Fiber-Optic Sensor with a Chitosan/Poly(vinyl alcohol) Cladding for the Determination of Ethanol in Alcoholic Beverages†

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A fiber-optic sensor having a chitosan/poly(vinyl alcohol) blended membrane as the cladding and a Teflon protective coating was fabricated to determine ethanol in alcoholic beverages. The response was explained on the basis of an increase in the critical angle of the optical fiber by ethanol, which was induced by a shrinkage of the cladding membrane, leading to an increase in the refractive index. Crosslinking of the cladding membrane with glutaraldehyde enhanced the sensor sensitivity in the 0 - 70 v/v% ethanol-content range. The Teflon coating removed interferences from sugars and organic acids commonly present in alcoholic beverages. The response time was within 2 min for 50 v/v% ethanol and the relative standard deviation was within 1.7% in 20 repeated determinations during a 2-week period. A linear relationship was obtained between the response and the ethanol content in the 0 - 70 v/v% range. The sensor was applied to the determination of ethanol in shochu, sake, wine, whiskey and beer and the results were consistent with the certified values and values obtained by the conventional specific-gravity method.

Keywords  Fiber-optic sensor, chitosan/poly(vinyl alcohol) cladding, ethanol determination, alcoholic beverage

Many types of sensors, particularly biosensors, have been devised to determine ethanol in alcoholic beverages, instead of the conventional specific-gravity and titrimetric methods; typical sensors are those having alcohol dehydrogenase-immobilized electrodes.1-3 Enzyme electrodes on which alcohol oxidase was immobilized were also employed for the determination of ethanol in wine4 and Japanese sake.5 In addition, fiber-optic biosensors using these enzymes have been newly designed.6-8 The advantages of these enzyme sensors are their high sensitivities, selectivities and simplicities. On the other hand, process controls in biotechnological industries, such as continuous alcoholic fermentations and beer brewing, require a technique for the on-line determination of ethanol. No enzyme sensors meet this need because of the short lifetimes of 1 - 2 weeks and the low measurable concentration ranges below about 1 wt%.

An optrode using a plasticized poly(vinyl chloride) membrane containing a trifluorooacetophenone derivative has been fabricated for the determination of ethanol.9 Although the sensor offers a high selectivity, a wide measurable concentration range of 0.5 - 35 v/v% and a short response time of 30 s, the safety of the reagents employed must be carefully examined when being applied to food industries. The direct optical determination of ethanol based on a surface plasmon measurement10 is interesting in this regard because this sensor has no reagent which elutes into a sample. However, the influences of the coexistents in practical samples are obscure.

Recently, we have tentatively prepared a fiber-optic ethanol sensor with a chitosan/poly(vinyl alcohol) (PVA) blended membrane as the cladding.11 The defect of the sensor was that the response was affected by sugars and organic acids present in alcoholic beverages. Here, we describe that by coating the cladding with a Teflon membrane we can remove this defect. Further, the principle of the response is discussed on the basis of studies on the refractive index of the blended membrane.

Experimental

Reagents

Chitosan (95% deacetylation) was kindly supplied by Katokichi Co., Kagawa, Japan. PVA (mw=72000) and an amorphous fluoropolymer (Teflon AF 1600) were purchased from Du Pont Japan Ltd. Co. An organic fluorine solvent (Fluorinert FC72) and a nonionic detergent (Fluorad FC-431) were obtained from Sumitomo 3M. Glutaraldehyde (GA; electron microscopic grade) from Wako Pure Chem. Industries and 3-glycidoxypropyltrimethoxysilane from Shin-etsu Chem. Co. were used as received. Distilled ethanol was mixed in intended proportions with water purified by reverse osmosis (Millipore Milli RO15) and deionization. All

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other chemicals were of reagent or better grade and were used without further purification.

**Fabrication of the probe**

A quartz optical fiber (Showa Electric Wire & Cable, SHOWGUIDE SF-S800/1000) was cut to a 80-mm length and the nylon protective coating was peeled so as to give a quartz rod. In order to remove a 50-mm midsection of the quartz cladding from the rod, the rod was immersed in 46% hydrofluoric acid for 45 min at room temperature after both distal ends of the rod had been covered with poly(vinyl chloride). The partly stripped rod was dried in vacuo at 100°C and then allowed to react with 10v/v% 3-glycidoxypropytrimethoxysilane in dry benzene at 85°C under Ar for 12 h. The rod was washed with methanol, immersed in 13 cm³ of 0.25 M acetate buffer (pH 6) containing 150 mg of chitosan and kept at 60°C for 48 h. After being washed successively with 2v/v% aqueous acetic acid, 0.37 M aqueous NH₃ and deionized water, the chitosan-modified rod was dipped in 10 cm³ of 1v/v% aqueous acetic acid containing 200 mg of chitosan and 200 mg of PVA; it was then air-dried overnight at 30°C to give a chitosan/PVA cladding membrane. The membrane was thoroughly washed with 1 M NaOH and water and then crosslinked with GA. The thus-obtained rod was dipped in an ethyl acetate solution of a nonionic detergent Fluorad FC-431 (0.02 wt%); then, after air-dried, it was immersed in 10 ml of a Fluorinert FC72 solution containing 336 mg of Teflon AF 1600 and air-dried again to give a Teflon protective coating. The cladding and Teflon coating membranes were about 1 µm and 0.1 µm thick, respectively, as measured using a Hitachi Model S-2400 electron microscope. Both membranes covered the core smoothly and no breaking was observed.

The thus-obtained optical fiber was fixed in the center of a flow-through-type double jacket Teflon cell with an epoxy resin, as shown in Fig. 1. Both distal ends of the cell were polished with emery paper (#800) and alumina (Fujimi Metapolish No. 4).

**Apparatus and procedure**

The cell was mounted in a JASCO UVIDEC-610A spectrophotometer and incident light was focused with a spherical simple lens of 20-mm focal length (Sigma Koki, SLB-10-20PM) on one edge of the optical fiber. A sample was introduced into an inner cell containing 336 mg of Fluorinert FC72 solution containing 13 cm³ of aqueous ethanol containing 2v/v% aqueous acetic acid and 0.1 µm thick, respectively, as measured using a Hitachi Model S-2400 electron microscope. Both membranes covered the core smoothly and no breaking was observed.

The thus-obtained optical fiber was fixed in the center of a flow-through-type double-jacket Teflon cell with an epoxy resin, as shown in Fig. 1. Both distal ends of the cell were polished with emery paper (#800) and alumina (Fujimi Metapolish No. 4).

**Results and Discussion**

**Response of the sensor**

Figure 2 shows the typical response curve of the sensor. When aqueous ethanol was introduced to the cell, the intensity of the transmitted light through the core decreased and gave a constant value within 2 min. The light intensity returned to the initial value within 5 min upon replacing the sample by water. Although the response was affected by the setting position of the cell, a reproducible response was obtained once the cell was set: the relative standard deviation was within 1.7% in 20 replicate measurements for 50% ethanol during 2 weeks. Moreover, the sensor manifested no decrease in sensitivity, even after 100 measurements for over 6 months. Unless the Teflon membrane was coated, the durability was poor, though the response time was as short as 40 s; in addition, sugars and organic acids interfered with the response, as is described later.
Optimum conditions for the preparation of the cladding

The calibration curve varied with the composition of the cladding membrane and the extent of crosslinking with GA (Fig. 3). No response was observed when the cladding was prepared with only chitosan or PVA, while the mixture of these two polymers showed a steep rise in the response above an ethanol concentration of 70v/v%. This response was much improved by crosslinking of the cladding with GA; the sensitivity in the low ethanol-content range was most greatly enhanced by the crosslinking when the cladding was composed of equivalent weights of chitosan and PVA and was crosslinked for 12 h at 50°C. A linear relationship was obtained between the response and the ethanol content in the range of 0 - 70v/v%. No response was observed without the chitosan/PVA cladding. An original optical-fiber whose quartz cladding was not removed with hydrofluoric acid also showed no response. These facts suggest that the response depends on a refractive index change of the chitosan/PVA cladding, which brings about an alteration in the critical angle of the optical fiber.

Swelling and refractive indices

The chitosan/PVA cladding membrane can be expected to swell in water because of the high hydrophilicity. Figure 4 shows the relationship between the swelling index and the ethanol content. Irrespective of the extent of crosslinking with GA, the membranes which had previously swollen in water gradually shrank upon coming in contact with ethanol and the index eventually approached 1. This decrease in volume caused an increase in the refractive index of the membrane as shown in Fig. 5. At 0v/v% of ethanol, the refractive index of the membrane crosslinked with GA for 12 h was higher than those of the untreated or insufficiently crosslinked membranes. The order of the refractive index at 100% of ethanol was reversed, though the refractive indices of all the three membranes increased with an increase in the ethanol content; that is, the refractive index of the highly crosslinked membrane varied in a narrow range of 1.43 to 1.50, while the refractive index of the membrane untreated with GA increased most largely from 1.40 to 1.54. These results account for the response curves in Fig. 3 (described below), if the response of this sensor obeys the theory of total internal reflection spectroscopy.

As depicted in Fig. 1, b, total reflection in an optical fiber occurs when incident light strikes the cladding at an angle (θ) greater than the critical angle (θc), which is defined as sin θc = n1/n2, where n1 is the refractive index of the core and n2 is that of the cladding. In the case of the optical fiber used here, the θ of light propagating through
the core \( (n_1 = 1.460) \) is larger than 82.96°, since the quartz cladding \( (n_2 = 1.449) \) is left at the distal end. Thus, if the refractive index of the chitosan/PVA membrane is smaller than 1.449 when the membrane swells in 100% water and, moreover, is unattainable to this value even after the membrane shrinks with ethanol, no change in the transmitted light intensity would be observed. When the refractive index exceeds 1.449, \( \theta_e \) becomes larger than 82.96° and, thus, a part of the propagating light goes out of the core, thus causing an increase in the absorbance. This simple interpretation probably holds for a fiber untreated with GA, which showed no response practically below 70v/v% ethanol content, though the refractive indices of the membranes used in Fig. 5 can not be directly compared to those of the sensor claddings because the thicknesses of the cladding membranes are fairly thin and, thus, the cladding membranes are assumed to differ from separately prepared membranes to the extent of the crosslinking. It seems likely that the refractive index of the highly crosslinked cladding membrane which responded efficiently to lower contents of ethanol changed successfully in the range of 1.449 to 1.460. The refractive indices of the chitosan and PVA membranes swollen in water were 1.41, smaller than 1.449 and 1.54, larger than \( n_1 \), respectively. It should thus be emphasized that the combination of these two polymers and crosslinking permitted us to offer a cladding which exhibits a refractive index change that is suitable to the present fiber-optic sensor.

The chitosan/PVA membrane had no absorption at 500 nm. Further, an electron-microscopic analysis showed that the core was closely covered by a cladding of about 1 µm in thickness and that the core-cladding interface was as smooth as a mirror. These facts suggest that the response of this sensor can be based on the principle of total internal reflection.

**Determination of ethanol in alcoholic beverages and the effect of a Teflon coating**

The ethanol contents in alcoholic beverages determined using the present fiber-optic sensor (Sensor 1) and a sensor having no Teflon coating (Sensor 2) are summarized in Table 1. In either sensor, the chitosan/PVA cladding was crosslinked with GA at 50°C for 12 h. Beer was subjected to an analysis after being degassed by sonicating for a few minutes. Other samples were introduced into the cell without any pretreatment. The sensor having a bare cladding exhibited larger responses to Japanese sake, wine and whiskey than those expected from their certified values. These beverages usually contain small amounts of sugars, amino acids and other coexistents. The influences of glucose, glutamic acid and lactic acid on the response are shown in Fig. 6, where the concentrations of these coexistents were adjusted to the values observed in ordinary Japanese sake. All of the coexistents gave constant positive errors, suggesting that the sensor may also respond to these coexistents. These interferences were removed by coating the Teflon protective membrane on the cladding; the differences in

<table>
<thead>
<tr>
<th>Alcoholic beverage</th>
<th>Concentration of ethanol, v/v %</th>
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<tbody>
<tr>
<td></td>
<td>Certified value</td>
</tr>
<tr>
<td>Shochu A</td>
<td>20</td>
</tr>
<tr>
<td>Shochu B</td>
<td>25</td>
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<tr>
<td>Shochu C</td>
<td>35</td>
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<tr>
<td>Sake</td>
<td>15–16</td>
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<tr>
<td>White wine</td>
<td>14</td>
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<tr>
<td>Red wine</td>
<td>14</td>
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<tr>
<td>Whiskey</td>
<td>43</td>
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<tr>
<td>Beer</td>
<td>5</td>
</tr>
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</table>

a. In this sensor, the Teflon-coated cladding was used. b. In this sensor, the bare cladding was used. c. Not determined.
the response between the presence and absence of the coexistences were within ±4% in the range of ethanol content used in Fig. 6. As can be seen in Table 1, the ethanol contents in alcoholic beverages were also in fair agreement with the certified values and values obtained by the specific-gravity method. The amorphous fluoropolymer, which has been known to have the lowest dielectric constant of any plastic materials, certainly hindered the water-soluble non-volatile compounds from penetrating into the cladding. The Teflon protective coating, thus, made practical the present fiber-optic sensor. Importantly, the Teflon coating would also play an important role in protection against the contamination of a sample with the sensor probe reagents and, consequently, would allow us to employ the present sensor to control alcoholic fermentation processes.

References


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