Rapid Communications

Fabrication of Amperometric Glucose Sensor Using Glucose Oxidase-Cellulose Nanofiber Aqueous Solution

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Cellulose nanofiber aqueous solution, which remained virtually transparent for more than one week, was prepared by using the clear upper layer of diluted cellulose nanofiber solution produced by wet jet milling. Glucose oxidase (GOx) was easily dissolved in this solution and GOx-immobilized electrode was easily fabricated by simple repetitious drops of GOx-cellulose solution on the surface of a platinum-iridium electrode. Glucose sensor properties of the obtained electrodes were examined in phosphate buffer solution of pH 7.4 at 40°C. The obtained electrode provided a glucose sensor response with significantly high response speed and good linear relationship between glucose concentration and response current. After an initial decrease of response sensitivity for a few days, relatively constant sensitivity was obtained for about 20 days. Nevertheless, the influence of electroactive compounds such as ascorbic acid, uric acid and acetaminophen were not neglectable.

Keywords Cellulose nanofiber, enzyme immobilization, glucose sensor

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Immobilization of the enzyme is one of the most critical processes for the fabrication of an enzyme sensor, since it will highly influence properties of the obtained sensor. Various procedure have been proposed for the preparation of a glucose sensor, such as covalent attachment,1,3 cross-linking,1,4–14 hydrogel entrapment,15,17 polymer film entrapment,6,17–20 and the combination of two or more methods.21–24

On the other hand, the concept of green chemistry has infiltrated various fields of the manufacturing industry. It is recommended to employ safer solvents, reagents and materials. Cellulose is a low cost, biodegradable material with significant high strength and stability due to strong intra- and intermolecular hydrogen bonds within and among individual chains. Yabuki et al. reported that cellulose is an excellent enzyme-immobilizing material and the researchers were able to produce a glucose sensor with long-term stability of more than 11 months.25 Since cellulose is commonly insoluble in water and organic solvents, they employed ionic liquid to obtain a cellulose solution. Recently, cellulose nanofiber (CNF) dispersion solution, which was produced using wet jet milling, are available on the market. In this study, CNF was applied for the immobilization of the enzyme by simple entrapment within the CNF matrix. Glucose oxidase (GOx) was employed as the enzyme, and glucose sensor properties of the obtained GOx-immobilized electrode were investigated. First, 2 wt% CNF (BiNFi-s Cellulose WMa-10002, polymerization degree: 650, viscosity: 3000 mPa s) dispersion aqueous solution, which was kindly supplied from Sugino Machine, was diluted 20 times with water, and ultrasonic treatment was performed for 15 min. After it was kept standing for two days, the solution was separated into a clear upper layer and cloudy lower layer. The clear upper layer was confirmed to have approximately 500 mg/L of cellulose and the collected upper layer remained visually transparent for more than a week. GOx (265 U/mg, purified from Aspergillus niger) was added in clear 500 mg/L CNF solutions and CNF/GOx solutions with different GOx concentrations, 250 and 500 mg/L, were prepared. Then, 0.5 μL of these solutions were dropped on the surface of a platinum-iridium (Pt 90%-Ir 10%, 2.14 mm²) wire electrode by a microsyringe 16 times at an interval of 10 min. After GOx/CNF solution application, electrodes with GOx-CNf film were dried for at least 24 h at room temperature before they were inserted in aqueous media. With these procedures, we expected that electrodes having 4.0 μg of CNF and 2.0 μg of GOx, and 4.0 μg of CNF and 4.0 μg of GOx, would be prepared using CNF/GOx solutions with 250 mg/L GOx and 500 mg/L GOx, respectively. Three individual electrodes were prepared with each solution. The thickness of GOx-CNf films, formed on the electrode prepared using 500 mg/L CNF solutions containing 250 mg/L GOx and 500 mg/L GOx, were estimated to be about 3 and 4 μm, respectively, based on SEM observation. All response current measurements of obtained GOx-immobilized electrodes were performed at 40°C in a 0.1 M phosphate buffer solution of pH 7.4 containing 0.1 M NaCl by measuring the electrooxidation current at a potential of 0.60 V (vs. Ag/AgCl) for hydrogen peroxide detection. Electrodes were stored in phosphate buffer (pH 7.4) at 4°C when not in use.

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Figure 1 shows SEM images of cellulose film prepared using (a) clear separated 500 mg/L CNF solution and (b) 40 mg/L CNF dispersion solution without separation. CNF film obtained using clear solution consisted of uniformed size fibers, while that using CNF solution without separation contained thick fibers. This indicates that most of the thick cellulose fibers are included in the cloudy lower layer, and the clear CNF upper solution is made up of fine fibers.

Figure 2 shows the typical amperometric response of the GOx-immobilized electrode to the addition of glucose up to 22.4 mM. GOx/CNF solution containing 250 mg/L GOx was used for the electrode preparation. The response speed of the obtained electrode was significantly high and a steady-state response current was presented within a second of each glucose addition. High response speed was obtained also on the electrode prepared using the solution containing 500 mg/L GOx (Fig. 3). Although, the reason for this high response speed has not been proven, our guess is that it is due to the property of the enzyme-entrapping CNF film, which has a pore size small enough to immobilize the enzyme but large enough to allow the transportation of glucose, oxygen and hydrogen peroxide without any inhibition. The sensitivity of GOx-immobilized electrodes prepared using 250 and 500 mg/L GOx/CNF solutions were nearly the same. Good linear relationship between the glucose concentration and the response current was obtained for both electrodes within a range lower than 11.2 mM (correlation coefficient, r² > 0.992).

Figure 4 shows the long-term stability of the electrodes prepared using GOx/CNF solutions containing (a) 250 mg/L GOx and (b) 500 mg/L GOx, respectively. After an initial decrease in response for a few days, the response remained relatively stable for about 20 days. It was unexpected that the sensitivities of the electrode prepared from CNF solution with 250 mg/L GOx and with 500 mg/L GOx (twice the amount) would be approximately the same from the initial time to 29 days after preparation. It is clear that the amount of GOx immobilized on the electrode was significantly higher on the electrode prepared using a higher concentration of GOx than that using a lower concentration GOx when it was prepared. However, the sensitivity of the high GOx loaded electrode can be the same or even lower than that of the low GOx loaded electrode, since the sensitivity of the GOx-immobilized electrode depends on the oxidation current of hydrogen peroxide.
from GOx/CNF aqueous solution remained stable in aqueous media after completely drying once. Nevertheless, an initial decrease in sensitivity was clear on both electrodes and its improvement was recommended. Our expectation was that the amount of CNF in CNF/GOx solution was not high enough to entrap GOx firmly on the electrode. We hope that the increase of CNF ratio in CNF/GOx solution may improve the sensor stability. On the other hand, the introduction of CNF film between and/or after the CNF/GOx film can be another strategy.

The interference of electroactive compounds existing in biological fluids on the glucose response of obtained GOx-immobilized electrodes was examined in the presence of their physiological maximum levels. Ascorbic acid (0.11 mM), uric acid (0.48 mM) and acetaminophen (0.17 mM) notably interfered with the determination of glucose (5.6 mM) as the signal ratios of the interferent to glucose reached above 20, 300 and 900%, respectively.

The introduction of permselective inner films, such as cellulose acetate and γ-polyglutamic acid, was essential for practical use. Considering that the obtained CNF aqueous solution was visually stable, we hope that enzyme-CNF solution can be applied as an eco-friendly enzyme ink of ink-jet printing for enzyme sensor fabrication. Such work is now in progress.

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References

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