R. Sambles, K. A. Than, J. A. Edgar, *J. Immunol. Methods*, **1994**, *175*, 149.
5. M.-C. Dubs, D. Altschuh, and M. H. V. Van Regenmortel, *J. Chromatogr.*, **1992**, *597*, 391.