Slab Optical Waveguide with Octadecylsilane Coated Surface for Absorption Detector of Flow Analysis

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A K^+ doped glass slab optical waveguide (SOWG) was coated with octadecylsilane (ODS) to separate and concentrate analytes onto the SOWG with hydrophobic interaction between analytes and ODS. As an absorption detector of flow analysis, the ODS–SOWG detector was almost insensitive to a blue dye (bromothymol blue) which is not adsorbed to the surface of the SOWG. On the other hand, it was highly sensitive to another blue dye of coomassie brilliant blue G-250 (CBB) which is firmly adsorbed onto the SOWG surface.

A slab optical waveguide (SOWG) is a dielectric slab covered with a thin layer whose refractive index is higher than that of a substrate (Fig. 1). Incident light into the thin layer propagates through the SOWG with minimum attenuation. The SOWG has been developed as one of the most important devices for integrated optical circuits in optoelectronics during past two decades. The SOWG has also been recognized by several chemists as a useful tool for highly sensitive spectroscopic measurement of a thin film and its surface.[1–3] When an SOWG is applied to surface analysis, the evanescent wave of propagated light is used for the excitation of an analyte. In this manner, the principle of this technique is similar to that of an attenuated total reflection (ATR) technique in infrared spectroscopy. However, as the guiding layer of the SOWG can be much thinner than that of an ATR element, the number of reflections of incident light at the boundary can be by orders of magnitude greater in the SOWG than in the ATR element. Thus, the SOWG can provide us a much more sensitive method for spectroscopic measurements of surface phenomena. Recently, Degrandpre et al. and Saavedra and Reichert discussed the characteristics of the SOWG as a flow cell of liquid phase absorption spectrometry.[4,5] Itoh et al. applied the SOWG to an opto-biosensor for ethanol.[6] Moreover, Choquette et al. developed an immunosensor using an Ag^+ doped glass SOWG.[7]

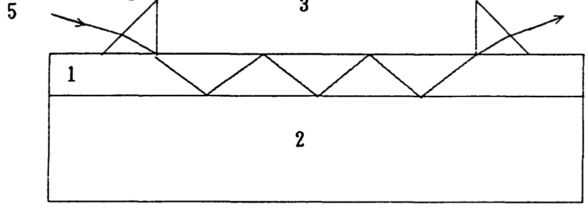

Fig. 1. Schematic diagram of slab optical waveguide (SOWG). 1. guiding layer, 2. substrate, 3. air, 4. coupler prisms, 5. incident light. When the refractive indices of a guiding layer, a substrate and the air are expressed as n_1, n_2, and n_3 respectively, the following equation must be satisfied in a SOWG; $n_1 > n_2, n_3$.

In this work, a K^+ doped SOWG was fabricated by an ion-exchange process of a slide glass in molten potassium nitrate.[3,8] Then, the SOWG was coated with octadecylsilane (ODS) to separate and concentrate analytes onto the SOWG with hydrophobic interaction between analytes and ODS. The ODS has been commonly used as a stationary phase in reversed phase high performance liquid chromatography (HPLC) and can easily be introduced to glass surface.[9,10] Moreover, the K^+ doped glass SOWG has been known to prepare easily in an ordinary chemical laboratory and to show relatively high transmissivity of incident light.[3,8] Thus, the combination of the K^+ doped glass SOWG and the ODS was chosen in this study. The ODS–SOWG was used for an absorption detector of flow analysis, and the characteristics of the SOWG detector were examined with two kinds of dye standard solutions.

Experimental

Octadecylsilane-Coated Slab Optical Waveguide. SOWGs were fabricated by an ion-exchange process of commercial sodalime slide glasses in molten potassium nitrate at 673 K for times ranging from 30 min to 2 h.[3,8] In general, a SOWG only propagates the lights of discrete incident angles due to boundary conditions.[1] The SOWGs in this study supported one to three propagation modes. In particular, the SOWG with a 30 min ion-exchange process supported one mode and gave the best sensitivity in absorption measurements. Thus, the 30 min SOWG was used throughout the following experiments. Octadecylsilane was then introduced onto the surface of the SOWG by the procedure of Kingston and Gerhart with minor modification.[10] The SOWG was heated in concentrated HCl for 4 h under reflux, washed with distilled water and dried at 363 K for 1 h. The dried SOWG was heated with 10 vol% octadecyltrichlorosilane in toluene for 10 h under reflux, and was washed with toluene then with ethanol. The bound chlorosilane was hydrolyzed with 50 vol% aqueous acetonitrile for 2 h at room temperature. After washing with ethanol and drying at 333 K for 1 h, the SOWG was heated with 10 vol% trimethylsilane in toluene under reflux for 2 h to protect remaining silanol groups, followed by final washings with toluene, ethanol and water.

Measurement System. A diagram of the measurement system is shown in Fig. 2. The flow cell was made on the SOWG with a poly(tetrafluoroethylene) (PTFE) block and poly-

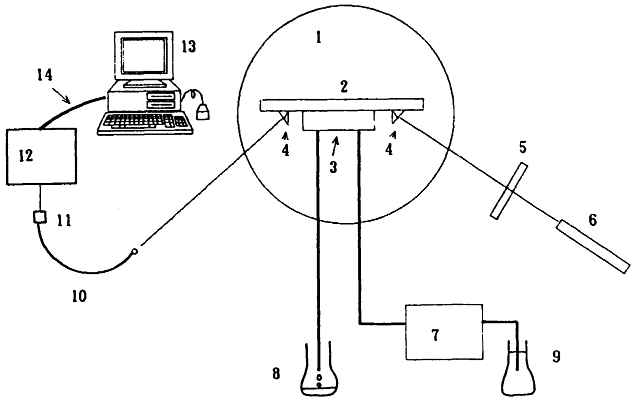

Fig. 2. Schematic diagram of the measurement system. 1. rotation stage, 2. SOWG, 3. flow cell, 4. coupler prisms, 5. polarizer, 6. He–Ne laser, 7. HPLC pump, 8. waste, 9. solvent reservoir, 10. optical fiber, 11. photodiode, 12. digital electrometer, 13. personal computer, 14. GP–IB.

(tetrafluoroethylene-co-hexafluoropropylene) (FEP) film and PTFE (0.05 mm and 0.50 mm thick respectively) spacers. The cell length was 1.5 cm and the cell volume was 25 μl(=mm^3) (3 mm (W)×15 mm (L)×0.55 mm (H)). Distilled water was sent with an HPLC pump and sample solution was introduced through a silicone septum with a syringe. A He–Ne laser (632.8 nm, 2 mW, and random polarization) was used as a light source. The SOWG was mounted upon a 360° rotational stage with X–Y–Z translation to determine the angle of incident light for each propagation mode. The laser light was coupled into the SOWG with a prism coupler.[1] The refractive index of the coupler prisms (CL-01 from Kogaku Giken Co. Ltd., Japan) was 1.8785 at 632.8 nm. A polarizer was placed between the coupler and the laser to select the polarization of propagated light. The out-coupled beam was collected with an optical fiber and the light intensity was measured with a photodiode. The photocurrent was monitored with a digital electrometer and the signal fed into a personal computer through GP-IB.

Absorbance Standard Solutions. Blue color dyes of bromothymol blue (BTB) and coomassie brilliant blue G-250 (CBB) from Wako Chemicals Co. Ltd., Japan, were used for absorbance standards. BTB dye was dissolved in 0.05 M (M=mol dm^{-3}) phosphate buffer (pH 8.02) and CBB was dissolved in distilled water. The BTB solutions used in the SOWG measurements were 0.63 to 2.5 mM, which gave absorbances of 8.2 to 32.8 at 633 nm with a conventional spectrophotometer of a 1 cm cell respectively. Such absorbance values in a 1 cm cell were obtained by measuring the absorbances of the diluted BTB solutions. On the other hand, 5 to 40 μM of CBB solutions were used, which gave absorbances of 0.09 to 0.74 at 633 nm in a 1 cm cell respectively.

Results and Discussion

Characteristics of K$^+$ Doped SOWG as Absorption Cell. When the SOWG detector is used for absorption measurements of liquid samples, Lambert–Beer's law has to be satisfied as follows;

$$A_{\mathrm{SOWG}} = \varepsilon c L_{\mathrm{eff}}, \qquad (1)$$

A_{SOWG}: Absorbance obtained with the SOWG detectors,
ε: Molar absorption coefficient of an analyte,
c: Concentration of an analyte (M) in liquid sample,
L_{eff}: Effective cell length (cm) of the SOWG detector.

In this equation, the adsorption of analytes onto the SOWG surface is assumed to be negligible. In an ordinary spectrophotometer with a 1 cm cell, on the other hand, Lambert–Beer's law is expressed as

$$A_{1\,\mathrm{cm}} = \varepsilon c L \qquad (2)$$

$A_{1\,\mathrm{cm}}$: Absorbance with a 1 cm cell,
L (=1 cm): Cell length.

From Eqs. 1 and 2,

$$L_{\mathrm{eff}} = A_{\mathrm{SOWG}} / A_{1\,\mathrm{cm}}. \qquad (3)$$

Since the evanescent wave of propagating light is solely absorbed by liquid samples in the SOWG, the L_{eff} is much shorter than its physical cell length, and is dependent upon properties of the SOWG such as the refractive index profile of the guiding layer.[1] Thus, the L_{eff} value is an important parameter to evaluate the SOWG as an absorption detector. The L_{eff} is experimentally obtained for the nonODS–SOWGs based on Eq. 3. BTB standard solutions were used for the measurement because BTB is not adsorbed onto glass surface.[4] BTB solution (0.5 cm^3) was injected into the flow system (1.0 cm^3 min^{-1}) to obtain the maximum possible absorbance. Figure 3 shows the results on the TM$_0$ (transverse magnetic) mode of K$^+$ doped SOWGs of 30 min and 1 h ion-exchange processes respectively. The SOWG with a 30 min ion-exchange process supported only one mode, and its TM$_0$ mode provided the longest L_{eff} of 21 μm. The L_{eff} value remained unchanged with the ODS coating. The experimental value was in good agree-

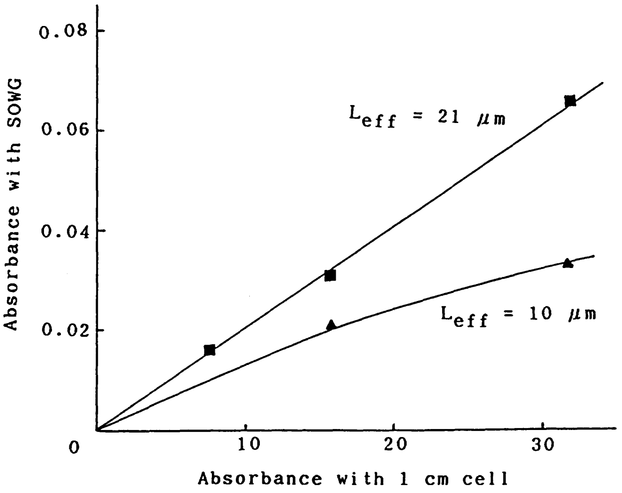

Fig. 3. Absorbance response of SOWG without ODS to BTB solution. Sample volume; 0.5 cm^3, flow rate; 1.0 cm^3 min^{-1}; —■— TM$_0$ mode of the SOWG with a 30 min ion-exchange process; —▲—TM$_0$ mode of the SOWG with a 1 h ion-exchange process.

ment with the calculated one (28.4 μm), which was obtained by using an inverse Wentzel–Kramers–Brillouin (WKB) method and a ray optics method.[11,12] The number of reflections of the propagated light at the liquid/SOWG interface (N_{ref}) was also estimated to be about 170 times/cm in the 30 min ion-exchanged SOWG. A full description on the characterization of the K$^+$ doped SOWGs will be given elsewhere. The N_{ref} value corresponds to the degree of amplification in sensitivity with the SOWG. In the case of an ATR technique, the N_{ref} value is between 1 to ca. 10. Thus, the SOWG detector in this study was by one to two orders of magnitude as sensitive as the ATR technique. However, even the longest L_{eff} of 21 μm is one five hundredth as long as 1 cm cell. Thus, the SOWG detector is almost insensitive to liquid sample itself.

Detection of CBB with ODS-SOWG Detector. The K$^+$ doped SOWGs with and without ODS were applied to the absorption measurements of CBB standards in the flow system. Figure 4 shows the absorbance response of the SOWG detectors to 0.5 cm^3 of the CBB standard solution volume. As seen in this figure, quantitative response was obtained with the ODS-SOWG. CBB molecules were adsorbed onto the ODS-SOWG surface so firmly that 1 M HCl solution was necessary to remove CBB from the surface. On the other hand, nonlinear response was obtained with the nonODS-SOWG. CBB molecules seemed to be rather weakly adsorbed onto the nonODS-SOWG because the absorption signal returned to the baseline soon after the injection of the CBB solution. As seen in the same figure, A_{SOWG} values with the ODS-SOWG were slightly greater than corresponding $A_{1\,cm}$ values.

Now the apparent cell lengths L_{app} (cm) is defined as

$$L_{app} = A_{SOWG}/A_{1\,cm}. \tag{4}$$

Equation 4 is the same form as Eq. 3. Thus, the L_{app} is equal to the L_{eff} for an analyte whose adsorption onto the SOWG surface is negligible. On the contrary, the L_{app} becomes longer than the L_{eff} when the analyte is adsorbed onto the SOWG surface. Moreover, the A_{SOWG} for the adsorbed analyte is expressed as[1)]

$$A_{SOWG} = \varepsilon nf/S, \tag{5}$$

n: Total amounts of an analyte (mol) concentrated on the SOWG surface,
f: A constant which depends on the parameters of the SOWG,
S: The area of the SOWG surface (dm^3×cm^{-1}).

From Eqs. 2, 4, and 5,

$$L_{app} = (n/c) \times (f/S). \tag{6}$$

The L_{app} is dependent on neither the molar absorption coefficient nor the concentration of an analyte in the sample solution, because the n value in Eq. 6 should be proportional to the c value in lower concentration range. On the other hand, the L_{app} is dependent upon the affinity of analytes to the SOWG surface, physical parameters of the SOWG cell and the measurement conditions. Thus, the L_{app} can be a practical parameter to evaluate the sensitivity of the SOWG when compared with a conventional spectrophotometer. Moreover, when the measurement conditions are fixed, the differ-

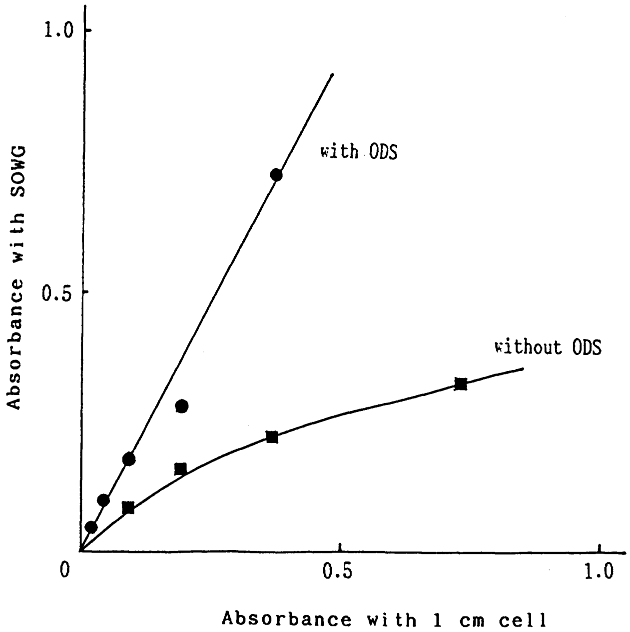

Fig. 4. Absorbance response of SOWG with and without ODS to CBB solutions. Sample volume; 0.5 cm^3, flow rate; 1.0 cm^3 min^{-1}. The propagated light of m=0 (TE$_0$+TM$_0$) of the K$^+$-doped SOWG with a 30 min ion-exchange process was used for the absorption measurements.

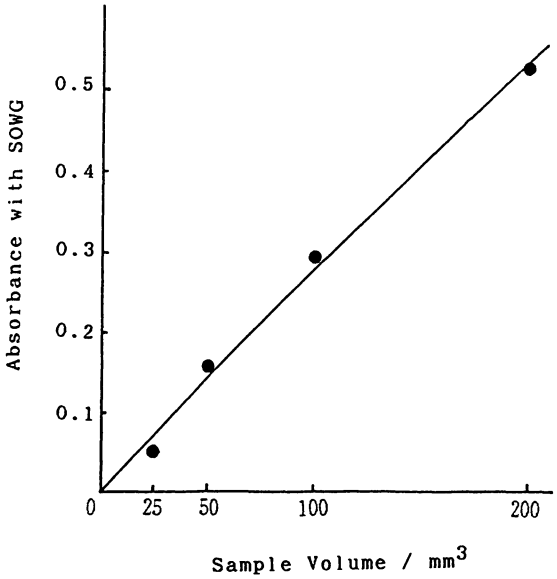

Fig. 5. Dependence of absorbance of CBB solution upon sample volume with ODS-SOWG detector. Flow rate; 1.0 cm^3 min^{-1}, absorbance in a 1 cm Cell of CBB solution; 0.62, mode of propagated light; TM$_0$.

ence of the L_{app} among various compounds can be ascribed to the difference of their affinity to the SOWG surface. So the L_{app} is also useful to evaluate the selectivity of the SOWG detector. Figure 5 shows the dependence of A_{SOWG} for CBB solution upon the sample volume. As seen in this figure, CBB solution of 200 μl gave almost the same A_{SOWG} as $A_{1\,\mathrm{cm}}$, i.e. the L_{app} is ca. 1 cm. This value is about 500 times greater than that of BTB ($=L_{\mathrm{eff}}$, 21 μm). Consequently, the absorbance with the ODS-SOWG detector is about 500 times greater for CBB solution than for BTB solution, when both the BTB and the CBB solutions show the same absorbance with a conventional spectrophotometer with a 1 cm cell. The theoretical consideration based on literatures,[1,11,12] makes it possible to estimate the f value in the Eq. 6 for the ODS-SOWG used in this work. The amounts of CBB molecules adsorbed onto the SOWG surface for the 200 μl sample in Fig. 5 should be ca. 4.1×10^{-11} mol (ca. 0.56% of CBB in the 200 μl sample solution), as the f value is 400, the S value is 4.5×10^{-5} dm^3 cm^{-1} and the c value is 37 μM in Eq. 6. Thus, the absolute sensitivity of the SOWG detector (defined as 1% absorption) is estimated to be 2.3×10^{-13} mol for CBB. The detail of calculation process will be given elsewhere. In spite of the insensitivity to BTB solution, the ODS-SOWG was highly sensitive to CBB. This feature can provide the SOWG detector with selectivity. Only analytes adsorbed onto the SOWG surface can be detected with high sensitivity, while sample matrix in the solution may not interfere with the SOWG measurements.

Conclusion. The ODS coating made it possible to detect trace amounts of CBB quantitatively. Moreover, the ODS-SOWG detector gave the absorbance for CBB about 500 times as great as that for BTB with the sample volume of 200 μl. Although the present work was done on model compounds, the ODS-SOWG could be a sensitive and selective opto-chemical sensor.

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