J. of Korean Oil Chemists' Soc.,
Vol. 32, No. 4. December, 2015. 724~730
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2015.32.4.724

Diagnostic *Ex-Vivo* Assay of glucose Using Diabetic-Control Circuits

Suw Young Ly[*,†] · Chang Hyun Lee[**] · Hai-Soo Yoo[***]

[*]*Biosensor Research Institute, Seoul National University of Science & Technology*
172 Gongreung 2 dong, Nowon gu, Seoul, South Korea 139-743.
[**]*Division of General Education, Pyeongtaek University, Gyeonggi-do 405-701, Korea*
[***]*East Sea Research Institute/KIOST*
(Received November 13, 2015; Revised December 28, 2015; Accepted December 30, 2015)

Abstract : For *ex-vivo* diabetic control, the voltammetric diagnosis of glucose (GU) was conducted with a modified carbon nanotube paste electrode, using handheld analytical circuits. The optimum analytical conditions were attained within the 0.5-4.0 ug/L working range and at the 0.06 ug/L detection limit, which system was interfaced to the feedback circuits and was applied to human urine for diabetic-patient diagnosis. It can be used for *ex-vivo* flow control analysis, vascular flow detection and other medicinal assays. The equations of the patients' urine are y=36.65x+12.13 and R^2 =0.987, those of the healthy person of y= 2.5x+10.9 and R^2 =0.928 (patients: 118 ug/L; healthy person: 12.34 ug/L).

Keywords : Voltammetry, glucose, diabete, urine, Hg nanotube electrode

1. Introduction

The detection of glucose (GU) is important for human diabetic assay as most of the *in-vivo* energy generated by the oxidation of GU is used for the work that is necessary to maintain the ionic balances associated with synaptic transmission [1]. Diagnostic systems, however, require *in-vivo* blood extraction in the vascular tract, and limitless blood suction is very painful for a diabetic patient. Moreover, *ex-vivo* urine assay is not ideal for common use as it demands high sensitive detection limits and is very expensive. Various electroanalytical sensor techniques were recently developed, such as those using the bienzyme carbon paste electrode [2], gold electrode [3], digital microfluidic biosensor [4], polylysine calcium alginate [5], HRP GOD layered assembly [6], modified sol gel glass [7], ormosil-based electrocatalytic biosensor [8], microplate [9], FIA system [10], acrylated amperometric glucose biosensor [11], and enzymes [12]. Some of these methods, however, are not easy to use as they do not have low detection limits and require complex modification techniques, and as they involve devices that are too big for home use. Simpler and more sensitive *ex-vivo* analytical methods are thus needed by diabetic patients. As such,

†Corresponding author
(E-mail: suwyoung@snut.ac.kr)

in this study, a simple, easy-to-use, and inexpensive device using mercury immobilized onto a carbon nanotube sensor and compact voltammetric [13-19] systems was developed. It can also detect low concentration ranges, which means that not much blood is required from the diabetic patients who will use it. Its circuit can first be interfaced with the feedback control systems, which can be controlled through a fluid convection and other medicinal pumping system.

2. Experiment Design

2.1. Preparation of the electrode

For the sensor preparation, mercury (Hg) immobilized onto a carbon nanotube paste electrode (HNE; prepared by mixing 40% carbon nanotube powder, 40% solid mercury, and 20% mineral oil), bismuth immobilized onto a carbon nanotube paste electrode (BNE; prepared by mixing 40% carbon nanotube powder, 40% solid bismuth powder, and 20% mineral oil), and a carbon nanotube paste electrode (NE; prepared by mixing 80% carbon nanotube powder and 20% mineral oil) were prepared. The paste was homogenized in a mortar for 30 min then inserted into a plastic syringe needle with a 3.0 mm diameter and a 100 mm length, and a copper wire was connected to the voltammetric workstation. An Ag/AgCl electrode and a platinum wire electrode served as the reference electrode and auxiliary electrode, respectively. A three-electrode cell was used to monitor the voltammetric signal.

2.2. Reagents

All the experiment solutions were prepared from 18M ohm cm^{-1} double-distilled water. A 0.1M $NH_4H_2PO_4$ solution with a pH strength of 4.75 served as a supporting electrolyte solution. Analytical-grade standard glucose (Aldrich Chemical Co.) was also prepared. All the experiment solutions were exposed to dissolved oxygen, and an electrode cleaning time was not necessary for every measurement.

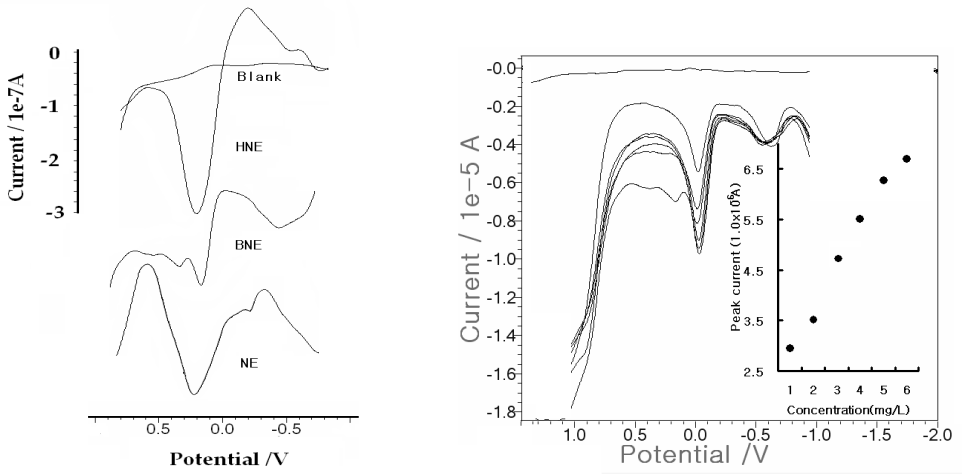
2.3. Experiment Procedure

Electrochemical workstations were used with the new Bioelectronics-1 system, which was constructed at the authors' institute (computerized, handheld, +-2.0 V potential range, 10 pA measuring current, rechargeable battery or external power, and USB port interface with a PC). The instrument is as big as a typical cellular phone and can be used in bioassay by individuals at home as well as in a laboratory. The common parameter for +-2.0V cyclic voltammetry (CV) was a scan rate of 100 mVs^{-1}, and the common parameters for square-wave (SW) stripping voltammetry were set at the optimized conditions. Hg immobilization was performed through a cyclic scan with an initial potential of +1.6 V, a switching potential of 0.6 V, and a scan rate of 0.5 mVs^{-1}, with a tan cyclic repeat to stabilize the electrode surface. Since the voltammetric response of GU is dependent on the electrolyte solutions and the hydrogen ionic strength, various types of electrolyte solutions were tested. The phosphate solution yielded the best results.

3. Results and Discussion

3.1. Cyclic voltammetry

First, the electrodes' properties were determined using common and specially prepared sensors. Fig. 1(A) shows the three sensitive sensor types. HNE, BNE, and NE were compared by adding 300-mg/L GU using the optimum stripping parameters. Under these conditions, the peak current was obtained at $3.0x10^{-7}$A HNE, $1.2x10^{-7}$A BNE, and $2.4x10^{-7}$A NE. Here, HNE was found to be more sensitive than the others and obtained a sharp peak width. The HNE concentration effects were thus examined. Fig. 1(B) shows the result of the effects of high-concentration



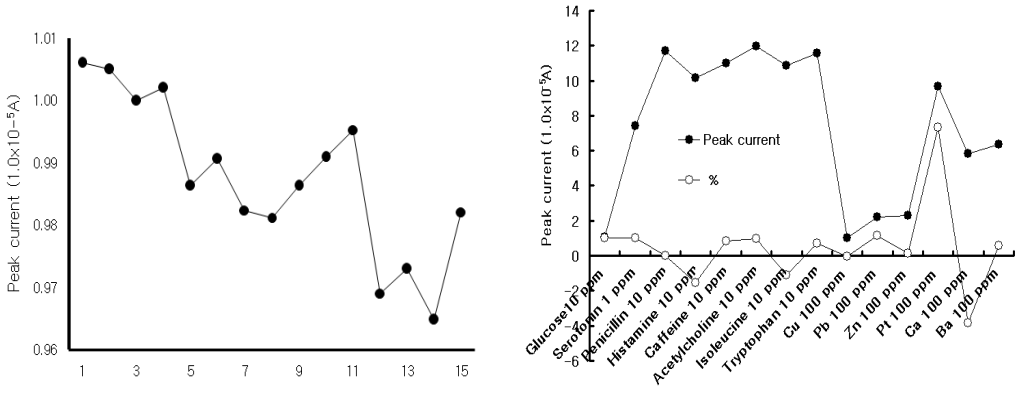
(A) (B)

Fig. 1. (A) Three types of electrodes were compared to select the sensitivity using 300-mg/L GU added under the optimum parameters. (B) SW concentration effects for the HNE of 10- to 250-mg/L GU added under the optimum conditions, 5.34 pH, 0.1M H_3PO_4 electrolyte solution.

GU using HNE. Before the experiment, 1000-mg/L glucose standard was used. As shown in the figure, the blank electrolyte was simple, and some glucose was applied. A peak current increase was detected. Here, 10-, 20-, 50-, 100-, 200-, and 250-mg/L GU were added. The peak current reached 2.942, 3.513, 4.753, 6.217, 6.274, and 6.999 x 10^{-6}A, respectively. The more Gu that was applied, the greater the peak current increase. This result shows that the device that was developed in this study can detect GU in the blood. Very low detection limits are required, however, in *ex-vivo* fluid assay. Thus, the optimum analytical conditions were examined (data not shown), and their statistical application was likewise investigated.

3.2. Statistics and Interference

Fig. 2(A) shows the experiment statistics for the 10-ugL^{-1} GU that was tested 15 times using the final SW conditions. The peak current first reached 1.006×10^{-6} and then deceased to 0.96 x 10^{-6} in the second and third repetitions. It increased slightly to 1.002 × 10^{-6} in the fourth repetition but decreased again to 0.9863 × 10^{-6} in the fifth repetition, however, in the 12th repetition, increased slightly to 0.973 × 10^{-6} in the 13th repetition. It then went up to 0.9821 × 10^{-6} in the 15th repetition and thereafter became stable. Under the aforementioned conditions, various analytical-interference ions were examined by adding other metals and analog neurotransmitters to the medium containing 0.1-mgL^{-1} GU, for the tenfold spiking of 1-mgL^{-1} Cu, Pb, Zn, Pt, Ca, and Ba. This yielded results of 100, -2, 116, 12, 735, -384, and 56%, respectively. The analytical interferences were effectively corrected using the standard addition methods. Under these conditions, the usable working range was examined via SWSV and CV.



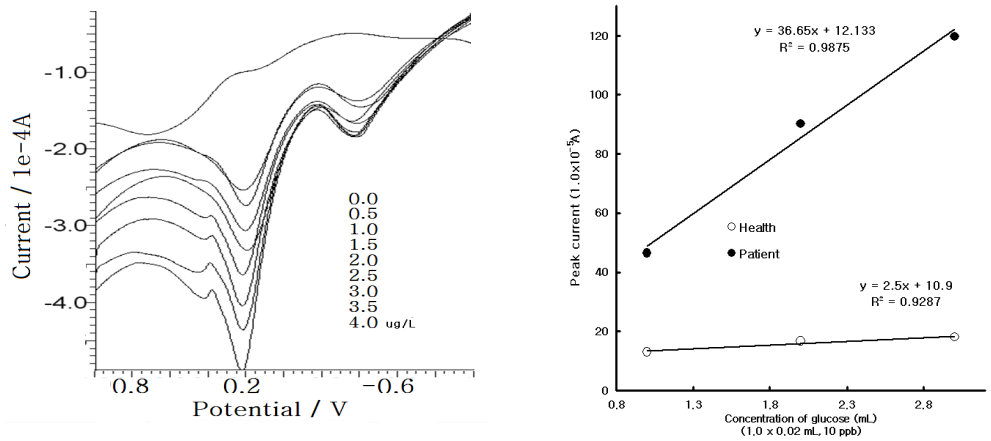
(A) (B)

Fig. 2. (A) Statistics for the 10-ug/L GU added. (B) Result of the interference by various metals and neurotransmitters using the optimum parameters (5.34 pH, 0.1M H_3PO_4 electrolyte solution).

3.3. Working range and application in patients' urine

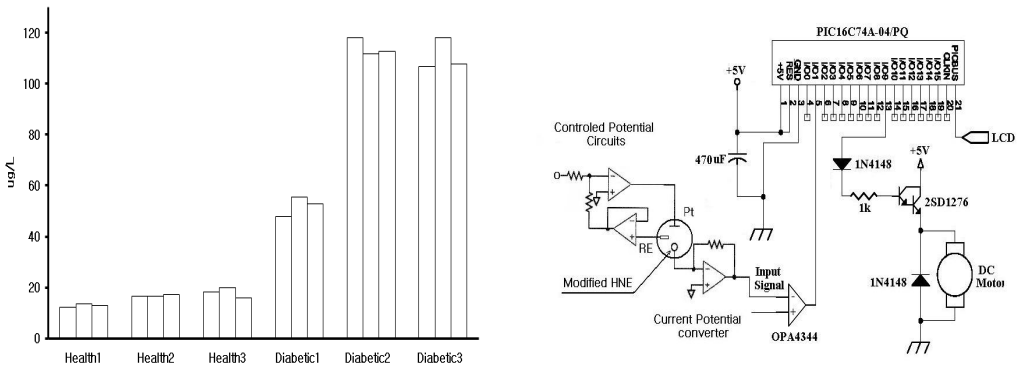
As the concentration of GU in urine is low, the developed device can detect the GU therein. As such, an experiment was conducted involving low-concentration GU. When 0.5-, 1-, 1.5-, 2-, 2.5-, 3-, 3.5-, and 4-ug/L GU was added, the peak current reached 1.144, 1.269, 1.297, 1.448, 1.712, 1.947, 2.182, and 2.656 × 10^{-4} A, respectively. It was observed that the peak current gradually increased with the addition of more GU. As it is known that HNE can detect a low concentration of GU, an experiment was conducted using a healthy person's and two patients' urine. Fig. 3(B) shows the results of such applications. The right graph shows the result of the patients' urine, and the left graph shows the results of the healthy person's urine. In this figure, the first curve represents the blank electrolyte solution where the 0.01-mL urine sample was added (1-ml urine in 100-ml distilled water). To this water was added 0.02- and 0.03-mL standard glucose in a 10-ml ammonium phosphate. Thereafter, the developed device was used, and GU was detected. Equations were formulated based on the result. The equations of the patients' urine are y=36.65x+12.13 and R^2 =0.987, and those of the healthy person are y= 2.5x+10.9 and R^2 =0.928 (patients: 118 ug/L; healthy person: 12.34 ug/L). These equations show that the developed device can detect GU in the urine and can be easily used to measure a patient's GU at home.

Analytical application was performed using patients' and normal urine, via the standard addition methods. Fig. 4(A) shows the statistical results of the three samples of patients' and a healthy person's urine. Here, the GU contents of the patients' urine were three times those of the normal group's urine. The developed techniques can be used for diagnostic assay in medicinal urine and blood analysis. Under the aforementioned conditions, the advanced feedback systems were examined. For *ex-vivo* control, the PIC current amplification circuits, DC flow control motor systems, and voltammetric circuits were interfaced. Fig. 4(B) shows the fabrication of



(A) (B)

Fig. 3. (A) The SW result of a low concentration within the range of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 ug/L. (B) The equation of the urine of a healthy person and of patients.



(A) (B)

Fig. 4. (A) Statistics of the urine of a healthy person and of tree patients using the optimum parameters. (B): The amplification inversion circuit.

the circuits, where the voltammetric output current was connected to the amplification inversion circuit. Here, the voltammetric peak current can be controlled in the flow systems of the rotating DC potential motors, which can be controlled for urine solutions.

4. Conclusions

HNE is made with carbon nanotube and Hg-immobilized paste. Its optimal parameters were found to be as follows: 0.025 V SW amplitude, 15 Hz frequency, -2 V initial

potential, and 0.004 V increment potential. Under these conditions, the detection limit of 0.06 ug/L GU was attained. HNE can thus detect GU not only in blood but also in urine. It overcomes the limitations of the other parallel devices, which are the fact that they are expensive and are difficult to use at home due to their sizes. The developed device is small and can detect a low GU concentration; it can thus be used at home and with any flow technique.

References

1. D. Schubert, Glucose metabolism and Alzheimer's disease, *Ageing Research Reviews* (4)240-257(2005)2. T. Huang, A. Warsinke, O. V. K. Skorobogat'ko, A. Makower,. T. Kuwana, F.W. Scheller and A Bienzyme Carbon Paste Electrode for the Sensitive Detection of NADPH and the Measurement of Glucose-6-phosphate Dehydrogenase, *Electroanalysis.* 11(5)295-300(1999).
2. T. Huang, A. Warsinke, O. V. K. Skorobogatko, A. Makower, T. Kuwana and F.W. Scheller, A Bienzyme Carbon Paste Electrode for the Sensitive Detection of NADPH and the Measurement of Glucose-6-phosphate Dehydrogenase, *Electroanalysis* 11(5)295-300(1999).
3. S. Suye, T. Matsuura, T. Kimura, H. Zheng, T. Hori, Y. Amano and H. Katayama, Amperometric DNA sensor using gold electrode modified with polymerized mediator by layer-by-layer adsorption, *Microelectron Eng* (81) 441-447(2005).
4. V. Srinivasan, V. Pamula, M. Pollack and R. Fair, A digital microfluid Biosensor for Multianalyte detection, *IEEE* 329-333 (2003).
5. R. Russel, M. Pishko, C. Gefrides and G cote, A Fluopescent Glucose Assay using Pol-L-Lysine And Calcium Alginate Microencapsuled tric-succinyl-concanavalin A and Fitc-Dextran, *IEEE* 2858-2860 (1998).
6. T. Ferri, S. Maida, A. Poscia and R. Santucci, A Glucose Biosensor Based on Electro-Enzyme Catalyzed Oxidation of Glucose Using a HRP-GOD Layered Assembly, *Electroanalysis,* 13 (14) 1198-1202(2001).
7. P. C. Pandey, S. Upadhyay and H. C. Pathak, A new glucose sensor based on encapsulated glucose oxidase within organically modified sol--gel glass, *Sensor Actuat B-chem* (60)83-89(1999).
8. P. C. Pandey, S. Upadhyay, I. Tiwari, and V. S. Tripathi, A Novel Ormosil Based Electrocatalytic Biosensor for Glucose Ethanol Based on Dehydrogenase Modifed Electrode, *Electroanalysis* 13(10) 820-825 (2001)
9. J. S. Velterop and F. Vos, A Rapid and Inexpensive Microplate Assay for the Enzymatic Determination of Glucose, Fructose, Sucrose, L-Malate and Citrate in Tomato (*Lycopersicon esculentum*) Extracts and in Orange Juice, 2001 John Wiley & Sons, Ltd. ,*Phytochem. Anal.* (12)299-304 (2001).
10. S. A. M. Marzouk, H. E. M. Sayour, A. M. Ragab, W. E. Cascio, S. S. M. Hassan, A Simple FIA-System for Simultaneous Measurements of Glucose and Lactate with Amperometric Detection, *Electroanal* 2000 (16) 1304- 1311(2000).
11. C. Puig-Lleixa, C. JimeÂneza, J. Bartrolõ and A. polyurethane Ð photopolymeric membrane for amperometric glucose biosensor construction, *Sensors Actuat-chem* (72) 56-62(2001).
12. V. M. T Spackman and A. H Cobb, An enzyme-based method for the rapid determination of sucrose, glucose and fructose in sugar beet roots and the effects of impact damage and postharvest storage in clamps, *Sci Food Agric,* 80-86(2001).

13. Othman A. and Farghaly, Direct and simultaneous voltammetric analysis of heavy metals in tap water samples at Assiut city: an approach to improve the analysis time for nickel and cobalt determination at mercury film electrode, *Microchem J* (75) 119-131(2003).
14. S. C. C. Monterroso, H. M. Carapuça, J. E. J. Simão and A.C. Duarte, Optimisation of mercury film deposition on glassy carbon electrodes: evaluation of the combined effects of pH, thiocyanate ion and deposition potential, *Anal Chim Acta* (503) 203-212(2004).
15. Helena M. Carapuça, Sandra C.C. Monterroso, L. S. Rocha and A. C. Duarte, Simultaneous determination of copper and lead in seawater using optimised thin-mercury film electrodes in situ plated in thiocyanate media, *Talanta* (64)566-569(2004).
16. J. M. Zen, F.shienhsu, N. Y. Chi, S. Y. Huang and M.J. Chung, Effect of model organic compounds on squrare-wave voltammetric stripping analysis at the Nafion/ chelating agent mercury film electrodes, *Anal Chim Acta* (310)407-417 (1995).
17. J. T. Wu, Y. Huang, J. Z. Zhou, J. Luo and Z. h. Lin, Electrochemical behaviors of DNA at mercury film electrode, ***Bioelectrochem Bioenerg*** (44). 151-154 (1997).
18. N.B.F. Zakharchuk þ and K.Z. Brainina, *The Surface Morphology of Mercury Plated Glassy-Carbon Electrodes and Stripping Voltammetry of Heavy Metals*, *Electroanal.* 10(6), 379-386(1998).
19. P. Kostecka, L. Havran, H. Pivonkova and M. Fojta, Voltammetry of osmium-modified DNA at a mercury film electrodeApplication in detecting DNA hybridization, *Bioelectrochemistry* 63, 245-248(2004).