

## Research Article

# A Simple and Facile Glucose Biosensor Based on Prussian Blue Modified Graphite String

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This work describes the string sensor for the simple and sensitive detection of glucose which is based on Prussian blue (PB) modified graphite utilizing dipping. First, PB modified graphite (PB-G) strings are characterized by physical and electrochemical techniques to optimize the PB-G layer thickness. Then, glucose oxidase (GOx) is immobilized on PB-G string electrode with biocompatible chitosan overlayer (Chi/GOx/PB-G). The Chi/GOx/PB-G string electrode exhibits a sensitivity of  $641.3 \mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$  to glucose with a linear range of 0.03 to 1.0 mM ( $R^2 = 0.9957$ ) and a rapid response time (<3 s). Moreover, the Chi/GOx/PB-G string electrodes are less sensitive to common interference materials such as ascorbic acid, uric acid, galactose, and acetaminophen than to glucose. The Chi/GOx/PB-G string electrodes also show excellent reproducibility (<5% RSD). Therefore, our Chi/GOx/PB-G string electrodes can be simple, robust, and reliable tools for glucose sensing which can avoid complicated and difficult multistep fabrication processes. In addition, we expect that they have many potential applications in fields ranging from health care to food analysis, in particular where single use is favorable.

## 1. Introduction

Current demands for point-of-care testing devices for clinical diagnostics and on-site environmental monitoring have attracted great attention in terms of developing flexible, lightweight, low-cost, and wearable sensors [1, 2]. In particular, smart fabrics with biomonitoring functionality have a lot of potential in health care, sports, and defence applications. Although conductive metal wires and other rigid fibers have been utilized for the wearable electronics, these materials do not provide textiles with sufficient biological compatibility, flexibility, durability, and mobile comfort as well as smart biosensing [3].

Prussian blue (PB,  $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ ) has been widely used as an “artificial peroxidase” in electrochemical sensors [4, 5]. PB itself has been recognized as the most advantageous low-potential transducer for hydrogen peroxide over all known

systems [6]. PB can be applied to various electrodes including graphite, glassy carbon, and gold electrode to avoid the disturbance from interference species [7–9]. However, the fabrication of sensors can require several steps and each step might lead to variation in the sensor performance. Although inkjet or screen printing is an attractive deposition technology for the fabrication of PB modified sensor, it still requires the film substrates and special instruments such as inkjet or screen printer. Dipping technique is the most convenient way of the processing of fabric, yarn, or string because it is simple and of low cost and has rapid fabrication process without needing special instrument.

In this work, we represent a new type of glucose sensor fabricated by flexible binding string utilizing simple dipping. A binding string was coated with PB modified graphite (PB-G) ink. And the surface of the PB-G string was immobilized by glucose oxidase (GOx) and a biocompatible

chitosan membrane. This fabrication method is fast, simple, robust, and of low cost to manufacture. We investigated the performance of our PB-G string electrodes including their sensitivity, selectivity, and reproducibility as a glucose sensor.

## 2. Materials and Methods

**2.1. Materials.** The black binding string composed of polyester spun was purchased from a local stationary store. PB-G ink (C2070424P2, 43% solid content with 48.7 ohms/square resistivity) was purchased from GWENT (Mamhilad, Pontypool, UK). Acetic acid ( $C_2H_4O_2$ ), ascorbic acid (AA,  $C_6H_8O_6$ ), acetaminophen (AAP,  $C_8H_9NO_2$ ), chitosan, D-(+)-glucose ( $C_6H_{12}O_6$ ), galactose ( $C_6H_{12}O_6$ ), glutaraldehyde ( $C_5H_8O_2$ ), GOx (180,200 units/g), human serum (4.89 mM, H4522) potassium ferricyanide ( $K_3Fe(CN)_6$ ), and uric acid (UA,  $C_5H_4N_4O_3$ ) were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Potassium chloride (KCl) was obtained from Junsei Chemical Co., Ltd. (Chuoku, Tokyo, Japan). Bovine serum albumin (BSA) was obtained from Bovogen (Melbourne, VIC, Australia). All aqueous solutions were prepared using distilled water with a resistivity of  $18.3\text{ M}\Omega\text{ cm}^{-1}$ .

**2.2. Preparation of PB-G String Electrode.** The string electrode was prepared utilizing a commercial black binding string. Firstly, bare string was washed with distilled water and dried at room temperature. And simple dipping was performed by completely immersing the string into PB-G ink five times for 5 seconds on each dipping. The string was cured in a convection oven (BF-80N, Biofree, Seoul, Korea) at  $100^\circ\text{C}$  for 20 min to remove some solvents. After curing, the conductive strings were air-dried, and the electrical resistance was measured. Resistance measurements were recorded by multimeter probes (Fluke-115, Fluke, WA, USA) at opposite extremities of a 1 cm PB-G string. To expose approximately 10 mm of the string as a sensing part (3 mm in diameter, 10 mm in length), the remainder of the string length was coated with blue insulating ink.

**2.3. Enzyme Immobilization on PB-G String Electrode for the Detection of Glucose.** The PB-G string electrode was dipping in  $40\text{ }\mu\text{L}$  GOx solution (40 mg/mL containing 10 mg/mL BSA stabilizer) and dried under ambient conditions. Subsequently, this surface was covered with  $60\text{ }\mu\text{L}$  of 1 wt% chitosan solution in 0.1 M acetic acid. Finally, the PB-G string electrode was crosslinked with  $20\text{ }\mu\text{L}$  of 2.5% glutaraldehyde. Following these steps, the PB-G string electrodes covered with GOx and chitosan membrane (Chi/GOx/PB-GS) were rinsed and immersed in a 0.1 M phosphate buffer solution (PBS, pH 7.4) for 20 min to remove monomeric residues from the electrode surface as well as any nonbound enzyme. After thorough washing, the Chi/GOx/PB-GS electrode was stored at  $4^\circ\text{C}$  before use.

**2.4. Characterization and Electrochemical Measurement.** The morphology of the PB-G string electrode was characterized using a field emission scanning electron microscope (FE-SEM; S-4800, Hitachi, Tokyo, Japan). An energy dispersive X-ray spectroscope (EDX; 7200-H, HORIBA, Northampton,

England) was used to examine the elemental composition of PB-G string.

All electrochemical experiments were carried out in a three-electrode system controlled by a Compactstat (Ivium Technology, Eindhoven, Netherland). An Ag/AgCl (sat. KCl) and a platinum wire were employed as reference and counter electrode, respectively. A 0.5 M KCl solution and 0.1 M PBS (pH 7.4) were utilized as supporting electrolytes in cyclic voltammetry for PB characterization and chronoamperometry for glucose detection, respectively.

## 3. Results and Discussion

We utilized dipping method because of its simplicity and simple integration with existing processing steps. Figure 1(a) shows the photograph and SEM image of bare binding string which was made up of a network of many fiber strands. While the surface of string after the first dipping possessed many cracks, it was fully covered with PB-G without any defects or cracks after the fifth dipping (Figure 1(b)). As shown in Figure 1(c), the resistances of PB-G coated strings decreased and reached a plateau as the dipping number increased. We investigate the effect of the dipping number on the electrochemical property of PB-G string electrode. Cyclic voltammetry was performed in a 0.5 M KCl solution at a scan rate of  $50\text{ mV s}^{-1}$  in the potential range from  $-0.2$  to  $+1.0\text{ V}$ . PB is electrochemically reduced to form Prussian white (PW) and the sharpness of PB/PW redox peaks in cyclic voltammograms can be used as an indicator of the quality of PB layers [6]. As shown in Figure 1(d), a pair of redox peaks can be seen which are due to the oxidation and reduction of PB. This data showed that peak current increased with the dipping number. Therefore, the PB-G coating on string was more densely packed after the fifth dipping, and the resistance as well as the amounts of PB was sufficient to act as an electrode for electrochemical sensing.

Typically, glucose biosensors utilize enzyme such as GOx. As GOx based amperometric detection of glucose commonly requires a relatively high potential ( $>+0.65\text{ V}$ ) to measure the hydrogen peroxide product, it is subject to be disturbed from various electroactive interferences. PB has been referred to as an “artificial peroxidase” [14]. It is electrochemically reduced to form Prussian white (PW), which is capable of catalyzing the reduction of hydrogen peroxide at low potentials (around 0 V versus Ag/AgCl). This characteristic allows it to work well in the presence of a wide range of interferences [15]. In addition, biopolymer chitosan is a kind of matrix for enzyme immobilization with attractive properties that include excellent film-forming ability, high permeability toward water, good adhesion, and biocompatibility [16]. Therefore, we constructed the Chi/GOx/PB-GS electrode for the detection of glucose, as shown in Figure 2(a). Figure 2(b) represents the mechanism of the catalytic  $H_2O_2$  reduction mediated by PB.

To investigate the effect of glucose concentration, we measured the current response of the Chi/GOx/PB-GS electrode to successive injection of glucose in 0.1 M PBS solution (pH 7.4) at an applied potential of  $-0.05\text{ V}$  versus Ag/AgCl. Figure 3(a) shows the amperometric response of the Chi/GOx/PB-GS electrode through successive addition of

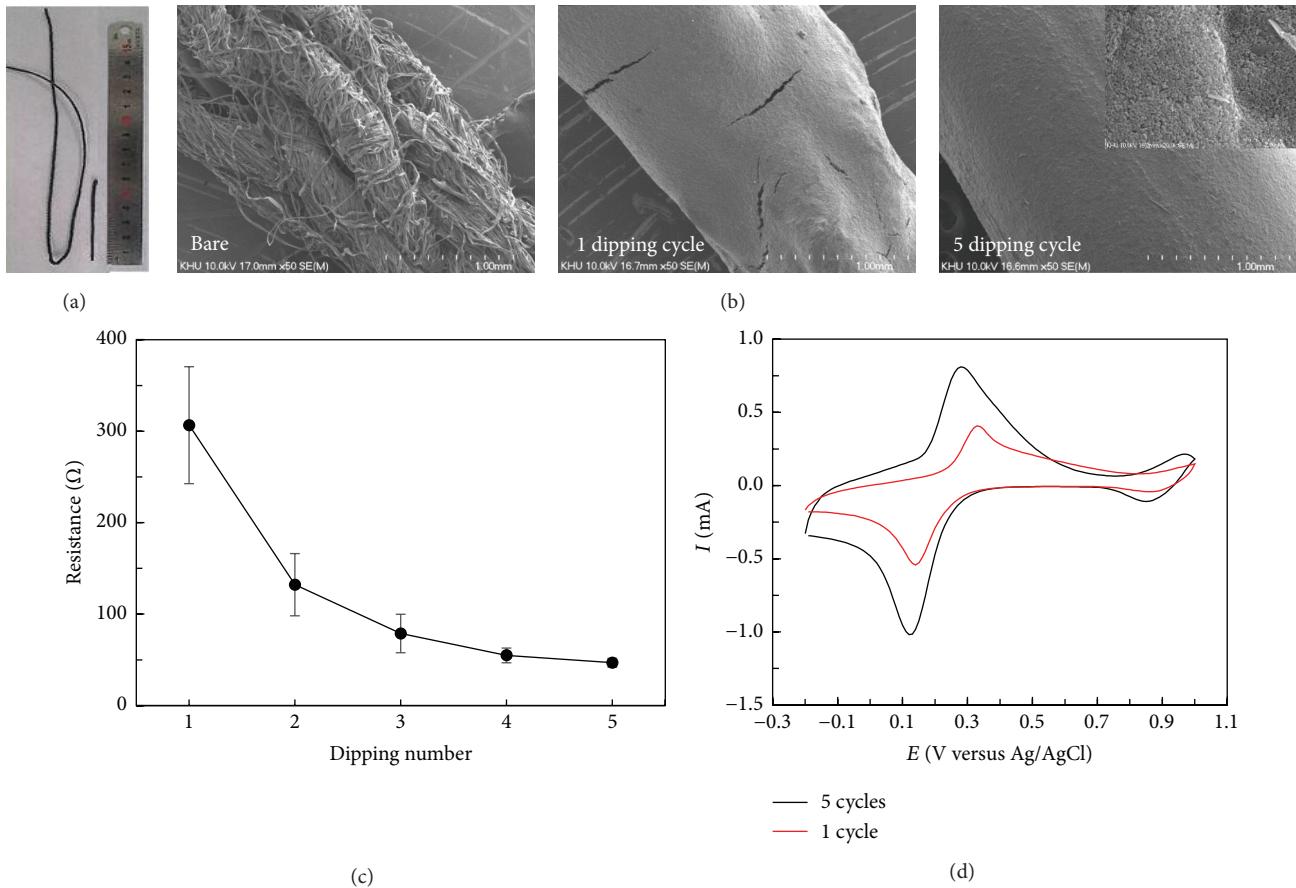


FIGURE 1: (a) Photographs of bare black binding string and (b) SEM images of bare string before and after one and five dipping cycles with the Prussian blue modified graphite (PB-G). (c) Change in electrical resistance of PB-G coated string according to number of dipping cycles. (d) Cyclic voltammograms of the PB-G coated string electrodes after one and five dipping cycles in 0.5 M KCl solution at a scan rate of 50 mV s<sup>-1</sup>.

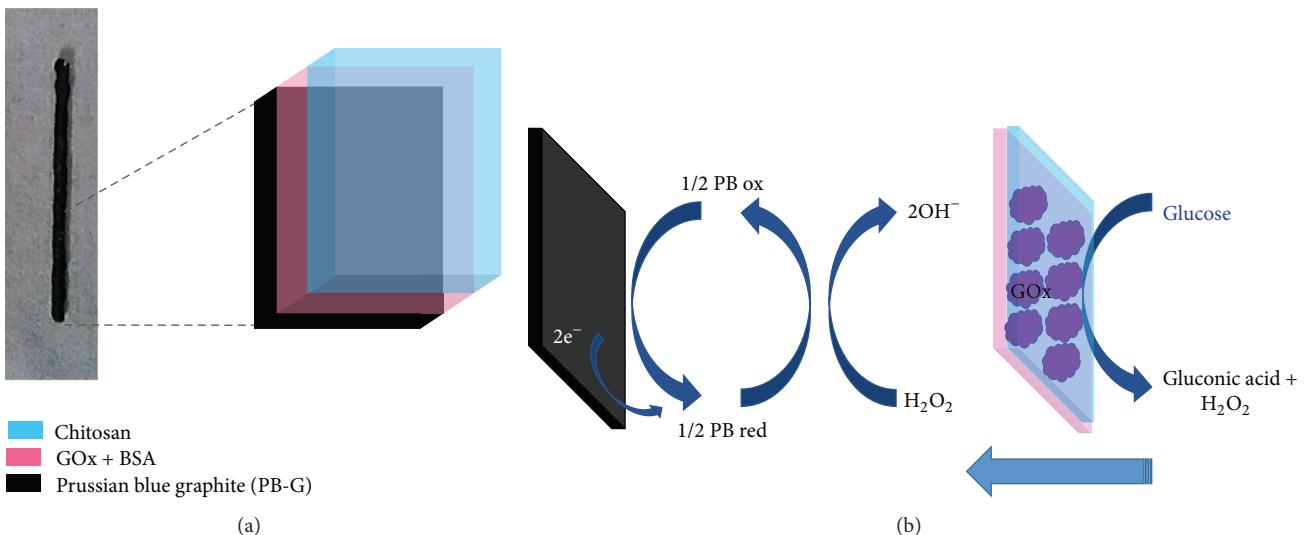


FIGURE 2: (a) Schematic illustration of the Prussian blue modified graphite string electrode coated with glucose oxidase and chitosan biopolymer for the detection of glucose (Chi/GOx/PB-GS). (b) The mechanisms of the catalytic hydrogen peroxide reduction mediated by PB in Chi/GOx/PB-GS electrode.

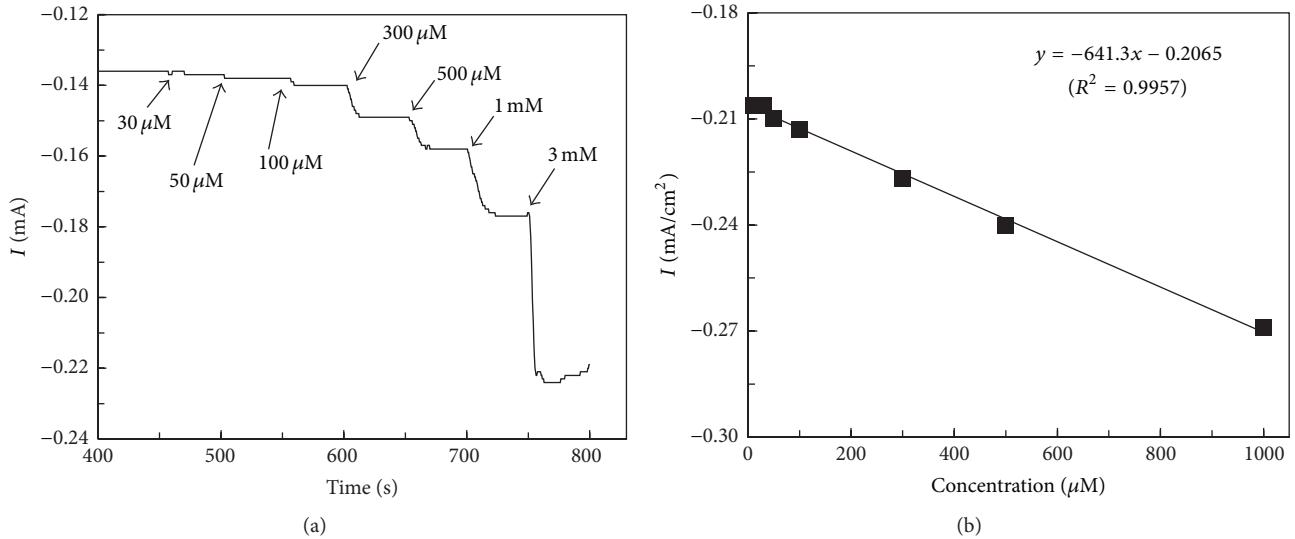


FIGURE 3: (a) Amperometric response of the string-based glucose biosensor toward successive addition of glucose from 30  $\mu\text{M}$  to 3 mM in 0.1 M PBS solution (pH 7.4) at -0.05 V. (b) The calibration curve (current versus concentration) of glucose.

TABLE 1: Comparison of the analytical performance characteristics of the string-based glucose biosensor with other Prussian blue modified electrode.

Electrode	PB modification method	Sensitivity ( $\mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$ )	Linear range (mM)	LOD ( $\mu\text{M}$ )	Response time (sec)	References
Chi/GOx/PB-G string electrode	Dipping	641.3	0.03~1.0	10	<3	This work
GOx/Chi-PB/Chi-Au electrode	Electrodeposition	—	0.002~0.4	0.4	<10	[10]
GO-GCE/PB/GOx/Chitosan	Electropolymerization	408.7	0.005~1.2	0.12	<5	[11]
GOx/PB-FCNF/GCE	Chemical process and casting	35.94	0.02~12	50	5	[12]
GOx/Ferri-COs-SPCEs	Drop-coating	0.677 ( $\mu\text{A}\cdot\text{mM}^{-1}$ )	~33.3	1380	<20	[13]

\*PB: Prussian blue; GOx: glucose oxidase; Chi: chitosan; GO: graphene oxide; GCE: glassy carbon electrode; PB-FCNF: Prussian blue nanostructures/carboxylic group-functionalized carbon nanofiber nanocomposites; Ferri-COs: hexacyanoferate (III) mixed with chitosan oligomers; SPCE: screen printed carbon electrode.

glucose. When adding glucose to the PBS solution, the Chi/GOx/PB-GS electrode quickly produced a steady state current within 3 s. The response of the Chi/GOx/PB-GS electrode was linear with respect to glucose concentration up to 1 mM ( $R^2 = 0.9957$ ), with a detection limit of 10  $\mu\text{M}$  ( $S/N = 3$ ) and a detection sensitivity of  $641.3 \mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$ , as shown in Figure 3(b). The performance of our Chi/GOx/PB-GS electrode is compared to that of other Prussian blue modified electrode for glucose detection in Table 1. Our electrode exhibits good performance in terms of sensitivity, response time, and linear range.

The selectivity of our Chi/GOx/PB-GS electrode was evaluated by the presence of physiological levels of the relevant electroactive constituents of blood including ascorbic acid, uric acid, galactose, and acetaminophen. The physiological level of glucose in normal serum is 3~8 mM and the levels of these interfering species are no more than 0.1 mM. As shown

in Figure 4(a), the interference currents by AA, UA, GA, and AAP were negligible, unlike the strong response by glucose. The current response by interference species was less than 3% compared to the response of glucose. The high selectivity of Chi/GOx/PB-GS electrode was attributed to the low operating potential by PB. Figure 4(b) represents the reusability of Chi/GOx/PB-GS electrode from the current response of 0.1 mM glucose in 0.1 M PBS solution (pH 7.4). The relative standard deviation (RSD) was 7.3% for ten repeated determinations of 0.1 mM glucose. The stability of Chi/GOx/PB-GS electrode was also investigated by measuring the current response to 0.03 mM glucose under 4°C storage conditions. As shown in Figure 4(c), there was no significant change in the current response of every test for five days. The reproducibility of the Chi/GOx/PB-GS electrode was also investigated by evaluating the amperometric response to 0.1 mM glucose in a 0.1 M PBS solution (pH 7.4) utilizing

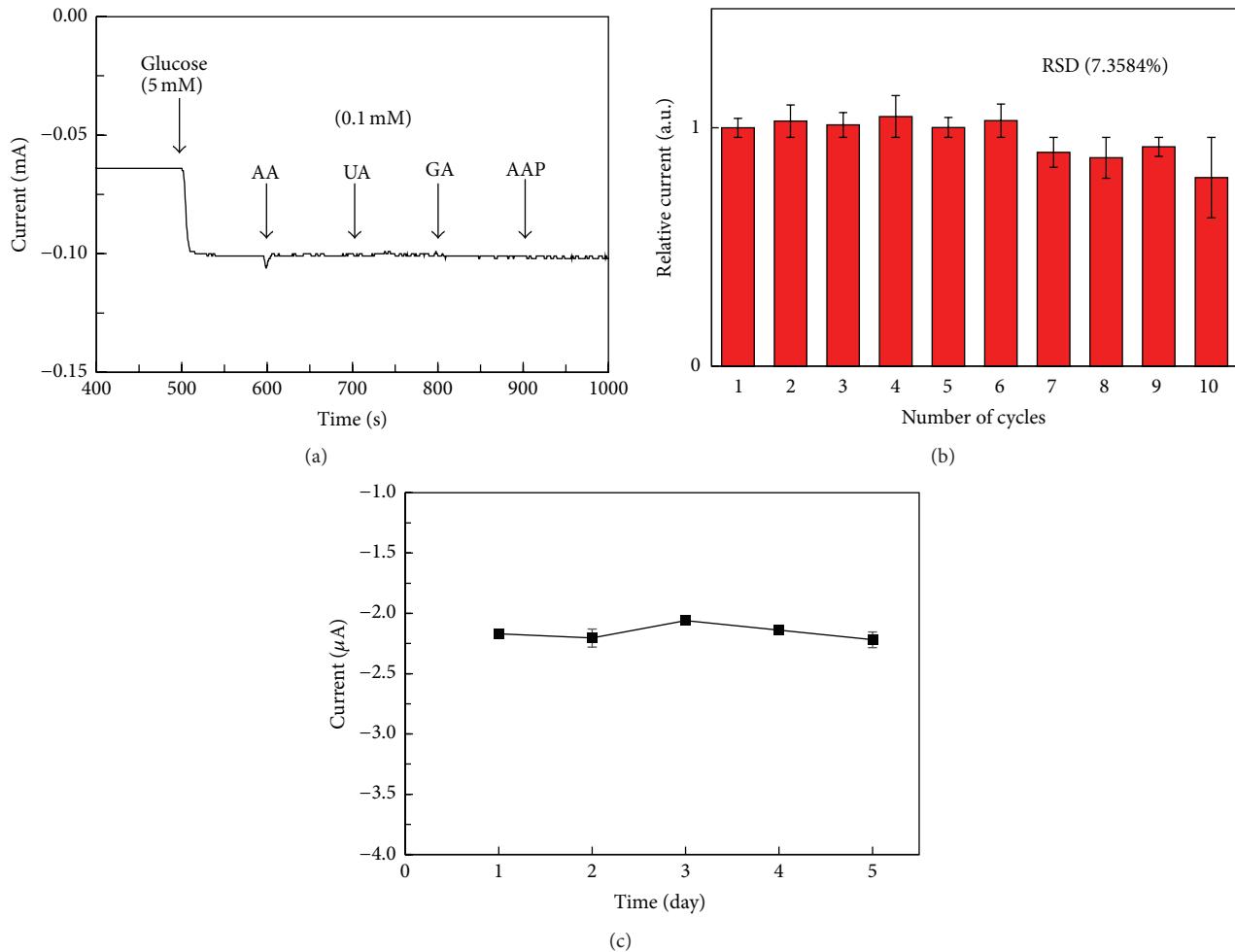


FIGURE 4: (a) Amperometric response of the string-based glucose biosensor after addition of 5 mM glucose and 0.1 mM of each interfering species including ascorbic acid (AA), uric acid (UA), galactose (GA), and acetaminophen (AAP) in 0.1 M PBS (pH 7.4). (b) Reusability test of the string-based glucose biosensor using a response to 0.1 mM glucose in 0.1 M PBS (pH 7.4) solution. (c) Stability test of the string-based glucose biosensor by measuring the current response to 0.03 mM glucose after 4°C storage for 5 days.

six electrodes on different fabrication dates. As a result, the Chi/GOx/PB-GS electrode was highly reproducible (RSD 4.5%).

To investigate the feasibility of the biosensor for practical applications, the sensor was applied to the detection of glucose in human serum. The recovery of glucose was determined by standard addition method with addition of pure glucose to the solutions containing the serum samples [17]. The concentration of glucose in human serum sample and the recovery results were summarized in Table 2. As shown in Table 2, the results showed that our string-based glucose sensor gave recoveries in the range of 89–106%. Therefore the Chi/GOx/PB-GS electrode could be suitable for real sample analysis.

#### 4. Conclusion

We have demonstrated a simple and facile glucose sensor based on PB modified graphite string. It can be fabricated

TABLE 2: Determination of glucose in human serum sample and the recovery test using glucose biosensor.

Samples	Concentration (mM)	Added glucose (mM)	Recovery (%)
1	4.89	0.3	106
		0.5	94
		1.0	95
2	4.89	0.3	103
		0.5	89
		1.0	98

through a fast and simple dipping process. The Chi/GOx/PB-GS electrode exhibited good sensitivity and selectivity, stable reproducibility, reusability, and a rapid response for glucose detection. This work successfully demonstrated the feasibility

of Chi/GOx/PB-GS electrode. Therefore, as-prepared Chi/GOx/PB-GS electrode keeps a good balance between high sensor performance and low cost and simple fabrication.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

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## References

- [1] S. Dong, J. Xi, Y. Wu et al., "High loading MnO<sub>2</sub> nanowires on graphene paper: facile electrochemical synthesis and use as flexible electrode for tracking hydrogen peroxide secretion in live cells," *Analytica Chimica Acta*, vol. 853, no. 1, pp. 200–206, 2015.
- [2] M.-C. Chuang, Y.-L. Yang, T.-F. Tseng, T. Chou, S.-L. Lou, and J. Wang, "Flexible thick-film glucose biosensor: influence of mechanical bending on the performance," *Talanta*, vol. 81, no. 1-2, pp. 15–19, 2010.
- [3] B. S. Shim, W. Chen, C. Doty, C. Xu, and N. A. Kotov, "Smart electronic yarns and wearable fabrics for human biomonitoring made by carbon nanotube coating with polyelectrolytes," *Nano Letters*, vol. 8, no. 12, pp. 4151–4157, 2008.
- [4] V. D. Neff, "Electrochemical oxidation and reduction of thin films of Prussian Blue," *Journal of The Electrochemical Society*, vol. 125, no. 6, pp. 886–887, 1978.
- [5] A. M. Farah, N. D. Shooto, F. T. Thema, J. S. Modise, and E. D. Dikio, "Fabrication of prussian blue/multi-walled carbon nanotubes modified glassy carbon electrode for electrochemical detection of hydrogen peroxide," *International Journal of Electrochemical Science*, vol. 7, no. 5, pp. 4302–4313, 2012.
- [6] A. A. Karyakin, "Prussian blue and its analogues: electrochemistry and analytical applications," *Electroanalysis*, vol. 13, no. 10, pp. 813–819, 2001.
- [7] B. Haghghi, H. Hamidi, and L. Gorton, "Electrochemical behavior and application of Prussian blue nanoparticle modified graphite electrode," *Sensors and Actuators B: Chemical*, vol. 147, no. 1, pp. 270–276, 2010.
- [8] S. Xing, H. Xu, G. Shi, J. Chen, L. Zeng, and L. Jin, "A simple and sensitive method for the amperometric detection of trace chromium(VI) based on Prussian blue modified glassy carbon electrode," *Electroanalysis*, vol. 21, no. 15, pp. 1678–1684, 2009.
- [9] N. B. Li, J. H. Park, K. Park, S. J. Kwon, H. Shin, and J. Kwak, "Characterization and electrocatalytic properties of Prussian blue electrochemically deposited on nano-Au/PAMAM dendrimer-modified gold electrode," *Biosensors and Bioelectronics*, vol. 23, no. 10, pp. 1519–1526, 2008.
- [10] X. Wang, H. Gu, F. Yin, and Y. Tu, "A glucose biosensor based Prussian blue/chitosan/hybrid film," *Biosensors and Bioelectronics*, vol. 24, no. 5, pp. 1527–1530, 2009.
- [11] Y. Zhang, X. Sun, L. Zhu, H. Shen, and N. Jia, "Electrochemical sensing based on graphene oxide/Prussian blue hybrid film modified electrode," *Electrochimica Acta*, vol. 56, no. 3, pp. 1239–1245, 2011.
- [12] L. Wang, Y. Ye, H. Zhu et al., "Controllable growth of Prussian blue nanostructures on carboxylic group-functionalized carbon nanofibers and its application for glucose biosensing," *Nanotechnology*, vol. 23, no. 45, Article ID 455502, 2012.
- [13] S.-H. Lee, H.-Y. Fang, and W.-C. Chen, "Amperometric glucose biosensor based on screen-printed carbon electrodes mediated with hexacyanoferrate-chitosan oligomers mixture," *Sensors and Actuators B: Chemical*, vol. 117, no. 1, pp. 236–243, 2006.
- [14] F. Ricci and G. Palleschi, "Sensor and biosensor preparation, optimisation and applications of Prussian Blue modified electrodes," *Biosensors and Bioelectronics*, vol. 21, no. 3, pp. 389–407, 2005.
- [15] S. Cinti, F. Arduini, D. Moscone, G. Palleschi, and A. J. Killard, "Development of a hydrogen peroxide sensor based on screen-printed electrodes modified with inkjet-printed prussian blue nanoparticles," *Sensors*, vol. 14, no. 8, pp. 14222–14234, 2014.
- [16] L. Han, S. Tricard, J. Fang, J. Zhao, and W. Shen, "Prussian blue @ platinum nanoparticles/graphite felt nanocomposite electrodes: application as hydrogen peroxide sensor," *Biosensors and Bioelectronics*, vol. 43, no. 1, pp. 120–124, 2013.
- [17] R. Ahmad, M. Vaseem, N. Tripathy, and Y.-B. Hahn, "Wide linear-range detecting nonenzymatic glucose biosensor based on CuO nanoparticles inkjet-printed on electrodes," *Analytical Chemistry*, vol. 85, no. 21, pp. 10448–10454, 2013.

