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Disposable Amperometric Glycated Hemoglobin Sensor for the Finger Prick Blood Test

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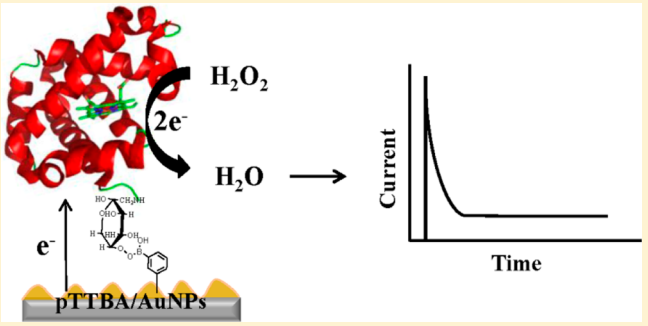
ABSTRACT: The analysis of glycated hemoglobin (HbA_{1C}) content in blood samples is crucial for the diagnosis of diabetes, and it still demands to practically use plenty of a blood sample and a complicated procedure. Hence, we report the development of a disposable amperometric HbA_{1C} sensor for the finger prick blood test through a simple treatment of a drop of blood. To fabricate the sensor probe, the conducting polymer, poly(terthiophene benzoic acid) (pTTBA), was electrochemically grown onto the gold nanoparticles (AuNPs) coated-screen printing electrode, followed by the covalent attachment of aminophenyl boronic acid (APBA) to pTTBA as a host to capture HbA_{1C} in the sample. The catalytic reduction response of hydrogen peroxide by HbA_{1C} itself captured on the sensor probe was monitored as an analytical signal. The experimental parameters for the HbA_{1C} analysis were optimized in terms of concentration of H_2O_2, pH, temperature, applying potential, and interferences. Under the optimized conditions, the linear dynamic range of HbA_{1C} by amperometry was determined to be from 0.1 to 1.5% and the detection limit was to be 0.052 ± 0.02%. The reliability of the proposed HbA_{1C} sensor was evaluated through the comparison of the results among the conventional method, the impedance method, and the proposed amperometry using a drop of a human peripheral blood sample.



Diabetes is a serious health concern worldwide.[1,2] High blood glucose has been reported to be the main cause for most complications associated with diabetes, such as diabetic nephropathy, diabetic retinopathy, atherosclerosis, and other perivascular diseases.[3,4] A fundamental diagnosis method for diabetes is to monitor the level of glycated hemoglobin (HbA_{1C}) that is formed from nonenzymatic glycosylation of hemoglobin following exposure to a high plasma concentration of glucose,[5] and the amount of HbA_{1C} is the most significant index that presents an accurate long-term average blood glucose level.[6] The level of HbA_{1C} reflects the average of the varying glucose concentration from the preceding 2 to 3 months due to the life span of red blood cells. Thus, HbA_{1C} analysis enables the precise monitoring of blood glucose level.[7] The normal level of glycosylated hemoglobin is less than 6%, and levels greater than 9% indicate poor glycemic control.[8] The "Standards of Medical Care in Diabetes" recently published by the American Diabetes Association (ADA) recommends the use of HbA_{1C} as a diagnostic criterion for diabetes.[9] Therefore, the ratio of HbA_{1C} to total hemoglobin may become a vital clinical index for evaluations of long-term glycemic control for diabetes and related diseases.

Currently, there are several clinical methods to analyze HbA_{1C}, such as ion-exchange chromatography,[10] boronated affinity chromatography,[11] electrophoresis,[12] piezo-electric methods,[13,14] surface plasmon resonance (SPR),[15,16] and fluorescence[17,18] after pretreatment of the blood sample. These methods possess certain disadvantages including the long time for the analysis, high cost, the need for labeling, and inaccurate results that may occur with variations in red blood cells. Otherwise, the electrochemical analysis for clinical diagnosis possess distinct advantages, such as excellent sensitivity, selectivity, simplicity, relatively low cost, and the potential for miniaturization and automation.[19,20] However, few reports have investigated the determination of HbA_{1C} using only the electrochemical impedance method so far,[21,22] but it has also a disadvantage that demands a sophisticated instrument and a long measuring time. On the other hand, the amperometric method is a simple and short time measuring technique, thus it can be easily used to develop a handy-type HbA_{1C} meter for point-of-care. Until now, there is no report for an amperometric sensor for the analysis of HbA_{1C}.

Since aminophenyl boronic acid (APBA) interacts with sugar,[23] its ability to bind to glucose was exploited for glucose sensing and to capture glycated proteins,[21] although it was not only interacting with glucose but all saccharides.[24] Because of the lack of selectivity to only glucose, it is interesting to develop a selective HbA_{1C} detection method with APBA through combining with the other selective analytical tool. A possible way to give the selective detection of HbA_{1C} is to use the catalytic property of HbA_{1C} itself captured on the probe. The catalytic reduction of H_2O_2 by hemoglobin can be used for the

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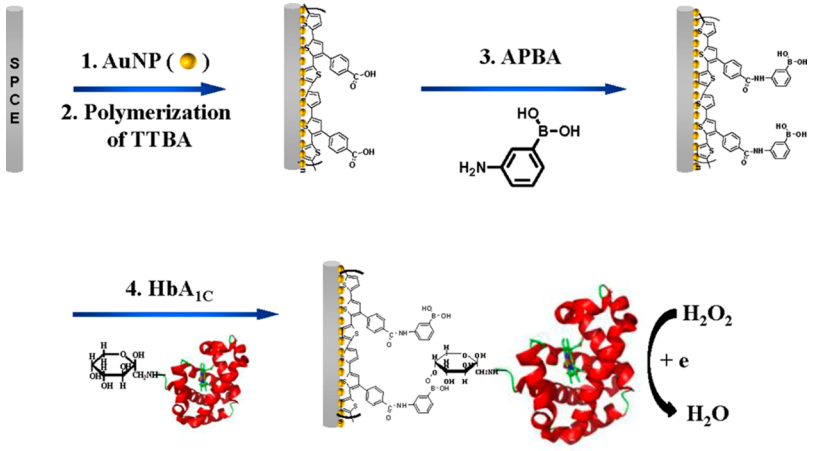
Scheme 1. Schematic Representation for the Sensor Fabrication



specific response of HbA_{1C} in amperometry, because hemoglobin (Hb) contains four iron heme groups that catalyze the reduction reaction of H_2O_2.[25] To develop a simple device for the selective amperometric HbA_{1C} detection, we propose a new biosensing protocol based on the interaction with APBA and the catalytic activity of HbA_{1C} itself toward H_2O_2 reduction. Thus, the selectivity of the proposed method can be achieved with both the construction of sensor probe and an adequate pretreatment of sample with removing plasma.

In the present study, a disposable biosensor for HbA_{1C} detection was fabricated using the APBA chemically bonded on the conductive polymer layer (pTTBA/AuNPs/SPCE), where AuNPs were electrodeposited on the screen print carbon electrode (SPCE) to enhance the stability and the sensitivity of sensor probe. AuNPs have been widely used in biosensors to enhance the sensitivity, due to their high electrocatalytic activities, high active surface area-to-volume ratios, and ease of chemical modification as well as structural and functional compatibility with biomolecules on the electrode.[26,27] The formation of each layer was characterized using cyclic voltammetry (CV), quartz crystal microbalance (QCM), X-ray photoelectron spectroscopy (XPS), electrochemical impedance spectroscopy (EIS), and chronoamperometry. Various experimental parameters, such as temperature, pH, H_2O_2 concentration, and applied potential were optimized, and the interference effect and detection limit of HbA_{1C} was subsequently discussed. In addition, the biosensor was applied to the analysis of HbA_{1C} in a finger prick blood sample through a simple filtration process with hemolysis of blood cells. The validity of the proposed method was evaluated through the comparison of results from conventional high-performance liquid chromatography (HPLC) with the treatment of blood samples by centrifugation.

EXPERIMENTAL SECTION

Materials. A terthiophene monomer containing a benzoic acid group, 2,2′:5′,5′′-terthiophene-3′-*p*-benzoic acid (TTBA), was synthesized using the Paal-Knorr pyrrole condensation reaction.[28] A lyophilized glycated hemoglobin (HbA_{1C}) reference, RM 405 was purchased from Fluka. A volume of 1 mL of deionized water was added to the lyophilized HbA_{1C} followed by gentle mixing for 15 min and then stored at 4 °C. The final concentration of HbA_{1C} was obtained to be 6.29 (±0.04) % in a 0.23 mM hemoglobin (Hb). Hemoglobin was purchased from Sigma Co. This HbA_{1C} solution was diluted to appropriate concentrations just before every measurement. Tetrabutylammonium perchlorate (TBAP, electrochemical grade) was purchased from Fluka. It was purified according to a general method and dried under vacuum at 1.33×10^{-3} Pa. Dichloromethane (99.8%, anhydrous, sealed under N_2 gas), di(propylene glycol) methyl ether, tri(propylene glycol) methyl ether, 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC), *N*-hydroxy succinimide (NHS), $HAuCl_4 \cdot 3H_2O$, and 3-aminophenylboronic acid (APBA) were purchased from Sigma Co. Aqueous solutions were prepared in doubly distilled water that was obtained from a Milli-Q water purification system (18 MΩ cm).

Instruments. The electrochemical experiments were performed in a three-electrode cell using an all-in-one screen printed carbon electrode. The SPCE and GCE were composed of modified working electrodes (area = 0.07 cm^2), Ag/AgCl (in saturated KCl) as a reference, and carbon as the counter electrode. Carbon and silver inks (Jujo Chemical, Japan) were used in the screen printing process. The SPCEs were printed on the polystyrene-based film using the screen printer (BANDO Industrial, Korea). The performance of modified SPCEs was confirmed with the same modified glassy carbon electrodes. Cyclic voltammograms (CVs), linear sweep voltammograms (LSVs), and amperograms were recorded using a potentiostat/galvanostat, Kosentech model PT-1 and EG & G PAR model PAR 273A. A quartz crystal microbalance (QCM) experiment was conducted using a SEIKO EG&G model QCA 917 and a PAR model 263A potentiostat/galvanostat. An Au-coated working electrode (area, 0.196 cm^2; 9 MHz; AT-cut quartz crystal) was used for the QCM experiment. The impedance spectra were measured with the EG&G Princeton Applied Research PARSTAT 2263 at an open circuit voltage from 100 kHz to 50 mHz at a sampling rate of five points per decade (ac amplitude, 10 mV). X-ray photoelectron spectroscopy (XPS) experiments were performed using a VG Scientific ESCALAB 250 XPS spectrometer with a monochromated Al Kα source with charge compensation.

Fabrication of Sensor Probe. The fabrication process of the glycated hemoglobin sensor is presented in Scheme 1. AuNPs were electrodeposited onto SPCE from a 0.5 M H_2SO_4 solution containing 0.001% $HAuCl_4$ using linear sweep voltammetry from 1.5 to 0.4 V before the polymerization of

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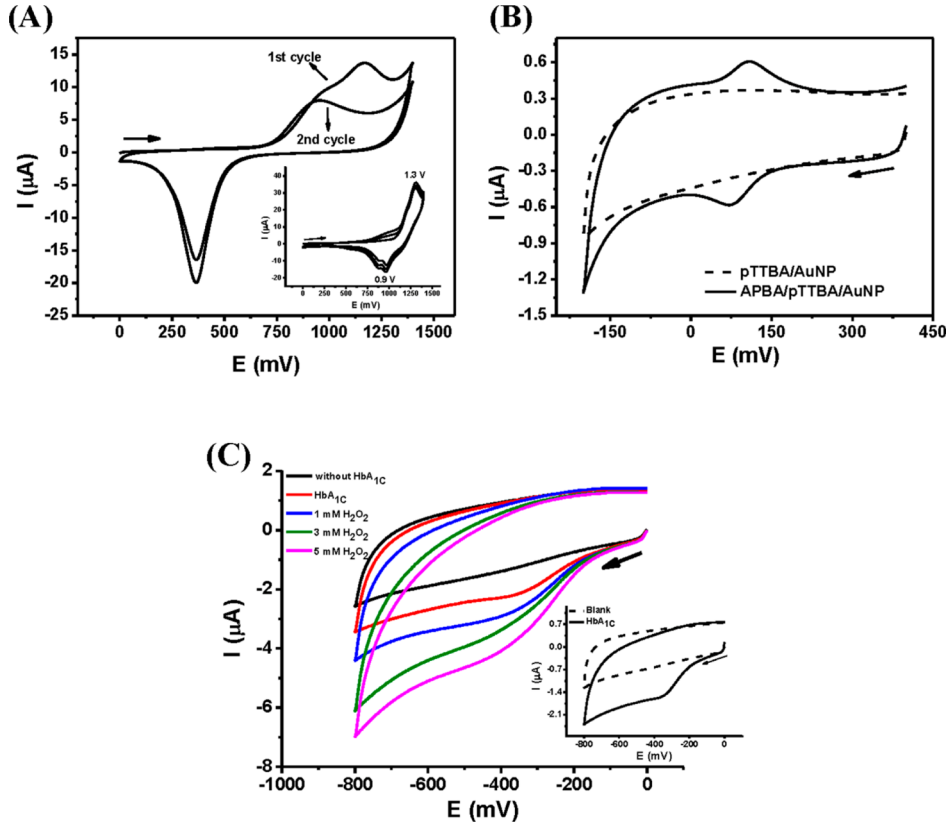

Figure 1. (A) CVs recorded for electropolymerization of TTBA on AuNPs/SPCE in 0.1 M PBS (pH 7.4) and AuNPs/GC in 0.1 M TBAP/CH_2Cl_2 (inset), (B) CVs recorded for pTTBA/AuNPs/SPCE (dashed line) and APBA/pTTBA/AuNPs/SPCE (solid line) in a 0.1 M PBS (pH 7.4), (C) CVs recorded for the catalytic reduction of H_2O_2 (0.0−5.0 mM) by HbA_{1C} captured probe in a 0.1 M PBS (pH 7.4). Inset: CVs recorded for the probe with and without HbA_{1C} in 0.1 M PBS (pH 7.4).

TTBA. The electrodeposition conditions were as follows: deposition time, 60 s; scan rate, 0.1 V/s; deposition potential, −0.6 V; and three-times potential cycling. The pTTBA layer was formed on AuNPs/SPCE according to our previously published method.[29,30] The polymer coated AuNPs/GC electrode was also fabricated for the control experiments of AuNPs/SPCE. The AuNPs/SPCE was dipped in a 1.0 mM TTBA containing solution at first, which was prepared in 1:1 of di(propylene glycol) methyl ether and tri(propylene glycol) methyl ether. After that, the polymer film was formed on the electrode through potential cycling twice from 0.0 to 1.4 V (Ag/AgCl) at a scan rate of 0.1 V/s in a 0.1 M PBS (pH 7.4). After polymerization, the pTTBA/AuNPs/SPCE was immersed for 12 h in a 0.1 M PBS (pH 7.4) containing 10.0 mM EDC and NHS to activate the carboxylic acid groups of the pTTBA layer. After that, the electrode was washed with a buffer solution and subsequently incubated in 10.0 mM 3-aminophenyl boronic acid (APBA) in 0.1 M PBS (pH 7.4) for 12 h at 40 °C. Finally, amine-terminated APBA was immobilized onto the pTTBA layer by amide bond formation, followed by the EDC activation step (APBA-pTTBA/AuNPs/SPCE).

Pretreatment of Human Whole Blood. The human whole blood was pretreated by centrifugation or syringe filtration. In the centrifugation method, the plasma was separated from 2 mL of the whole blood by centrifugation at 3000 rpm for 10 min at 4 °C. The supernatant (plasma) was discarded to remove glucose and other glycated proteins and molecules. The remaining red blood cells were washed three times with a physiological saline (0.9% NaCl solution) to remove the plasma completely. The washed red blood cells (0.5 mL) were lysed by addition of 0.5 mL of hemolyzing buffer solution (26 mM NaH_2PO_4, 7.4 mM Na_2HPO_4, and 13.5 mM KCN). The samples were diluted 10-fold with 0.1 M PBS (pH 7.4) for the amperometric measurement. In the filtration method, 2 μL of whole blood from a finger prick collected on the syringe filter (pore size, 0.45 μm) and then removed the plasma, after that the collected red blood cells on the filter were washed three times with a 0.9% NaCl solution. Hemoglobin was eluted from red blood cells on filter paper by addition of 18 μL of hemolyzing buffer solution, and impedance and amperometric measurements were performed after 15 min, respectively.

RESULTS AND DISCUSSION

Electrochemical Characterization of the Sensor Probe. As shown in Figure 1A, the pTTBA layer was formed through the anodic electropolymerization of the monomer drop-coated on the AuNPs/SPCE surface in a 0.1 M PBS (pH 7.4). At the first anodic scan, from 0.0 to 1.4 V, an oxidation peak at around 1.2 V due to the monomer oxidation to form the polymer and another redox peak of AuNPs was observed at 0.95/0.37 V. After polymerization, APBA was immobilized onto the pTTBA/AuNP/SPCE layer. For a comparative study of the AuNPs/SPCE electrode, the polymer layer was also formed on the AuNPs/GC by the electropolymerization of 1.0 mM TTBA in a 0.1 M TBAP/CH_2Cl_2 solution (inset of Figure 1A). The reverse scan to the negative potential showed a small cathodic peak at 0.9 V, which corresponded to the reduction of the

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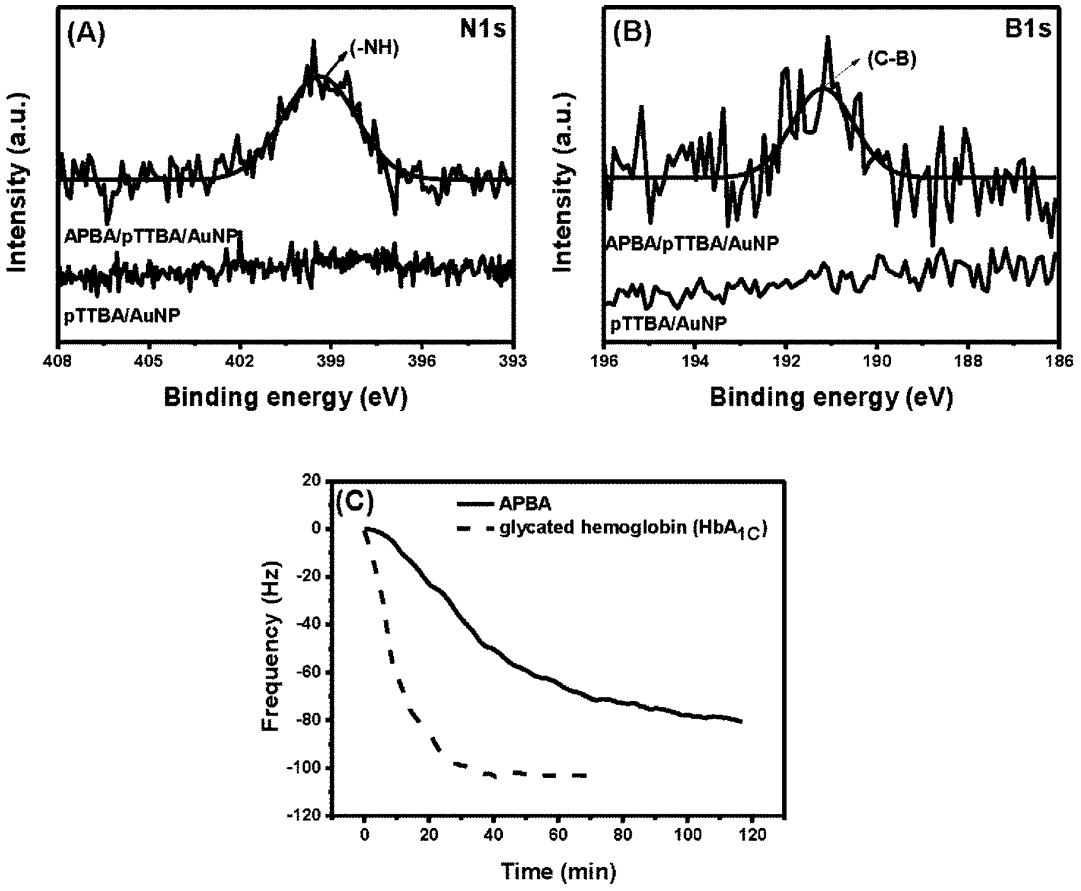

Figure 2. ESCA spectra of (A) N1s and (B) B1s peaks for pTTBA/AuNPs and APBA/pTTBA/AuNPs. (C) Frequency changes during immobilization of APBA onto the pTTBA/Au-coated electrode (solid line) and one of HbA_{1C} onto the APBA/pTTBA/Au-coated electrode (dashed line).

polymer film that had immediately formed on the electrode. The electrochemical behavior of the APBA-immobilized on the pTTBA/AuNPs/SPCE was examined using CV as shown in Figure 1B. No redox peak was observed when the CV was recorded for the pTTBA/AuNP/SPCE (dashed line) without APBA. However, a pair of redox peaks was observed at 0.076/0.107 V, which resulted from the redox process of the APBA bonded to the pTTBA, where the peak separation was determined to be approximately 0.031 V. These results indicated that the APBA monomer was successfully immobilized onto the pTTBA/AuNP/SPCE layer.

In the next step, HbA_{1C} was captured onto the APBA-pTTBA/AuNP/SPCE layer through cis-diol interactions between the diol group of glucose of HbA_{1C} and the boronic acid group. The inset of Figure 1C shows the CVs recorded for the HbA_{1C} captured on APBA modified electrode in 0.1 M PBS (pH 7.4) without H_2O_2. In this case, a pair of redox peaks of HbA_{1C} appeared at −0.34/− 0.16 V, which resulted from the redox process of the heme groups of HbA_{1C} captured on APBA. Otherwise, no redox peaks were observed when the CV was recorded for the APBA-pTTBA/AuNP/SPCE (dashed line) without HbA_{1C}. Figure 1C shows the catalytic reduction peak of H_2O_2 by the captured HbA_{1C} appeared at −0.37 V on the HbA_{1C}/APBA-pTTBA/AuNPs probe in 0.1 M PBS (pH 7.4) containing H_2O_2 (0.0−5.0 mM). In this catalytic process, the reduction current of H_2O_2 increases as the concentration of H_2O_2 increases, which is used as an analytical signal for the HbA_{1C} determination by amperometry.

XPS and QCM Analysis for APBA Modification. To elucidate the chemical bonding of APBA onto the pTTBA layer, the XPS spectra were analyzed in terms of Au4f, C1s, S2p, N1s (Figure 2A) and B1s (Figure 2B) peaks for AuNP, pTTBA/AuNP, and APBA-pTTBA/AuNP surfaces. The Au4f, C1s, and S2p spectra of AuNP and pTTBA/AuNP surfaces displayed the same result as they have been previously reported.[28] The N1s spectrum exhibits a distinct peak at 399.4 eV after APBA immobilization, while no peak appears for the pTTBA layer due to the absence of the nitrogen atom in the molecule. The B1s spectrum also exhibits a peak at 191.2 eV, corresponding to the C−B bond. These results indicated that APBA is successfully immobilized onto the pTTBA layer.

The amount of APBA immobilized onto the pTTBA-modified electrode was determined by the QCM experiment. Figure 2C shows the frequency change during the immobilization using 10.0 mM of APBA in a 0.1 M PBS (pH 7.4). The frequency decreased as the reaction progressed, indicating immobilization of APBA onto the pTTBA-modified electrode surface. In this case, the decrease in frequency did not reach a complete steady state before 2 h, due to the slow immobilization of APBA. At 2 h, the overall frequency change (Δf) was found to be 78.4 Hz, which corresponded to an increase in mass of 86.17 ng. In this case, the surface coverage of APBA was calculated to be 2.86×10^{-9} mol/cm^2. However, the change in frequency using the APBA attached probe was rapid in a solution containing 1.0% HbA_{1C}, indicating the HbA_{1C}−APBA interaction. The steady-state frequency change

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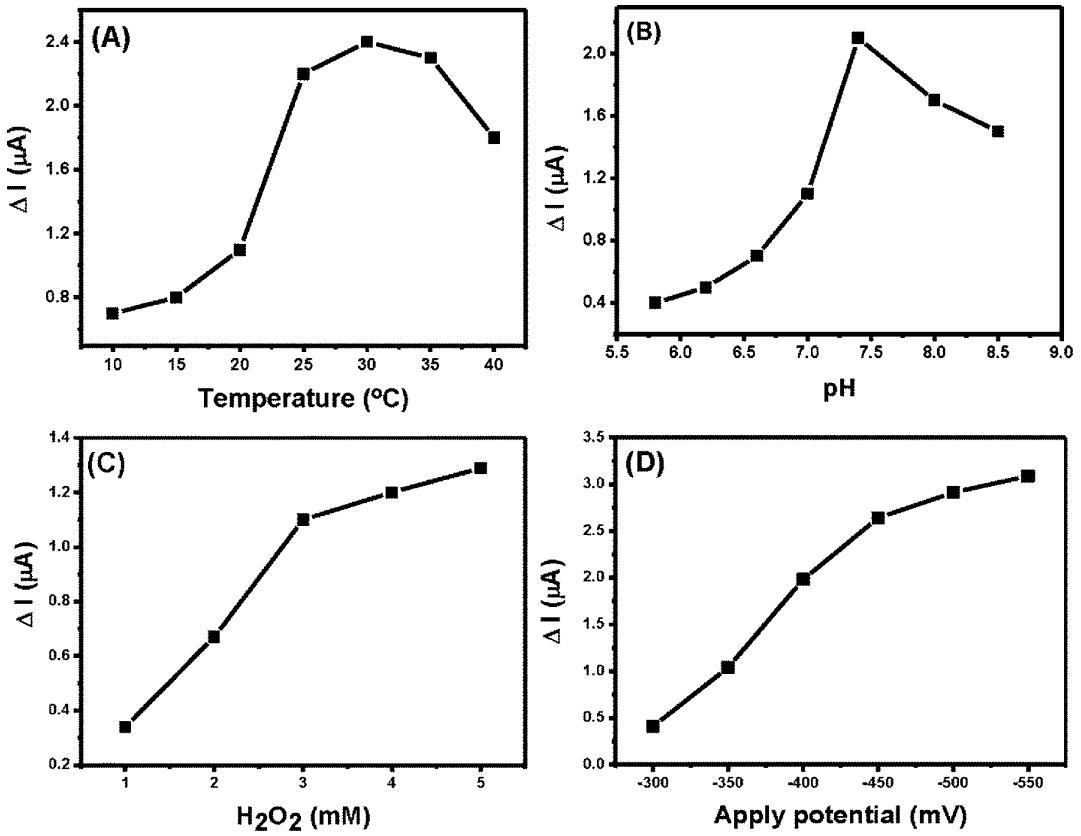

Figure 3. Optimization of experimental parameters in terms of (A) temperature, (B) pH, (C) concentration of H_2O_2, and (D) applied potential for catalytic H_2O_2 reduction.

was reached after approximately 20 min with a frequency change of 103.11 Hz. In this reaction, the mass change observed after the HbA_{1C}-APBA reaction was 113.34 ng, where the surface coverage of HbA_{1C} was calculated to be 1.03×10^{-10} mol/cm^2. Figure 2C shows that the time for HbA_{1C}–APBA interaction is clearly short, and it can, therefore, be used as the general detection method for HbA_{1C}.

Amperometric Analysis of HbA_{1C}. To optimize analytical conditions for amperometry, experimental parameters were investigated in terms of temperature, pH, the concentration of H_2O_2, and the applied potential. The effect of temperature on the current response of the electrocatalytic reduction of H_2O_2 by HbA_{1C} was studied from 10 to 40 °C as shown in Figure 3A. The peak current gradually increased with increasing temperature from 10 to 30 °C, and then it began to decrease as the temperature increased over 35 °C. The peak current decreased at the temperatures greater than 35 °C because of the thermal deactivation of HbA_{1C}. Thus, the optimum temperature was determined to be 30 °C. The effect of pH on the current response for HbA_{1C} detection was then performed between pH 5.8 and 8.5 (Figure 3B), where the response gradually increased as the pH increased from 5.8 to 7.4, but it decreased at pH values higher than 7.4. The maximum current response was observed at pH 7.4; hence, this value was used in all subsequent experiments. The effect of H_2O_2 concentration on the HbA_{1C} detection was investigated from 1.0 to 5.0 mM H_2O_2 (Figure 3C). The peak current increased gradually as the concentration of H_2O_2 increased from 1.0 to 5.0 mM, and it reached the steady state over 3.0 mM H_2O_2. Concentrations of H_2O_2 higher than 3.0 mM did not steeply increase in the current response. Therefore, 3.0 mM H_2O_2 was used for subsequent experiments.

The effect of applied potential on the current response of H_2O_2 catalytic reduction by HbA_{1C} was studied at the potential range from −0.3 to −0.55 V (Figure 3D). The response current increased as the potential decreased from −0.3 V, and the maximum response was obtained at −0.45 V. The application of potential less than −0.55 V did not improve the current response. Hence, the potential of −0.45 V was used for the final analysis.

The APBA-pTTBA/AuNP/SPCE was examined under the optimum condition for the determination of $HbA_{1C.}$ At first, the APBA-pTTBA/AuNP/SPCE was incubated for 5 min in the various concentrations of HbA_{1C} containing solution and then rinsed with 0.1 M PBS. The amperograms for the reduction of H_2O_2 were recorded using the sensor probe in 0.1 M PBS (pH 7.4) containing 3.0 mM H_2O_2. Figure 4A shows the calibration plot of HbA_{1C} analysis and amperometric responses, where the inset shows the chronoamperometric responses of H_2O_2 reduction at different concentrations of HbA_{1C} at −0.45 V vs Ag/AgCl. As the concentration of captured HbA_{1C} increase, the catalytic current of H_2O_2 increases because the reduction of H_2O_2 is specifically catalyzed by HbA_{1C}. The electrocatalytic response of H_2O_2 for the HbA_{1C} detection was linear from 0.1 to 1.5%. The linear dependence yielded a regression equation of ΔI_P (μA) = (0.34 ± 0.04) + (2.11 ± 0.07) [C] (%), and a correlation coefficient of 0.995. The HbA_{1C} detection limit of 0.052 ± 0.02% was determined from seven blank noise signals (95% confidence level, k = 3, n = 5). The reproducibility, expressed in terms of the relative standard deviation (RSD) was approximately 5.1% when five different SPCEs were used to detect 0.3% HbA_{1C}. This study shows that the HbA_{1C} sensor exhibits high sensitivity and stability. After the HbA_{1C}

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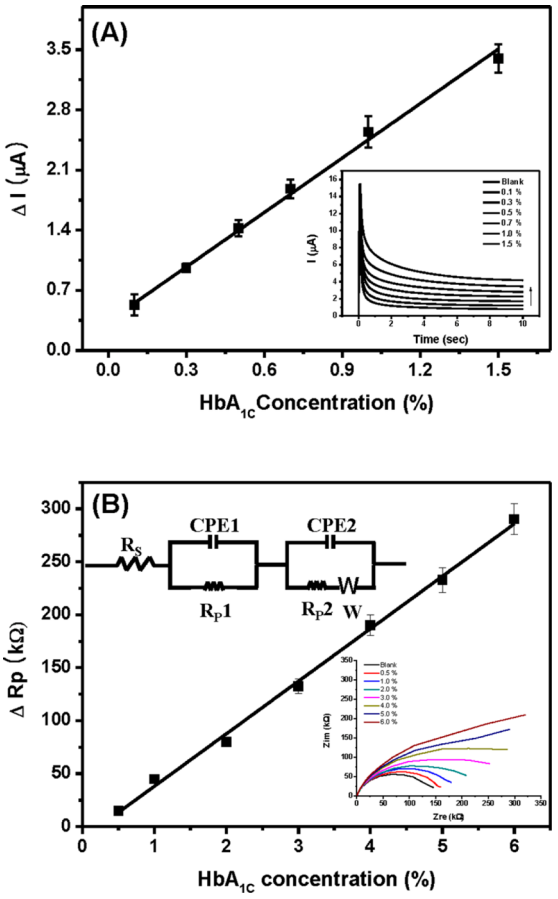

Figure 4. (A) Linear calibration plot and chronoamperometric response (inset) for HbA_{1C} (reference, RM 405) detection at −0.45 V vs Ag/AgCl in a 0.1 M PBS (pH 7.4) containing 3.0 mM H_2O_2. (B) Linear calibration plot for HbA_{1C} (reference, RM 405) detection. Inset: Nyquist plots and equivalent circuit of impedance measurements with various concentrations of HbA_{1C}.

measurements, the sensor activity was restored by dipping the electrode in a sodium acetate buffer (pH 4.0). When it was stored at 4 °C, the sensor retained more than 92% of its response to HbA_{1C} after 1 month.

Impedance Analysis of HbA_{1C}. Impedance spectroscopic experiment was performed for the characterization of APBA-pTTBA/AuNP/SPCE at an open circuit voltage and also for the analysis of HbA_{1C} to compare with the amperometric result. Before the impedance measurements, the APBA-pTTBA/AuNP/SPCE was incubated for 5 min in the various concentrations of HbA_{1C} solution and then rinsed with 0.1 M PBS. The HbA_{1C}−APBA interaction occurred on the sensor surface and can provide the changes in the interfacial properties, which could produce the impedance response. The impedance increased with increasing concentrations of HbA_{1C} in 0.1 M PBS (pH 7.4). The Rp (Rp1 + Rp2) values were obtained by fitting the experimental data to the equivalent circuit using the Zview2 impedance software. Figure 4B shows the calibration plot for HbA_{1C} detection using Rp (Rp1 + Rp2) values. The Nyquist plot obtained for various concentrations of HbA_{1C} as shown in the inset of Figure 4B. The linear dynamic range of HbA_{1C} was determined to be from 0.5 to 6.0%, and the detection limit was to be 0.27% (k = 3, n = 5). The linear regression equation is expressed as R_p (kΩ) = (− 11.4 ± 4.2) + (49.5 ± 1.2) [C] (%), with a correlation coefficient of 0.997. The concentration level of dynamic range in amperometry is lower than that of the impedance method, and the sensitivity of amperometry is five times higher than that of the impedance method. Thus, it is possible to use a very small quantity of blood sample (<2 μL) in this proposed amperometry.

Interference Studies. The selectivity for the determination of HbA_{1C} was evaluated amperometrically and impedimetrically with untreated whole blood and pretreated blood samples obtained from healthy volunteers. In order to remove interfering species in the whole blood, such as glucose, glycated albumin, and other glycated protein, the samples were pretreated with centrifugation or cell lysis in a syringe filter. Until now, the HbA_{1C} concentration in the whole blood sample could not be determined by using the amperometric method, because it exhibited no selective amperometric response by HbA_{1C} due to the strong and fast interaction by other interfering species including glucose molecules. However, by a simple pretreatment of whole blood with syringe filtration, the HbA_{1C} was amperometrically determined to be 4.72 ± 0.12% as shown in Figure 5A. The Nyquist plots obtained for whole blood and pretreated blood samples were as shown in Figure 5B, where the ΔR_p values for whole blood and pretreated blood samples were 430.6 and 230.7 kΩ, respectively. The ΔR_p value of whole blood sample without pretreatment was 1.87 times higher than that of the pretreated blood sample, indicating that there were interferences by other glycated molecules or glucose existing in the blood matrix. As shown in Figure 5C, the results from the impedance method with pretreatment are coincident to that of the amperometric method, where the HbA_{1C} concentration by the impedance method was determined to be 4.89 ± 0.19%. These results confirm that the amperometric method can very precisely determine HbA_{1C} in blood samples with simple filtration, indicating the utilization of the present method to diagnose the diabetes in a handy type sensor device.

Real Sample Analysis. The reliability of the proposed sensor was also examined through the determination of HbA_{1C} concentrations in the blood samples of healthy volunteers (samples 3, 4, and 5) and diabetic patients (samples 1 and 2), which were obtained from Pusan National University Hospital in South Korea. The HbA_{1C} concentration in the blood samples were determined using two different pretreatment methods of the filtration and the centrifugation followed by the standard addition method. At first, the HbA_{1C} concentration in a drop of blood through simple filtration was determined from the calibration plot as shown in Figure 6. The pretreated of blood samples with filtration were diluted 10-fold. The HbA_{1C} concentrations of samples 1, 2, 3, 4, and 5, which were diluted 10-fold, were found to be 0.564 ± 0.009%, 0.776 ± 0.004%, 0.518 ± 0.002%, 0.538 ± 0.005, and 0.472 ± 0.012%, respectively. Therefore, the actual HbA_{1C} concentrations in the blood samples were 5.64 ± 0.09%, 7.76 ± 0.04%, 5.18 ± 0.02%, 5.38 ± 0.05%, and 4.72 ± 0.12% for samples 1, 2, 3, 4, and 5, respectively. The comparison of the HbA_{1C} concentrations in healthy volunteers (samples 3, 4, and 5) was performed through further analysis using the centrifugation method for 2 mL blood samples. The HbA_{1C} concentrations using the centrifugation method were 5.26 ± 0.03%, 5.41 ± 0.09%, and 4.92 ± 0.08%, respectively. The agreement between the two results provided by amperometry with filtration of 2 μL and centrifugation of 2 mL blood samples separately was evaluated through the paired t-test, in which the calculated t value (2.05) was less than the critical t value (2.31) at a 95% confidence level (n = 9). In addition, the results from the

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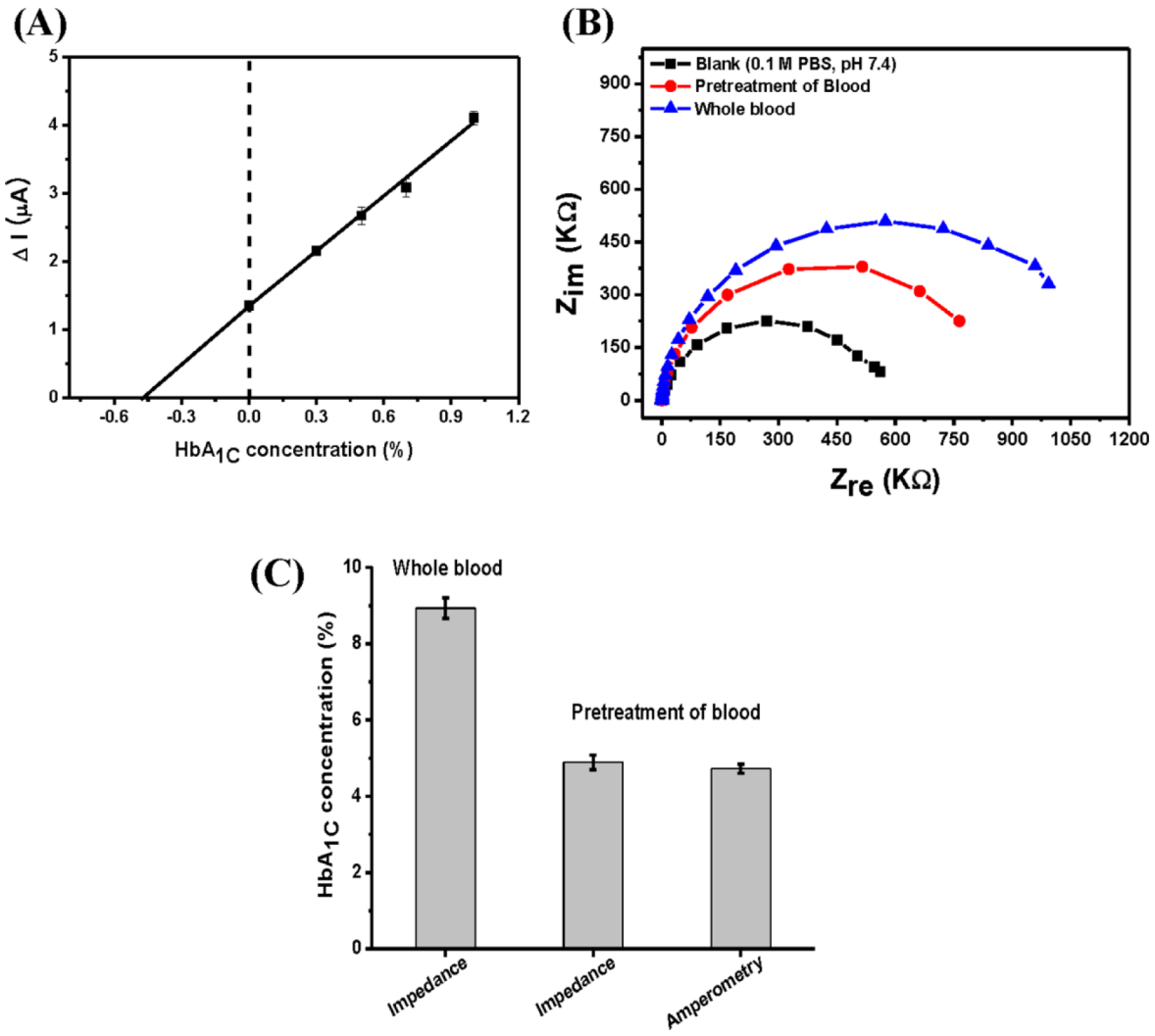

Figure 5. (A) Calibration plot for the HbA_{1C} detection in the pretreated blood samples with the standard addition method using chronoamperometry. (B) Nyquist plots of impedance measurements for blank (black), pretreated blood samples (red), and whole blood samples (blue) with the disposable sensor probe. (C) Comparison of HbA_{1C} analysis using impedance spectroscopy and chronoamperometry.

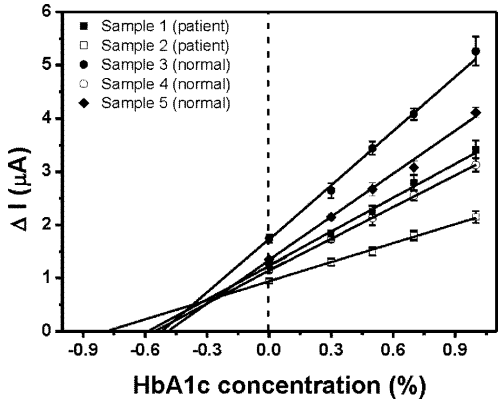

Figure 6. Calibration plots for the determination of HbA_{1C} in the real blood samples with the standard addition method using amperometry.

present work (samples 1 and 2) were comparable to results that were obtained from the HPLC experiment, where the HbA_{1C} concentrations in samples 1 and 2 were determined to be 5.7% and 7.5%, respectively. The results show good agreement between one using the proposed amperometric method for finger prick blood samples and another using the conventional HPLC method in hospitals.

CONCLUSIONS

Impedance spectroscopy and QCM experiment confirm clearly the interaction between APBA and HbA_{1C} in a 0.1 M PBS (pH 7.4). The catalytic property of HbA_{1C}, which was confirmed by voltammetry, made successful application to a disposable amperometric biosensor for glycated hemoglobin (HbA_{1C}) analysis. It was developed by exploiting the interaction between APBA and HbA_{1C} on the sensor probe and the specific catalytic reduction of H_2O_2 by HbA_{1C} itself. The proposed amperometric HbA_{1C} sensor was remarkably selective and stable, because the response of glycated hemoglobin captured on the sensor was selective and robust, since it was assembled on the highly stable conducting polymer layer. The developed disposable HbA_{1C} sensor was successfully applied to the analysis of finger prick blood samples through amperometry and the simple filtration method, in which results demonstrated excellent agreement with the conventional method.

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Notes

The authors declare no competing financial interest.

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