Korean J. Chem. Eng., 34(11), 2916-2921 (2017)
DOI: 10.1007/s11814-017-0224-9

pISSN: 0256-1115
eISSN: 1975-7220

Highly sensitive glucose biosensor using new glucose oxidase based biocatalyst

Marcelinus Christwardana, Jungyeon Ji, Yongjin Chung†, and Yongchai Kwon†

Graduate School of Energy and Environment, Seoul National University of Science and Technology,
232 Gongneung-ro, Nowon-gu, Seoul 01811, Korea
(*Received 19 June 2017 • accepted 9 August 2017*)

Abstract−Glucose, which is a primary energy source of living organisms, can induce diabetes or hypoglycemia if its concentration in blood is irregular. It is therefore important to develop glucose biosensor that reads the concentration of glucose in blood precisely. In the present work, we suggest new glucose oxidase (GOx) based catalysts that can improve the sensitivity of the glucose biosensor and make glucose measurements over a wide concentration ranges possible. For synthesizing such catalysts, a composite including pyrenecarboxaldehyde (PCA) and GOx is attached to substrate including carbon nanotube (CNT) and polyethyleneimine (PEI) (CNT/PEI/[PCA/GOx]). Catalytic activity and stability of the catalyst are then evaluated. According to the investigation, the catalyst shows excellent glucose sensitivity of 47.83 $\mu Acm^{-2}mM^{-1}$, low Michaelis-Menten constant of 2.2 mM, and wide glucose concentration detection, while it has good glucose selectivity against inhibitors, such as uric acid and ascorbic acid. Also, its activity is maintained to 95.7% of its initial value even after four weeks, confirming the catalyst is stable enough. The excellence of the catalyst is attributed to hydrophobic interaction, C=N bonds, and π-hydrogen interaction among GOx, PCA and PEI/CNT. The bindings play a role in facilitating electron transport between GOx and electrode.

Keywords: Glucose Biosensor, Diabetes, Hypoglycemia, Glucose Oxidase, Pyrenecarboxaldehyde

INTRODUCTION

Diabetes is a worldwide public health problem known as one of the leading causes inducing death and disability due to insulin deficiency and hyperglycemia [1]. Such diabetes is attributed to irregular blood glucose concentrations (the normal blood glucose concentration is 4.4-10 mM) [1,2]. People who develop diabetes are at higher risk for heart attack, kidney failure, lower limb amputation, stroke, or blindness. According to World Health Organization (WHO), the number of people with diabetes in the world has been reported as 422 million in 2014 and 1.5 million of them were dead [3]. Thus, finding out an easy and sensitive method detecting the blood glucose level is very critical.

As a way for the blood glucose measurements in humans, utilizing enzyme catalyst was started in 1962. In that time, Clark and Lyons proposed an initial concept of glucose enzyme electrode [4]. Since then, there have been various enzyme catalysts used for proper applications of the glucose enzyme electrode, and the glucose oxidase (GOx) became almost the final winner. Due to the remarkable progress of glucose biosensor using the GOx based catalyst, as time elapses, diabetes can be easily diagnosed.

The GOx has various advantages as the biosensor detecting glucose, such as excellent selectivity towards specific substrate, strong activity at near pH neutral and room temperature [5-7]. For this reason, many research groups have reported new results to replace non-enzyme based glucose biosensors [8,9]. Although there have been many advantages in glucose biosensor fabricated by the GOx based catalyst, the market assessment of the glucose biosensor was not easy due to still low performance and short stability of electrode, including the GOx based catalyst [10]. In particular, low GOx activity that was ascribed to low loading of GOx molecules was the main reason [11,12]. To get over the problems, various methods for improving immobilization of GOx molecules on the substrate and electrode have been suggested, and as the methods, non-covalent adsorption, encapsulation, covalent coupling, affinity bonding, and enzyme cross-linking have been attempted [13-16].

To further increase the performance and stability of the glucose biosensor, we suggest pyrenecarboxaldehyde (PCA) as a new material. PCA can play a role in collecting electrons and being a bridge between GOx and electrode [17]. In addition, PCA/GOx composite is synthesized by surface modification of GOx molecules using the PCA, and then the composite is directly attached to supporter material to increase electron transfer from the composite to supporter. As the supporter material combination, carbon nanotube (CNT) and polyethyleneimine (PEI) are considered due to their superior electron transfer capability (CNT) and excellent biocompatibility to prevent denaturation of GOx molcules (PEI). The CNT/PEI supporter is also capable of being embedded within the human body without any toxicity [18,19].

Regarding the PCA/GOx, we already proposed the composite structure in previous work [17]. In the work, we considered anodic catalyst for enzymatic biofuel cells (EBCs). About it, there are two bonding mechanisms between GOx and PCAs. First, active sites of GOx molecules (flavin adenine dinucleotide (FADs)) surrounded by hydrophobic pocket are connected physically with pyrene groups of PCAs by hydrophobic interaction, and second, aldehyde groups in PCAs are chemically bonded with amine group of GOx to create C=N bond. Furthermore, there are also two interactions between

†To whom correspondence should be addressed.
E-mail: yjchung@seoultech.ac.kr, kwony@seoultech.ac.kr

PCA/GOx composite and PEI/CNT substrate to prevent denaturation of GOx molecules. First, free aldehyde groups in PCA/GOx composites placed near hydrophobic pockets are chemically bonded by Schiff base formation reaction with free amine groups of CNT/PEI supporter to form C=N bonds, and second, electrostatically negative-charged free pyrene groups on GOx surface make π-hydrogen bond with positive-charged amine groups of PEI/CNT. By these four interactions amid all the components, GOx molecules can be strongly attached to the supporter, and electrons generated in the active sites of GOx molecules can be collected and transferred to electrode easily by π-conjugated effect [17].

However, in the previous work, the new CNT/PEI/[PCA/GOx] catalyst was only used for EBC [17]. With further research, in this study, we extended its utilization as a highly sensitive biosensor for sensing glucose. To verify performance of CNT/PEI/[PCA/GOx] as the catalyst for the glucose sensor, electrochemical characterizations such as cyclic voltammogram (CV), amperometric test, long-term stability and electrochemical impedance spectroscopy (EIS) tests were performed and such results were compared with that of other catalysts for the glucose biosensor.

EXPERIMENTAL SECTION

1. Materials

Multiwall carbon nanotubes (MWCNT) (purity is higher than 90%) were purchased from NanoLab (Brington, MA). Pyrenecarboxaldehyde (PCA, 99%), Glucose oxidase (GOx, from *Aspergillus niger* type X-S, 150.000 U g^{-1} solid), Horseradish peroxidase (HRP, 146 U mg^{-1} solid), o-dianisidine, Polyethylenimine (PEI) solution (50 wt%), Uric Acid (UA, 99%) were purchased from Sigma Aldrich (Milwaukee, WI, USA), while Ascorbic Acid (AA, 99.5%) was purchase from Samchun Chemicals (Gyeonggi-do, South Korea).

2. Fabrication of PCA/GOx Based Biosensor Catalyst

CNT/PEI/GOx catalyst was synthesized by electrostatic deposition. First, 25 mg of MWCNT, which was negatively charged, was dissolved in 5 mL of 2.5 mg mL^{-1} PEI that was positively charged in deionized (DI) water and then sonicated for 10 min; then the mixture was stirred for 1 h and centrifuged with 14,000 rpm for 7 min. Excess PEI was removed by using DI water. With the process, CNT/PEI was completed. Next, the CNT/PEI mixture was immersed in negatively charged GOx solution (5 mg mL^{-1} in 0.1 M PBS pH 7.4) for 20 min. In turn, the mixture was centrifuged for 7 min. During the process, sediment was formed and it was the CNT/PEI/GOx catalyst that was separated from supernatant [20].

To form CNT/[PCA/GOx] catalyst, PCA/GOx composite was initially synthesized. The composite was fabricated by mixing GOx of 5 mg and PCA of 2 mg and then the mixture was dissolved in 1 mL ethanol and immersed for 2 h. Such fabricated PCA/GOx composite was mixed with CNT of 5 mg. Afterward, the mixture was centrifuged 14,000 rpm for 7 min. During the process, sediment was formed and it was the CNT/[PCA/GOx] catalyst that was separated from supernatant.

To synthesize CNT/PEI/[PCA/GOx] catalyst, the PCA/GOx composite was immersed in CNT/PEI for 2 h. The mixture was then centrifuged at 14,000 rpm for 7 min. During the process, sediment was formed and it was the CNT/PEI/[PCA/GOx] catalyst that was separated from supernatant. Here, all the catalysts were stored in 0.01 M PBS (pH 7.4) and kept at 4 °C for storage when not in use.

3. Electrochemical Measurement of PCA/GOx Based Biosensor Catalyst

A potentiostat (Bio-Logic SP-240, USA) was used for electrochemical measurements. Half-cell tests like CV and amperometry were performed using Pt wire and Ag/AgCl (soaked in 3.0 M KCl) as counter and reference electrodes, while catalysts loaded on glass carbon electrodes (GCE) acted as working electrode. For loading of the catalysts, 5 μL of the catalytic ink (in 0.01 M PBs pH 7.4) dropped on the GCE was then dried at room temperature until completely dry. After that, 5 wt% Nafion solution was coated on the surface of modified GCE and catalyst ink to complete the working electrode. As for electrolyte, 1 M PBS (pH 7.4) was considered, while

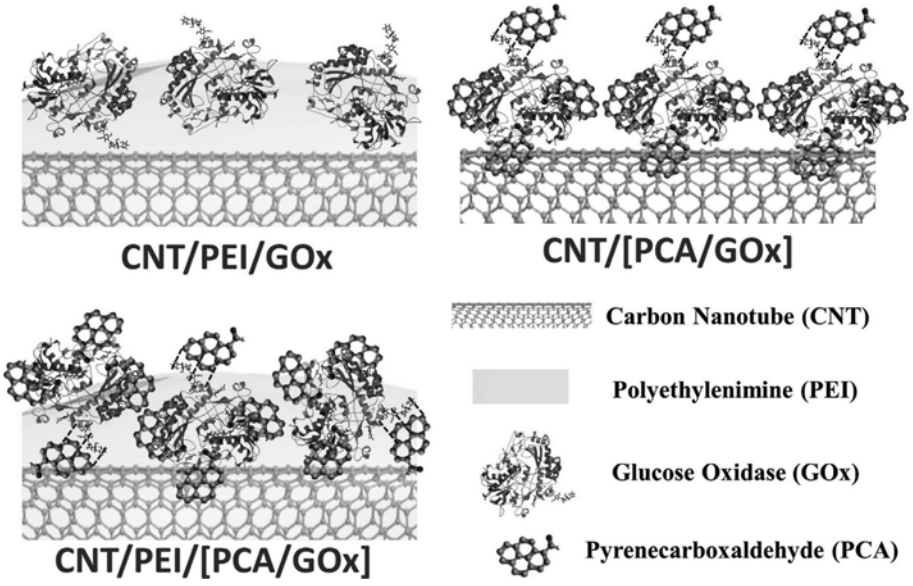

Fig. 1. Schematic illustrations showing fabrication of the CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts.

N_2 and air were fed to create specific atmosphere like N_2-purge state and air-state.

RESULTS AND DISCUSSION

1. Cyclic Voltammetry of Catalysts

A schematic showing redox reaction of GOx in three different catalyst structures (CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx]) is illustrated in Fig. 1. The FAD/$FADH_2$ redox reaction peak of the catalysts was measured by CV curves ($GOx(FAD)+2H^++2e^-\leftrightarrow GOx(FADH_2)$) and the results are represented in Fig. 2 [21]. According to Fig. 2, the redox reaction peak appeared in the potential range of −0.43~−0.47 V vs. Ag/AgCl in N_2-state (anaerobic) condition. The average potential ($\Delta V_{average}$) of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts are 0.454, 0.471, 0.443 V vs Ag/AgCl and peak current densities of CNT/PEI/[PCA/GOx] are 0.663 $mAcm^{-2}$ for oxidation peak and −0.688 $mAcm^{-2}$ for reduction peak, while those of CNT/PEI/GOx are 0.334 $mAcm^{-2}$ for oxidation peak and −0.342 $mAcm^{-2}$ for reduction peak and those of CNT/[PCA/GOx] are 0.418 $mAcm^{-2}$ for oxidation peak and −0.414 $mAcm^{-2}$ for reduction peak. It means that catalytic activity of CNT/PEI/[PCA/GOx] catalyst is the best, while that of CNT/PEI/GOx is lowest. In terms of peak potential, with addition of PEI, the peak current density was shifted a little bit due to local change of pH occurring in electrolyte by Schiff-Based formation [17].

These can be explained as that peak current density significantly increases with addition of PCA/GOx into CNT/PEI and an obvious evidence that PCA plays a role in increasing FAD/$FADH_2$ redox reaction rate of the CNT/PEI/[PCA/GOx] catalyst. This result well agrees with that of the reference paper in that excellence of the CNT/PEI/[PCA/GOx] catalyst is attributed to hydrophobic interaction, two C=N bonds and π-hydrogen interaction among GOx, PCA and PEI/CNT and the three interactions promote electron capturing and transferring, increasing the catalytic activity of the CNT/PEI/[PCA/GOx] catalyst [17].

This CV result was also supported by GOx activity that measures the amount of GOx immobilized in the corresponding catalytic structure. According to our previous result [17], the GOx activity was assesed using colorimetric assay based on the oxidation of odianisidine in a peroxidase-coupled system, while the percentage of GOx immobilized (ratio of output to output GOx concentration) in CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts was measured as 40, 51, and 63% of initial GOx of 5 mg mL^{-1} respectively.

2. Glucose Sensing Performance of Catalysts

To investigate the role for glucose biosensor of three GOx based catalysts, their amperometric responses were measured and the results are presented in Fig. 3(a)-(b). All the tests were done with air-state and sequential feeding of 0-20 mM glucose. According to Fig. 3(a), when the glucose concentration was 3 mM, peak current densities of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts were 38.4, 98.8 and 145.0 μAcm^{-2}. When

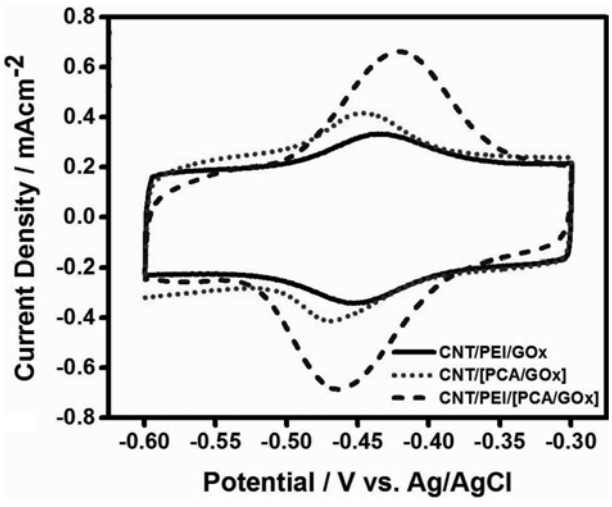

Fig. 2. Cyclic voltammograms of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts. For the tests, 1 M PBS (pH 7.4) was used as electrolyte under N_2-state and potential scan rate was 100 mV s^{-1}.

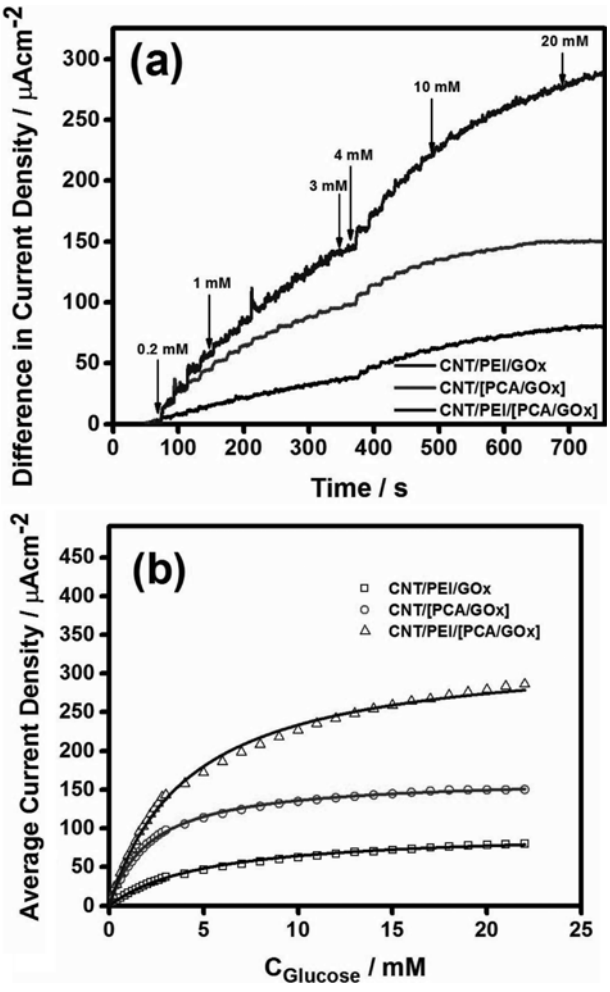

Fig. 3. (a) Amperometric responses of 0-20 mM glucose, (b) a relationship between glucose concentration and average current density, and also (c) Lineweaver-Burk plot of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts. For the tests, 0.01 M PBS (pH 7.4) was used as electrolyte under air-state.

glucose was further fed into electrolyte until its concentration reached 5 mM, which is similar glucose concentration to human blood, the peak current densities of the catalysts increased (in glucose of 5 mM, the peak current densities of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts are 50, 115 and 170 μAcm^{-2}) and the relationship between peak current density and glucose concentration was maintained until the glucose concentration reached 21 mM. In the glucose concentration, the peak current densities of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts were 80.6, 151.8 and 283.5 μAcm^{-2}. Such results mean that all the three catalysts are sensitive enough to be used as the glucose biosensor and, especially, the value of the CNT/PEI/[PCA/GOx] catalyst was highest, demonstrating that it can be a powerful catalyst to detect glucose within human blood in an effective manner [1,2].

To further evaluate effects of the catalysts on the performance of glucose biosensor, Michaelis-Menten constant (K_m), maximum current density (J_{max}) and sensitivity were measured [22-24]. To determine K_m and J_{max}, the Michaelis-Menten formula and Lineweaver-Burk plot were used as shown in Fig. 3(b). K_ms of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts were 5.06, 4.22, and 2.20 mM, and their J_{max}s were 82.37, 164.42, and 297.63 μAcm^{-2}. This trend indicates that even in terms of reaction kinetics that is expressed as K_m and J_{max} with sequential increase in glucose concentration, the CNT/PEI/[PCA/GOx] catalyst shows the best catalytic activity on glucose detection. Moreover, slopes of CNT/PEI/GOx and CNT/[PCA/GOX] catalysts were nearly saturated after 4 mM, but that of CNT/PEI/[PCA/GOx] catalyst consecutively increased up to the range of middle and high glucose concentration (4-21 mM). It implies that the CNT/PEI/[PCA/GOx] catalyst can be used as electrode for appropriate glucose biosensor that can detect blood glucose level of both normal persons and diabetes patients.

Measuring catalytic sensitivity in low concentration range of glucose (0-1 mM) is also important for proper detection of hypoglycemia [25], and searching for a proper electrode for a glucose biosensor is still an ongoing effort. According to the measurements, in CNT/PEI/[PCA/GOx] catalyst, glucose sensitivity measured in low glucose concentration was 47.83 $\mu Acm^{-2}mM^{-1}$ while that of CNT/PEI/CNT and CNT/[PCA/GOx] catalysts was 11.89 and 30.76 $\mu Acm^{-2}mM^{-1}$. These sensitivities are better than that of other competitive catalyst structures. For instance, glucose sensitivity of GODx/Ppy catalyst was 7 $nAmM^{-1}$ (or 0.23 $\mu Acm^{-2}mM^{-1}$) [26], that of GOD/CNTs/CS/GC catalyst was 0.52 μAmM^{-1} (or 7.43 $\mu Acm^{-2}mM^{-1}$) [27], that of GOx/ZnONT catalyst was 21.7 μAcm^{-2} mM^{-1} [28] and that of GOD/graphene/chitosan nanocomposite catalyst was 37.93 $\mu Acm^{-2}mM^{-1}$ [29]. In brief, the CNT/PEI/[PCA/GOx] catalyst showed the best performance in glucose level related to both diabetes and hypoglycemia patients.

Another important role of catalyst designed for the glucose biosensor is the glucose selectivity when glucose and inhibitors are mixed. To investigate it, sensitivity to ascorbic acid (AA) and uric acid (UA) as the inhibitors that are included in human blood was examined using the catalysts. For doing that, 1 mM of AA, 0.5 mM of UA and 1 mM of glucose were fed to the electrolyte one by one and amperometric responses of the catalysts were measured, as shown in Fig. 4. There were no specific responses when AA and UA were fed into electrolyte, whereas clear signals were detected when glucose was added. In addition, in the CNT/PEI/[PCA/GOx] catalyst, the amperometric response current measured by glucose addition was about 5 μA while that of CNT/[PCA/GOx] and CNT/PEI/GOx catalysts was only 1 and 2 μA. Regarding the AA and UA, the three catalysts did not respond. It means that all three catalysts have superior selectivity to glucose detection, and of them the glucose sensitivity of CNT/PEI/[PCA/GOx] catalyst was highest even when inhibitors existed.

Nyquist plots of the three catalysts using electrochemical impedance spectroscopy (EIS) were measured to gain charge transfer resistance (R_{ct}) of catalysts (Fig. 5). According to the measurements, R_{ct}

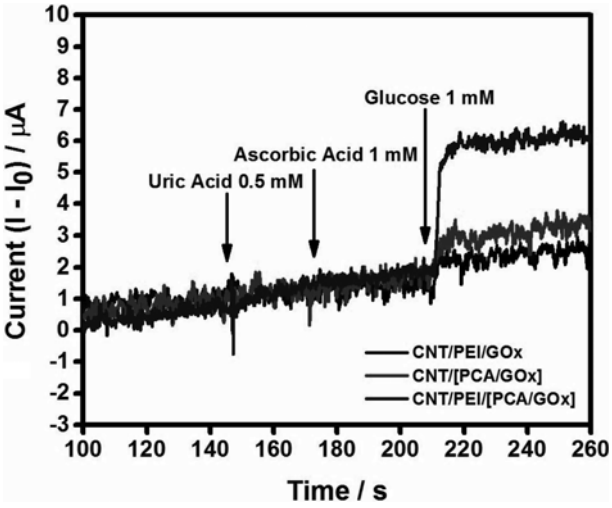

Fig. 4. Amperometric responses of biosensor run with additions of glucose (1 mM), UA (0.5 mM), and AA (1 mM) in 0.01 M PBS. Working potential was between −0.43 and −0.48 V. For the tests, 0.01 M PBS (pH 7.4) was used as electrolyte under air-state.

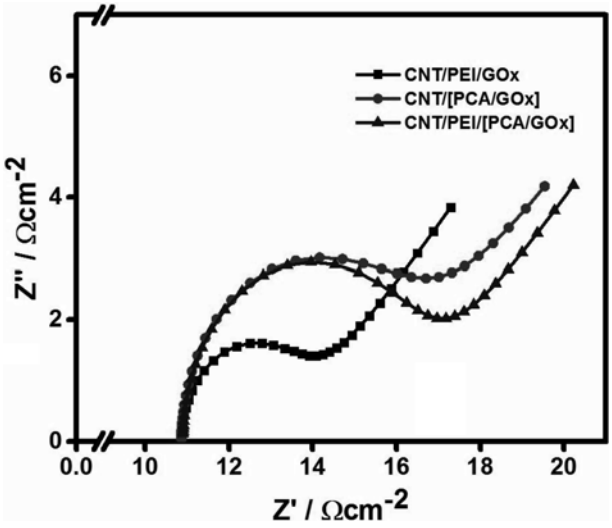

Fig. 5. Nyquist plots of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts. For the tests, 0.01M PBS (pH 7.4) was used as electrolyte under N_2-state.

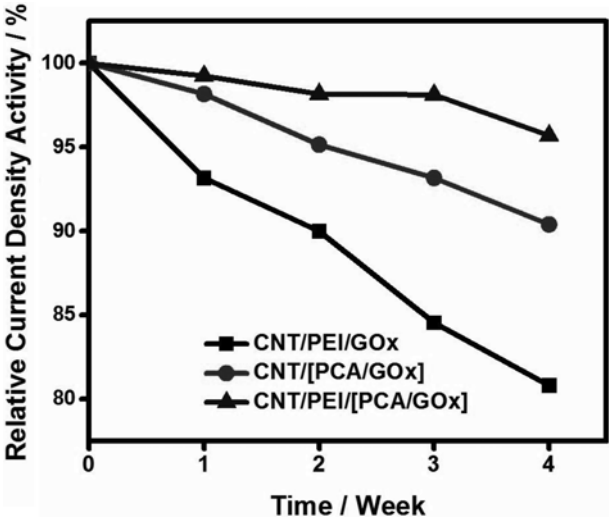

Fig. 6. Relative activity measurements of CNT/PEI/GOx, CNT/[PCA/GOx], and CNT/PEI/[PCA/GOx] catalysts that were measured over four weeks.

of CNT/PEI/[PCA/GOx] catalyst was 5.2 Ωcm^2, while that of CNT/[PCA/GOx] and CNT/PEI/GOx catalysts was 2.8 and 4.8 Ωcm^2. It indicates that PEI has a trade-off with both positive and negative effects. Namely, as a positive prospect, it can improve performance of the GOx and CNT based sensor catalysts because it promotes immobilization of GOx molecules and improves stability of the immobilized GOx molecules caused by its excellent biocompatibility and entrapping ability. However, in a negative prospect, due to its relatively low electrical conductivity, it may act as a barrier for electron transfer.

3. Stability of Biosensor Catalyst

Stability of the three catalysts was evaluated by measuring their immobilization over four weeks. During the four weeks, peak current densities of CNT/PEI/GOx, CNT/[PCA/GOx] and CNT/PEI/GOx catalysts were reduced to 19.2, 9.6 and 4.3% of their initial values. This stability result is better than that of other competitive catalysts. For example, it was reported that stability of PDDA/GOx/PDDA/CNT/GC catalyst was reduced 10% after four weeks [30], that of GOD/GNp/CS/GOD/GNp/PAA/Pt catalyst only maintained 90% of initial value after one month [31], and that of RGO/GOx catalyst was 6.4% reduced after 20 days [32]. It proves that the CNT/PEI/[PCA/GOx] catalyst has good capability to alleviate denaturation of GOx molecules that were already immobilized and, for that reason, the catalyst maintained its active state for a predetermined time.

CONCLUSIONS

To fabricate glucose biosensor that is highly sensitive to glucose and stable, and can detect a wide concentration range of glucose, a new biocatalyst was proposed. To synthesize the catalyst, a PCA/GOx composite was manufactured, and then it was attached to the surface of CNT/PEI that was considered supporting material. As a result, the CNT/PEI/[PCA/GOx] catalyst was formed. According to subsequent evaluations, the catalyst promoted electron transfer between GOx and electrode and kept its state with low denaturization of GOx molecules due to the following three reasons. First, the catalyst showed electron collection effect that was ascribed to hydrophobic interaction between hydrophobic pocket near GOx and pyrene rings of PCA second, the catalyst created new electron transfer pathway formed by π-conjugation between amine group of CNT/PEI and aldehyde group of PCA, and third, stability of the catalyst was well-maintanined due to π-hydrogen bond between pyrene rings of PCA and amine group of PEI.

To confirm effects of the three reasons on catalytic activity and stability of the catalyst, qnatitative measurments were implemented. In terms of detection range of glucose, the catalyst could detect glucose concentration of 0 to 21 mM, which corresonded to a wide range compared to other catalyst candidates. In terms of stability of the catalyst, it maintained its activity to 95.7% from its initial value for four weeks. When it came to catalytic activity for glucose biosensor, the catalyst showed high sensitivity (47.83 $\mu Acm^{-2} mM^{-1}$) and low Michaelis-Menten constant (2.2 mM). The catalyst had also excellent selectivity. When inhibitors, such as AA and UA were inserted with glucose, the catalyst only detected glucose. Such excellent features of the CNT/PEI/[PCA/GOx] as the catalyst for glucose biosensor indicate that high performance glucose biosensor can be developed, and the biosensor can be effectively used for the people who have hypoglycemia and diabetes as well as normal people.

ACKNOWLEDGEMENT

This study was supported by the Research Program funded by the SeoulTech (Seoul National University of Science and Technology).

REFERENCES

1. J. Wang, *Chem. Rev.*, **108**, 814 (2008).
2. Y. Chung and Y. Kwon, *Korean Chem. Eng. Res.*, **53**, 802 (2015).
3. World Health Organization (WHO) of United Nations (UN). Global Report on Diabetes sheet. Available online: http://www.who.int/diabetes/global-report/WHD16-press-release-EN_3.pdf (Accessed on May 09, 2016).
4. L. C. Clark Jr., C. Lyons and N. Y. Ann, *Acad. Sci.*, **102**, 29 (1962).
5. S. Cosnier, *Biosens Bioelectron.*, **14**, 443 (1999).
6. S. D. Minteer, B. Y. Liaw and M. J. Cooney, *Curr. Opin. Biotechnol.*, **18**, 228 (2007).
7. J. Kim, H. Jia and P. C. Wang, *Biotechnol. Adv.*, **24**, 296 (2006).
8. C. Zhou, L. Xu, J. Song, R. Xing, S. Xu, D. Liu and H. Song, *Sci. Rep.*, **4**, 7382 (2014).
9. H. Zhu, L. Li, W. Zhou, Z. Shao and X. Chen, *J. Mater. Chem. B.*, **4**, 7333 (2016).
10. M. Christwardana and Y. Kwon, *J. Power Sources*, **299**, 604 (2015).
11. S. C. Barton, J. Gallaway and P. Atanassov, *Chem. Rev.*, **104**, 4867 (2004).
12. E. H. Yu, U. Krewer and K. Scott, *Energies.*, **3**, 1499 (2010).
13. H. S. Choi, D. S. Kim, L. P. Thapa, S. J. Lee, S. B. Kim, J. Cho, C. Park and S. W. Kim, *Korean J. Chem. Eng.*, **33**, 3434 (2016).
14. R. A. Bohara, N. D. Thorat and S. H. Pawar, *Korean J. Chem. Eng.*,

33, 216 (2016).
15. Inamuddin, Beenish, Mu. Naushad, *Korean J. Chem. Eng.*, **33**, 120 (2016).
16. B. H. Jo, C. S. Kim, Y. K. Jo, H. Cheong and H. J. Cha, *Korean J. Chem. Eng.*, **33**, 1125 (2016).
17. M. Christwardana, Y. Chung and Y. Kwon, *NPG Asia Mater.*, **9**, e386 (2017).
18. Y. Chung, Y. Ahn, D.-H. Kim and Y. Kwon, *J. Power Sources*, **337**, 152 (2017).
19. M. Christwardana, Y. Chung and Y. Kwon, *Nanoscale.*, **9**, 1993 (2017).
20. K. Hyun, S. Han, W.-G. Koh and Y. Kwon, *J. Power Sources*, **286**, 197 (2015).
21. M. Wooten, S. Karra, M. Zhang and W. Gorski, *Anal. Chem.*, **86**, 752 (2014).
22. J. D. Cui, R. L. Liu and L. B. Li, *Korean J. Chem. Eng.*, **33**, 610 (2016).
23. K. Hyun, S. W. Han, W. G. Koh and Y. Kwon, *Int. J. Hydrogen Energy*, **40**, 2199 (2015).
24. M. Christwardana, K. J. Kim and Y. Kwon, *Sci. Rep.*, **6**, 3012 (2016).
25. E.-H. Yoo and S. Y. Lee, *Sensors*, **10**, 4558 (2010).
26. Y.-M. Uang and T.-C. Chou, *Biosens. Bioelectron.*, **19**, 141 (2003).
27. Y. Liu, M. Wang, F. Zhao, Z. Xu and S. Dong, *Biosens Bioelectron.*, **21**, 984 (2005).
28. T. Kong, Y. Chen, Y. Ye, K. Zhang, Z. Wang and X. Wang, *Sens. Actuators B.*, **138**, 344 (2009).
29. X. Kang, J. Wang, H. Wu, I. A. Aksay, J. Liu and Y. Lin, *Biosens. Bioelectron.*, **25**, 901 (2009).
30. G. Liu and Y. Lin, *Electrochem. Commun.*, **8**, 251 (2006).
31. B.-Y. Wu, S.-H. Hou, F. Yin, J. Li, Z.-X. Zhao, J.-D. Huang and Q. Chen, *Biosens. Bioelectron.*, **22**, 838 (2007).
32. B. Unnikrishnan, S. Palanisamy and S.-M. Chen, *Biosens. Bioelectron.*, **39**, 70 (2013).