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Disposable amperometric glucose sensor electrode with enzyme-immobilized nitrocellulose strip

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Abstract

Electrochemical properties of screen-printed carbon paste electrodes (CPEs) with a glucose oxidase-immobilized and hexamineruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$) containing nitrocellulose (NC) strip were examined. The NC strip (2×8 mm) placed on the CPEs printed on polyester (PE) film is tightly sealed using another PE film on the top with open edges on both sides. Samples containing macromolecules and particles (e.g. proteins and blood cells) are applied at one edge of the NC strip and reach the detection area, chromatographically separating small molecules (e.g. glucose, ascorbate, acetaminophen, and uric acid) of analytical interests. Since sample volumes and the amount of catalytic reagents (mediator and glucose oxidase) are precisely predefined by the dimension and pore size (8 μm) of the NC strip, the sensor-to-sensor reproducibility and accuracy of analysis are greatly improved. The use of $[Ru(NH_3)_6]^{3+}$ mediator, which exhibits characteristic substantially lowers the applied potential (0.0 V vs Ag/AgCl) for glucose determination and eliminates the interference from other oxidizable species, providing improved analytical results.

Keywords: Amperometric glucose sensor; Hexamineruthenium (III) chloride mediator; Nitrocellulose strip; Carbon paste electrode

1. Introduction

Accurate measurement of glucose level in blood has long been recognized as an important clinical test for diagnosing diabetes mellitus. It has been the main subject of numerous articles that deal with amperometric biosensors in the last three decades [1–6]. And many diabetic patients are now widely using the disposable-type screen-printed glucose sensor strips for self-monitoring their blood glucose levels [7].

Glucose is oxidized by the enzyme glucose oxidase (GOx) and the electrons involved in the redox reaction may be relayed through a mediator to the electrode, resulting in electric currents proportional to the level of glucose in sample solutions. The best known mediators for GOx are ferrocene and its derivatives because of their good

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electrochemical properties [8–11]: they exhibit well-defined, reversible, one-electron transfer voltammetry, adequate aqueous solubility, sufficient chemical stability, and little cross-reactivity with the enzymatic substrate in the absence of enzyme. The glucose sensors based on ferrocene derivatives, however, still suffer interference from easily oxidizable species, e.g. ascorbic acid, as the applied potentials required are not sufficiently low. Other mediators that were examined successfully with GOx include ferricyanide, N-methylphenazium, benzoquinone, and ruthenium compounds [12–15]. Morris et al. showed that hexamineruthenium (III) chloride, which exhibits quasi-reversible single-electron transfer electrochemistry at a gold electrode, could be used for the coulometric determination of glucose in a specially designed micro capillary fill device (volume: 20 μl) containing freeze-dried assay components [16]. Prior to their work, Murray et al. examined the possibility of using the same mediator with sulfite oxidase [17]. However, to the best of our knowledge, the electroanalytical performance and utility of $[Ru(NH_3)_6]^{3+}$ for glucose determination has not been examined on the screen-printing-type carbon paste electrodes.

Most disposable-type glucose sensors comprise two or three electrodes screen-printed on a plastic strip and a reagent layer containing assay components (GOx, mediator, buffer salts, etc.) on the electrodes, and use whole blood samples. Permselective membranes such as cellulose acetate, Nafion, Eastman-AQ, phospholipid are often placed on the top of the enzyme containing layer to filtrate the particulate and interfering species from the sample [18–21]. These approaches, however, substantially increase the time-to-first-result (TTFR) as the outer layer impede the sample permeation to the sensing site. Varying sample volumes applied to the strip sensor may result in inaccurate determination. To improve the accuracy, some commercial sensors employ micro capillary-fill channel on the sensing site. This type of device, however, is not efficient for eliminating the interference from hematocrit components in blood samples [22].

We recently reported that the use of screen-printed electrodes on nitrocellulose (NC) strip with two separate reagent zones effectively eliminates the interference from both easily oxidizable species and hematocrit in the glucose determination [23]. The sample applied directly onto the surface of the metal oxide band is used to destroy small oxidizable species (e.g. ascorbic acid, uric acid and acetaminophen), further chromatographically carried to the enzyme-containing band to produce hydrogen peroxide, and finally detected at the electrodes. Sample volumes, however, are not strictly controlled with this device. To exploit the advantages of the NC strip-based electrochemical sensor and the micro capillary-fill channel, we devised a new disposable-type glucose sensor by placing the NC strip (2×8 mm) impregnated with assay components (GOx, $[Ru(NH_3)_6]^{3+}$, buffer salts, etc.) in the capillary-fill channel. In this contribution, we report the electroanalytical properties of this new sensor device.

2. Experimental

2.1. Apparatus

Electrochemical measurement was performed with an EG&G PAR(Princeton, NJ, USA) model 273A potentiostat/galvanostat controlled by a IBM PC with an EG&G M270 software program. A semi-automated screen-printer MSP 150S (Minong, Korea) was used to produce disposable strip electrodes. The BioDot (CA, USA) CN3000 was used to cut the nitrocellulose membranes. Saturated calomel electrode (SCE) and Pt plate were used as reference and auxiliary electrodes, respectively, for electrochemical pretreatment of carbon paste electrode.

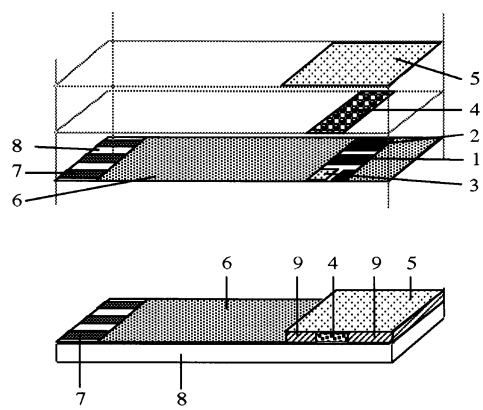
2.2. Materials and reagents

The sources of materials and reagents used in this experiment were as follows: glucose oxidase (GOx, EC 1.1.3.4, type VII-S, 180000 units g^{-1}, from aspergillus niger), β-D(+) glucose, ascorbic acid, uric acid, acetaminophen, *t*-octylphenoxypolyethoxyethanol (Triton X-100) and carboxylmethyl cellulose (CMC, low viscosity) from Sigma

(St. Louis, MO, USA); disodium hydrogenphosphate and sodium dihydrogenphosphate from Kanto Chemical Co. Inc. (Tokyo, Japan); hexammineruthenium (III) chloride from Aldrich (Milwakee, WI, USA); carbon paste (TU-15ST) and silver paste (LS-405) from Asahi Chemical Research Laboratory (Tokyo, Japan); insulator paste was from Seoul Chemical Research Laboratory (Shiheung, Korea); flexible polyester film from Korea 3M (Seoul, Korea); and nitrocellulose (NC) membranes from Whatman International Ltd.(Maidstone, England). All aqueous solutions were prepared with deionized water (18 MΩ cm). The glucose stock solution was prepared and allowed to stand 24 h before use to allow equilibration between the α and β anomers.

2.3. Disposable strip sensors fabrication

The strip-type electrodes on a polyester film were fabricated as illustrated in our previous report: briefly, as shown in Fig. 1, three lines of silver conductors (1 × 20 mm), two square carbon electrode sites (2 × 2 mm) on the top of the conductors, and a dielectric layer were screen printed sequentially exposing 2 × 8 mm channel area. The Ag/AgCl reference electrode was formed by applying 0.3 M $FeCl_3$ solution on the silver electrode. The carbon paste working electrodes were electrochemically activated in a saturated Na_2CO_3 solution at 1.2 V versus SCE for 5 min before assembling the whole strip.



1: working electrode
2: counter electrode
3: pseudo-reference electrode
4: GOx/Ru(III) immobilized NC
5: polyester film
6: insulator
7: connector
8: polyester substrate
9: two-side adhesive tape

Fig. 1. Exploded view of the disposable glucose sensor strip used in this work.

The enzyme solution was prepared by thoroughly mixing GOx, $[Ru(NH_3)_6]^{3+}$, 0.5 wt.% CMC and 0.2 wt.% Triton X-100 in 0.1 M PBS, pH 7.4. The NC membrane (2 mm in width), which was washed in 10 v/v.% methanol for 5 min, deionized water for three times, and dried at room temperature, was dipped in the prepared cocktail solution for 5 min. It was then allowed to dry in a decicator at room temperature. The NC membrane which contains GOx/$[Ru(NH_3)_6]^{3+}$ was placed on the electrodes exposed on the strip, and covered with another polyester film using double-side adhesive tape (Fig. 1).

2.4. Measurement of the sensor performance

To characterize the electroanalytical properties of the assembled sensor system, standard solutions containing 11.1 mM glucose were applied at one edge of the reagent strip and equilibriated for 30 s before applying a given potential. Applied potentials were varied from -0.1 to $+0.2$ V, and the corresponding chronoamperometric responses to the $[Ru(NH_3)_6]^{3+}$-meditated enzymatic glucose oxidation were recorded. Optimum applied potential (vs Ag/AgCl) that begins to result in near constant response was determined from the *I-E* curve (the anodic current values read at 30 s vs the applied voltage). The electrochemical properties of the thin-layer cell-type strip electrodes and were also examined with the cyclic voltammetry (CV). The effect of oxygen content was investigated by recording the sensor responses to 11.1 mM glucose solutions bubbled with nitrogen gas, air and oxygen for 30 min. The optimum working pH, and the enzyme and mediator compositions added to the reagent strip were determined by chronoamperometry as described in our previous report [24]. The current responses to 0–5 mM interfering substances, e.g. ascorbic acid, uric acid, acetaminophen, dopamine, salicylate and creatinine, were examined at a given potential. Calibration curves for glucose determination were

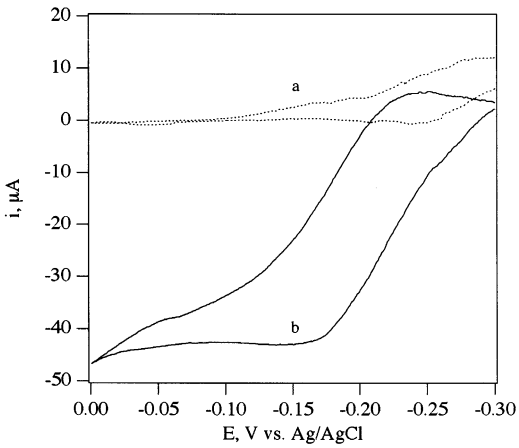


Fig. 2. Current responses of a 80 mM $[Ru(NH_3)_6]^{3+}$ in the presence of 8 mg dL^{-1} glucose oxidase (scan rate: 1 mV s^{-1}): (a) before; and (b) after the addition of 22.2 mM glucose to the sensor strip.

obtained by plotting the currents versus glucose concentrations of 0–33.3 mM.

3. Results and discussion

3.1. Cross-reactivity studies for the $[Ru(NH_3)_6]^{3+}$ mediator

The cross-reactivity of $[Ru(NH_3)_6]^{3+/2+}$ with glucose in the absence of GOx has been examined by measuring the changes in currents for $[Ru(NH_3)_6]^{2+}$ oxidation upon addition of glucose; it was negligible on the carbon paste electrode at potentials less than +0.3 V versus Ag/AgCl. On the other hand, as shown in Fig. 2, the sensor strip fabricated with NC membrane containing both GOx and hexaammineruthenium (III) exhibited a large increase in current upon addition of glucose, indicating that the ruthenium (III) compound is a good mediator for coupling the reaction between glucose and glucose oxidase. The CVs scanned in the absence and presence of $[Ru(NH_3)_6]^{3+}$ (Fig. 3a and b, respectively) upon addition of common interfering substance, i.e. 1 mM ascorbic acid, demonstrate that there is little cross-reactivity between those compounds; the peaks attributable to the oxidation of ascorbic acid and the reduction of hexaammineruthenium (III) are not affected. The same type of CVs were observed with other interfering substances.

3.2. Optimization of electroanalytical performance for the sensor system

As shown in Fig. 1, samples applied at one side of the glucose sensor are taken into the working electrode through the NC membrane while separating particulate and large molecules. The level of glucose in the sample is usually determined by chronoamperometric method. To find the optimum operating potential for the sensor, a series of chronoamperometric measurements for the 11.1 mM glucose sample was made and the change in currents at 30 s was recorded as a function of applied potential. In this experiment, we also examined the effect of dissolved oxygen on the catalytic current changes using the standard glucose samples bubbled with nitrogen, air and oxygen for 30 min (Fig. 4a–c, respectively). It is seen that the current became independent of the applied voltage at −0.03 V for curve b and c. The catalytic currents in the oxygen- or air-saturated samples are close to steady state value over 0.0 V versus Ag/AgCl. Considering that the oxidation of common interfering substances in glucose de-

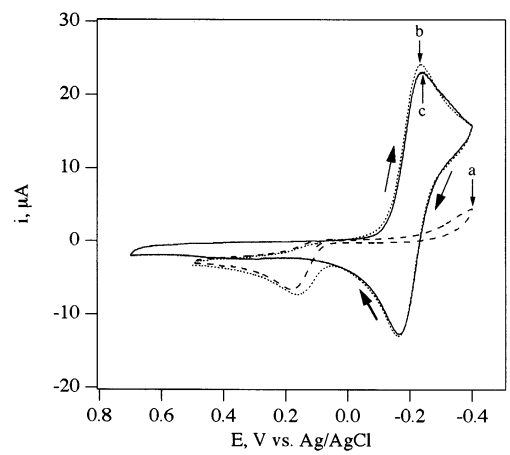


Fig. 3. Cyclic voltammograms of ascorbic acid (1 mM) in the (a) absence; (b) presence of $[Ru(NH_3)_6]^{3+}$ (3 mM); and (c) 3 mM $[Ru(NH_3)_6]^{3+}$ (scan rate: 60 mV s^{-1}, start potential: 0.0 V, switching potential: −0.4 V).

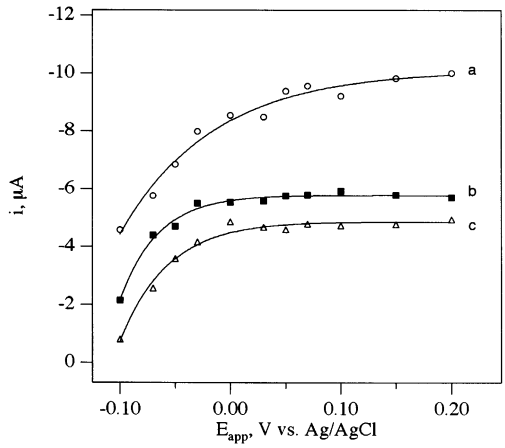


Fig. 4. Current response to 11.1 mM glucose samples containing different concentration of dissolved oxygen as a function of applied potentials. Glucose samples are bubbled with (a) N_2; (b) air; and (c) O_2 for 30 min.

termination near 0.0 V are not significant, further chronoamperometric measurements were made at this potential.

Fig. 4 also shows that the current changes in nitrogen flushed sample increase continuously over 0.0 V and are greater than those in the oxygen- or air-saturated samples. The catalytic current of the sensor in the O_2-saturated and air-saturated glucose solution (11.1 mM) was decreased by 43 and 35%, respectively, at 0.0 V compared to the value observed in the N_2 bubbled sample. The differences in the magnitude of catalytic current changes among three curves, i.e. the smaller Δi with increasing oxygen contents, at a given potential may result from the competing redox reaction between ruthenium mediator and oxygen for the reoxidation of reduced enzyme (i.e. $FADH_2$ of glucose oxidase) [25]. Nevertheless, the comparison of the graphs b and c in Fig. 4 suggests that the Δi difference caused by the varying oxygen contents in clinical samples, which contain near constant level of oxygen, at 0.0 V is much less significant than the errors that result from the oxidation of interfering substances.

The amount of the mediator, $[Ru(NH_3)_6]Cl_3$, used for the sensor system was optimized by measuring the catalytic current changes at constant enzyme (20 mg dL^{-1}) and glucose (11.1 mM) concentration; the maximum response to glucose was obtained when the mediator concentrations were between 80 and 100 mM. The decrease in current responses at higher concentrations indicates that the mediator tends to inhibit enzymatic catalytic reactions. Furthermore, the use of higher mediator concentration led to the increase in background currents. For these reasons, we used 80 mM of $[Ru(NH_3)_6]Cl_3$. The amount of GOx, 8 mg dL^{-1}, was also optimized using the same method. The influence of pH on this sensor was also investigated. Fig. 5 shows that the optimum pH for the proposed sensor system is 7.4.

3.3. Calibration plots and the effect of interferents

The calibration plot, current versus [glucose], was obtained by measuring the chronoamperomeric responses to varying concentration of glucose at 30 s. Fig. 6 shows the result: each point in the curve is the average of the ten measurements obtained from ten sensor strips. Thus, the standard deviation (S.D.) of each point ($< \pm 0.40$) represents the sensor-to-sensor reproducibility. The sample volume required for the

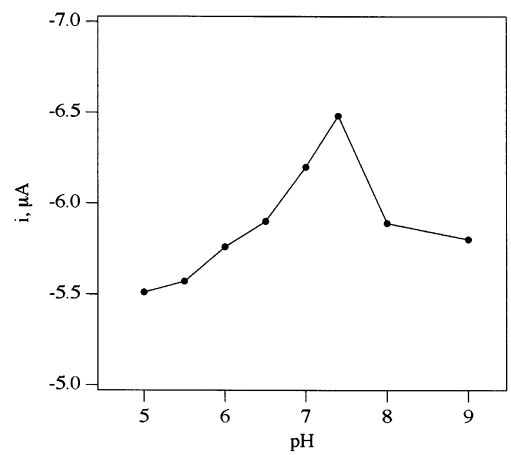


Fig. 5. Effect of pH on the current responses of $[Ru(NH_3)_6]^{3+}$ /GOx immobilized strip sensor.

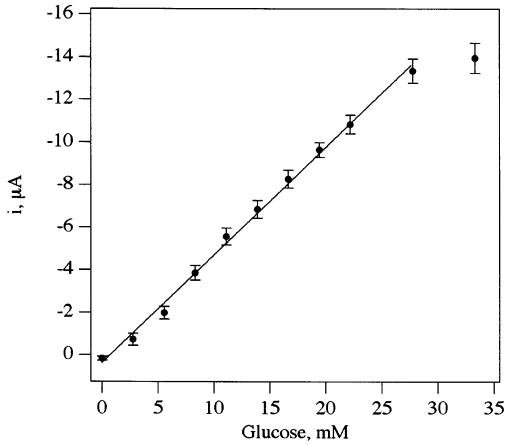


Fig. 6. Calibration plot for the glucose sensor. Each point on the curve represents the average of ten measurements obtained with ten different sensor strips.

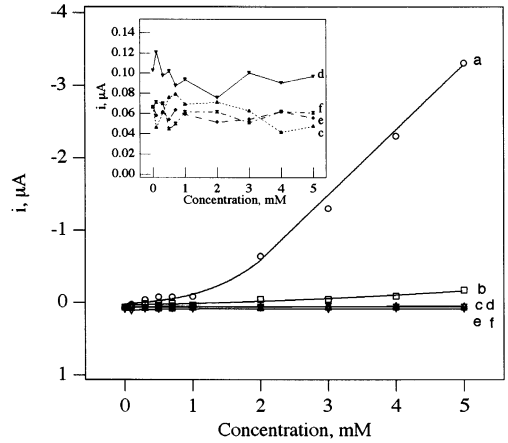


Fig. 7. Current responses of the disposable sensor strips to varying level of interferents (operating potential: 0.0 V vs Ag/AgCl): (a) ascorbic acid; (b) uric acid; (c) acetaminophen; (d) dopamine; (e) salicylate; and (f) creatinine.

sensor to exhibit highly reproducible response is less than 2 μl, which is determined by the dimension and pore size of the NC strip employed. The sensor exhibits excellent linear response up to 27.7 mM with the slope of 0.51 μA mM^{-1} and the correlation coefficient 0.996.

The current arising from the oxidation of the physiological interferents such as ascorbic acid, uric acid, acetaminophen, salicylate, dopamine, etc., causes a severe problem in electrochemical biosensors [26,27]. In human blood, the normal levels of ascorbic acid and uric acid are as high as 0.114 and 0.47 mM, respectively. Hence, we examined the effect of these interferents on the glucose determination using excessive concentrations (up to 5 mM). As shown in Fig. 7, the use of low applied potentials provides practically interference-free responses (< 1 mM). The sensor strip was also applied for the determination of glucose level in whole blood and serum samples. The sensor strip was also applied for the determination of glucose level in whole blood and serum samples. The traces b and c in Fig. 8 are the current responses for the whole blood and the plasma that was obtained after centrifugating the same blood sample. This result clearly demonstrates the advantage of using NC strip as the reagents carrier and hematocrit separator in strip-type biosensor systems [24].

4. Conclusion

In this study, we examined the electrochemical properties of screen-printed carbon paste electrodes (CPEs) assembled with a glucose oxidase-immobilized and hexaminerutheniun (III) chloride ($[Ru(NH_3)_6]^{3+}$) containing nitrocellulose (NC) strip. The use of $[Ru(NH_3)_6]^{3+}$ mediator, which exhibits characteristic substantially lowers

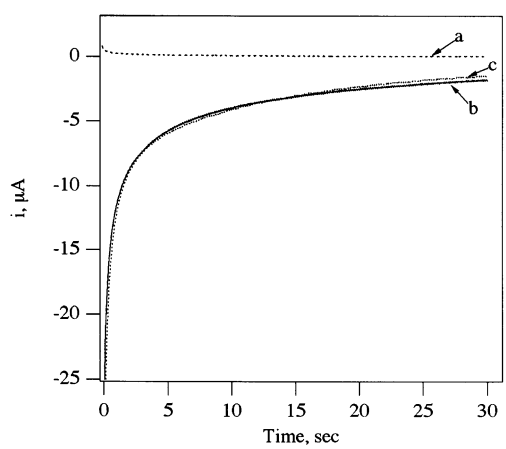


Fig. 8. Current response of the NC membrane-based glucose sensor in different sample matrices. (a) PBS (140 mM NaCl) with no glucose; (b) plasma sample separated from the whole blood; and (c) whole blood.

the applied potential (0.0 V vs Ag/AgCl) for glucose determination and eliminates the interference from other oxidizable species, providing improved analytical results. The NC strip (2×8 mm) placed on the CPEs printed on polyester (PE) film is tightly sealed using another PE film on the top with open edges on both sides. Small molecules (e.g. glucose, ascorbic acid, uric acid, acetaminophen, dopamine and salicylate) of analytical interests, which are chromatographically separated from macromolecules and particulate contained in the sample, are transferred to the working electrode through the NC membrane. Since the sample volumes and the amount of reagents (mediator and GOx) for the catalytic reactions are precisely predefined by the dimension and pore size (8 μm) of the NC strip, the sensor-to-sensor reproducibility and accuracy of determination are greatly improved.

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