Development of a Composite Optical Waveguide Sensor for Immunoglobulin G

Abliz Yimit,† Xiuzhu Huang, Yitai Xu, Takashi Amemiya, and Kiminori Itoh
Graduate School of Environment and Information Science, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama 240-8501
†*Department of Chemistry, Xinjiang University, Urumqi 830046, P. R. China*

(Received September 30, 2002; CL-020836)

Highly sensitive composite optical waveguide (COWG) immunosensors were developed for immunoglobulin IgG detection. The sensing layer of the protein A was coated onto the COWG surface with (γ-aminopropyl) triethoxysilane. The sensor has a short response time, reversibility and high sensitivity (as low as 70 pg/cm^3 IgG was easily detected).

Sensors capable of on site chemical or biochemical analysis are required in many areas including industrial process control, medical analysis, and environmental monitoring. Optical waveguide (OWG) sensors hold potential for applications in these areas. In particular, OWG based biochemical sensors have been widely studied, because the utilization of OWGs offers numerous advantageous features such as small size, robustness, and potential for remote sensing.

The principle of typical OWG biosensors is as follows: In biochemical affinity sensors the chemically selective coating contains receptor molecules onto the OWG surface that specifically or selectively binds certain ligands as analyte molecules. Thus, the refractive index of the medium near the OWG surface is changed. The effective refractive index change can be measured by various optical means. In fact, several optical sensors were developed by using the highly selective affinity reaction between protein A and IgG.[1–3] However, their detection limit is several ng/cm^3, and needs to be improved.

A highly sensitive thin film (TiO_2 film/K^+ ion-exchanged glass) COWG was developed in our laboratory. It was applied to a refractive index sensor,[4] and to a highly sensitive ammonia sensor.[5] In the present work, we applied this COWG to a highly sensitive immunoglobulin (antibody IgG) sensor.

The COWG sensing device consisted of a substrate, a waveguiding layer (thin film) and a sensing layer. The TiO_2 film/K^+ ion-exchanged glass COWG devices were prepared in the following way: A microscope glass slide (borosilicate: purchased from Matsunami Glass Co. Ltd.) was dipped into potassium nitrate melt at 400 °C for 30 min to obtain a K^+ ion-exchanged glass OWG, and was used as a substrate. A TiO_2 thin film was deposited onto the substrate by RF sputtering method, which gives a miniband TiO_2 film/K^+ ion-exchanged glass COWG (cf. Figure 1). A mask with a narrow gap, under which the substrate of K^+ ion-exchanged glass was mounted at a distance of 5–10 mm, gave the thin film of TiO_2 pattern of 5–8 mm wide striping with two 1–2 mm long tapered ends (slopes) as shown in Figure 1. These tapered structures work as optical couplers, which efficiently transfer guided light from the K^+ ion-exchanged layer ($n = 1.518$) to TiO_2 thin film layer and vice versa. The refractive index of the TiO_2 films was larger than 2.35 and the values of thickness were 20–30 nm, as determined by ellipsometry measurements.

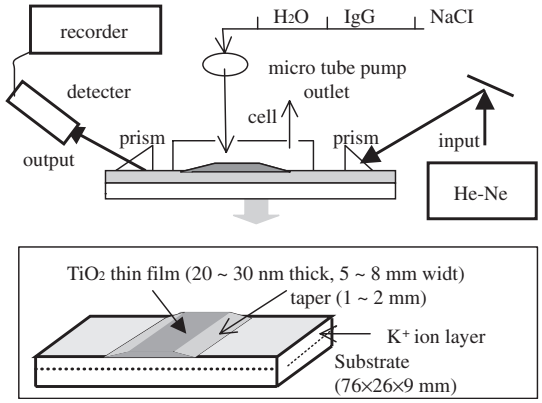

Figure 1. Structure of the composite optical waveguide (COWG) and the sensor system.

The sensing layer (protein A) was immobilized onto the COWG surface in the following manner: The COWGs were first modified with hydrogen peroxide (17.5%) for 20 h, and (γ-aminopropyl) triethoxysilane (γ-APTES, 5% in acetone) for 1 h. They were then air dried and placed into glutaraldehyde solution (GA, 5%, pH 7) for 3 h. Protein A (1 mg/cm^3, pH 7, 0.05 mol dm^{-3} phosphate buffer) was immobilized onto the COWG via the surface aldehyde, the remaining unreacted aldehyde was blocked with 0.1 mol dm^{-3} glycine.[6]

Figure 1 shows the IgG testing system. The COWG sensor device in a 0.9 cm^3 flow cell was mounted on a rotational stage equipped with X-Y-Z translation. A He–Ne laser (633 nm) was used as a light source. The laser beam was introduced into the COWG using a prism coupler (glass prism, $n = 1.75$; matching liquid of diiodomethane, $n = 1.74$), and emerged from it by the second prism coupler. The intensity of the output light was monitored with a photomultiplier detector and the signal was recorded with a pen recorder. Water, IgG and NaCl solution were injected into the cell through a separate injected port. All measurements were carried out under room temperature.

Figure 2 shows the COWG sensor response in distilled water, IgG solution (7 ng/cm^3) and NaCl solutions. When the water was drained off, solutions of IgG in phosphate buffer (pH 7) were then injected into the cell. The output light intensity (signal) decreased and became a stable value after a few minutes. The decrease in the signal may be caused by the IgG binding to the protein A, which increases refractive index of the COWG surface. The intensity of the evanescent wave at the COWG surface becomes very strong, and extremely sensitive to the surface condition. Increases of the refractive index of the COWG sensing layer will cause changes in the waveform of the guided light, and hence scattering attenuation becomes large.[4] When IgG bound to protein A was removed with 0.5 mol dm^{-3} NaCl solution and distilled water, the output light

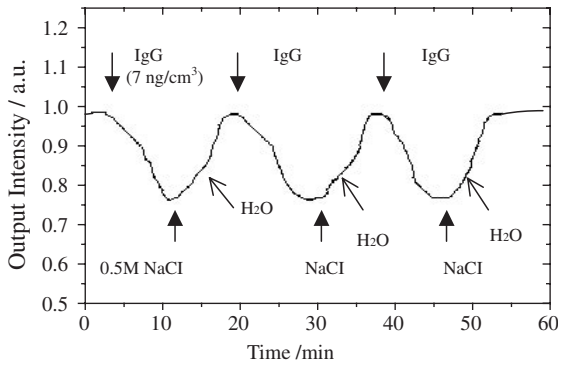

Figure 2. A typical sensor response of the IgG sensor.

intensity fully returned to its primary level.

Figure 3 shows the response of the COWG sensor to various concentrations of IgG. Attenuation of the output intensity,[7] defined as $\alpha = 10\log(P_{H_2O}/P_{IgG})$, were 2.9–0.4 dB/cm, corresponding to IgG concentration of 7 μg/cm^3–70 pg/cm^3. Under the described experimental conditions the COWG sensor easily detected 70 pg/cm^3 of IgG.

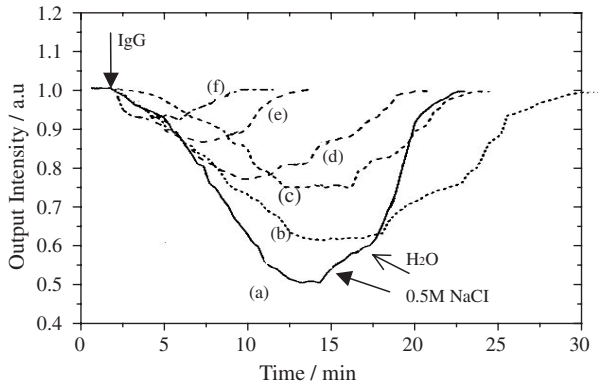

Figure 3. Temporal response of the sensor to different concentrations of IgG. (a) 7 μg/cm^3, (b) 700 ng/cm^3, (c) 70 ng/cm^3, (d) 7 ng/cm^3, (e) 700 pg/cm^3, (f) 70 pg/cm^3.

The $\log\alpha$ values thus obtained as a function of $\log[C_{IgG}]$ are plotted in Figure 4. A strong dependence of the sensor signal on the IgG concentrations were observed; $\log\alpha$ was linear to $\log[C_{IgG}]$ and we obtained the following relation:

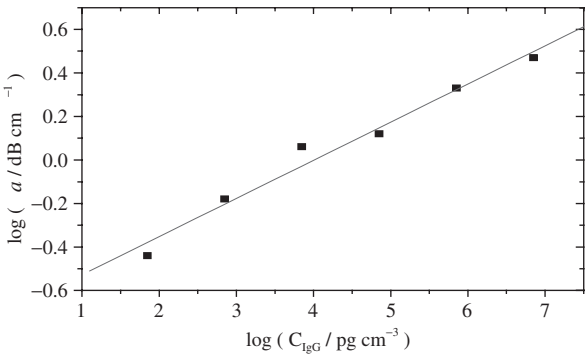

Figure 4. Relation between the attenuation of the guided light and the concentration of IgG.

$$\log\alpha = 0.2\log[C_{IgG}] + \text{constant.}$$
$$\alpha \propto [C_{IgG}]^{0.2}$$

The signal of the COWG (i.e., α) was proportional to the $[C_{IgG}]^{0.2}$. Thus, the signal is changed about 1.5 times, when IgG concentrations were changed 10 times.

By employing the COWGs as a detection method, we have successfully developed the protein A-TiO_2 film/K^+ ion-exchanged glass COWG immunosensor; it is fast in response, reversible and highly sensitive for IgG. In addition, the COWG sensor is inexpensive and simple in structure, which makes its fabrication easy.

References

1 Ph. M. Nellen and W. Lukosz, *Biosens. Bioelectron.*, **8**, 129 (1993).
2 R. Slavik, J. Hoola, J. Ctyroky, and E. Brynda, *Sens. Actuators, B*, **74**, 106 (2001).
3 N. F. Starodub, A. V. Nabok, V. M. Starodub, A. K. Ray, and A. K. Hassan, *Ukr. Biokhim. Zh.*, **73**, 55 (2001).
4 X. M. Chen, D. K. Qing, K. Itoh, and M. Murabayashi, *Opt. Rev.*, **3**, 351 (1996).
5 A. Yimit, K. Itoh, and M. Murabayashi, Proceedings of the 34th Chemical Sensor Symposium, Sendai 2002, Vol. 18, p 40–42.
6 H. Muramatsu, K. Kajiwa, E. Tamiya, and I. Karube, *Anal. Chim. Acta*, **188**, 257 (1986).
7 H. Nishihara, S. Haruna, and T. Suhara, ''Optical Integrated Circuits,'' (in Japanese), Ohm Publishing Co. Ltd., Japan (1993), p 263.