



Short communication

Direct electrochemistry of glucose oxidase and glucose biosensing on a hydroxyl fullerenes modified glassy carbon electrode



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ABSTRACT

Direct electrochemistry of glucose oxidase (GOD) was achieved when GOD-hydroxyl fullerenes (HFs) nano-complex was immobilized on a glassy carbon (GC) electrode and protected with a chitosan (Chit) membrane. The ultraviolet–visible absorption spectrometry (UV–vis), transmission electron microscopy (TEM), and circular dichroism spectropolarimeter (CD) methods were utilized for additional characterization of the GOD, GOD-HFs and Chit/GOD-HFs. Chit/HFs may preserve the secondary structure and catalytic properties of GOD. The cyclic voltammograms (CVs) of the modified GC electrode showed a pair of well-defined quasi-reversible redox peaks with the formal potential (E°) of 353 ± 2 mV versus Ag/AgCl at a scan rate of 0.05 V/s. The heterogeneous electron transfer constant (k_s) was calculated to be 2.7 ± 0.2 s⁻¹. The modified electrode response to glucose was linear in the concentrations ranging from 0.05 to 1.0 mM, with a detection limit of 5 ± 1 μ M. The apparent Michaelis–Menten constant (K_m^{app}) was 694 ± 8 μ M. Thus, the modified electrode could be applied as a third generation biosensor for glucose with high sensitivity, selectivity and low detection limit.

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1. Introduction

Direct electrochemistry between a redox protein and electrode provides a suitable model for understanding the electron transfer mechanisms in biological systems. It also helps to establish a platform for fabrication of electrochemical devices such as third generation biosensors (Armstrong and Wilson, 2000; Hill, 1996; Thevenot et al., 2001).

Glucose oxidase (GOD) is a structurally rigid glycoprotein with a molecular weight of about 150–180 kDa. It catalyzes the electron transfer from glucose to oxygen accompanying the production of gluconic acid and hydrogen peroxide (Hecht et al., 1993). Direct electrochemistry of GOD has been realized and studied when it

was immobilized on various types of modified electrodes, (Cai and Chen, 2004; Cui et al., 2011; Dai et al., 2009; Deng et al., 2008; Fu et al., 2009; Gu et al., 2011; Liu et al., 2007; Periasamy et al., 2011; Qiu et al., 2012; Tasviri et al., 2011; Wang et al., 2009; Yang et al., 2008; Zhai et al., 2011; Zhang et al., 2004; Zhang et al., 2011). However, these modifications may alter the conformation or structure of GOD (Calzolari et al., 2010; Shang et al., 2007), and result in poor selectivity and limited practical use of the modified electrodes.

Hydroxyl fullerenes (HFs) are water soluble fullerene derivatives with a formula of $C_{60}(OH)_n$. The number of –OH groups (n) is typically from 18 to 24. HFs are useful for the formation of gels, starburst polymers, and composites, and may be explored for biological activity (Chiang et al., 1996). HFs may link with a protein and form specific noncovalent complexes, and could be used to protect proteins (She et al., 1998).

In the present report, a novel GOD-HFs nano-complex was self-assembled and immobilized on a glassy carbon (GC) electrode. The electrode was then covered with a chitosan (Chit) membrane for protection and used to measure the direct electron transfer of

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GOD. Our results showed that the designed electrode can be used as a biosensor for detection of glucose with high sensitivity and selectivity.

2. Materials and methods

2.1. Materials

GOD (EC 1.1.3.4, from *aspergillus niger*), horseradish peroxidase (HRP, EC 1.11.1.7), guaiacol, Chit (0.5% in water), α -D-glucose, ascorbic acid (AA), uric acid (UA) and dopamine (DA) were from Sigma (Saint Louis, MO, USA). HF_s was purchased from Bucky (Houston, USA). All solutions were prepared in double-distilled deionized water. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of Chit/GOD-HFs modified GC electrode

The procedure for the preparation of the GC electrode was as previously described (Hong et al., 2013). Prior to coating, the GC electrode was mechanically polished twice with alumina. The electrode was then treated electrochemically in 0.2 M sulfuric acid. Thereafter, the working electrode was placed in a 50 mM PBS (pH 7.0), and an anodic potential of 1.70 V (versus Ag/AgCl) was applied for 3–5 min. After the electrode was washed, 3 μ l of the mixture of HF_s (2 mg/ml) and GOD (10 mg/ml) (ratio of volume: 1) was dropped onto the surface of the electrode, and dried for 24 h at 4 °C, and for protection, 2 μ l 0.5% Chit solution was dropped on the electrode surface, dried and stored at 4 °C.

2.3. Apparatus and measurements

All electrochemical experiments were carried out on a CHI650C (CHI Instrument, Austin, USA). An Ag/AgCl-saturated KCl, a Pt wire, and a GC electrode of 3 mm diameter (CHI Instrument, USA) were used as reference, counter, and working electrodes, respectively. The electrochemical measurements were carried out in N₂-saturated 50 mM sodium phosphate buffer solution (PBS, pH 7.0) at 25 ± 1 °C. The electro catalytic measurements were carried out after air bubbling (30 min, 300 ml/min).

After being mixed and incubated for half an hour, the far-UV spectra of GOD, GOD-HFs or Chit/GOD-HFs were performed on an Aviv Model 420SF circular dichroism spectropolarimeter (CD) (Lakewood, NJ, USA) at 25 °C, respectively, using rectangular quartz cells with a path length of 1.0 mm for far-UV (Farivar et al., 2010; Hong et al., 2012).

The catalytic rates of GOD, GOD-HFs and Chit/GOD-HFs were obtained, respectively, on a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Company, China), using a 1 cm path-length cell equipped with a thermostat holder and an external temperature controller (Shanghai Hengping Instrument Company, China) at 25 ± 0.1 °C, based on two step reactions: Step 1, GOD catalyzes glucose to produce H₂O₂ in the presence of O₂. Step 2, the guaiacol is oxidized by H₂O₂ produced in Step 1 in the presence of HRP and the reaction rate was monitored at 470 nm. The colored product from the Step 2 is tetraguaiacol with an extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ (at 470 nm), and the initial rate of guaiacol oxidation was measured by the rate of tetraguaiacol formation (Koduri and Tien, 1995).

Electron macrograph images of Chit/GOD-HFs sample were obtained using a TEM (JEM-1400, JEOL, Japan) operating at 80 kV.

3. Results and discussion

3.1. Characteristics of GOD, GOD-HFs and Chit/GOD-HFs

Fig. 1A and B shows the possible structure of GOD-HFs and TEM image of Chit/GOD-HFs, respectively. The TEM image (Fig. 1B) shows that GOD-HFs nano-particles may be formed in Chit membrane, with an average size of 20 nm. Fig. 1C is a schematic representation of the Chit/GOD-HFs modified GC electrode.

Fig. S1A shows the Far-UV-CD spectra of GOD, GOD-HFs and Chit/GOD-HFs in 50 mM PBS at pH 7.0. Fig. S1A inset table shows the detailed percentages of several secondary components of the GOD, GOD-HFs and Chit/GOD-HFs, which were obtained using Aviv software (CD spectra deconvolution). The percentage of helix decreased from 20% in GOD to 15.2% in GOD-HFs. However, after being modified with Chit, the percentage of GOD helix in Chit/GOD-HFs increased to 20.8%. Thus, the Chit membrane may help to preserve the GOD helix structure. The percentage of anti-parallel structure increased from 35.6% in GOD to 40.8% in GOD-HFs, and supports the possible structure schemed in Fig. 1A.

The catalytic rates of GOD, GOD-HFs, and Chit/GOD-HFs were compared through the initial reaction rate of guaiacol oxidation (Fig. S1B). The native GOD had the fastest initial reaction rate, while the GOD-HFs had the slowest reaction rate. This was mainly attributed to the conformational changes in GOD when combined with HF_s. However, the initial reaction rate was improved for Chit/GOD-HFs due to excellent biocompatibility of Chit membrane. The specific noncovalent linking (hydrogen bond, van der Waals or hydrophobicity interactions) between GOD and HF_s in Chit membrane can occur (She et al., 1998). This may help to preserve the structure and function of GOD, and lead to higher sensitivity and selectivity of GOD-HFs modified GC electrode for glucose.

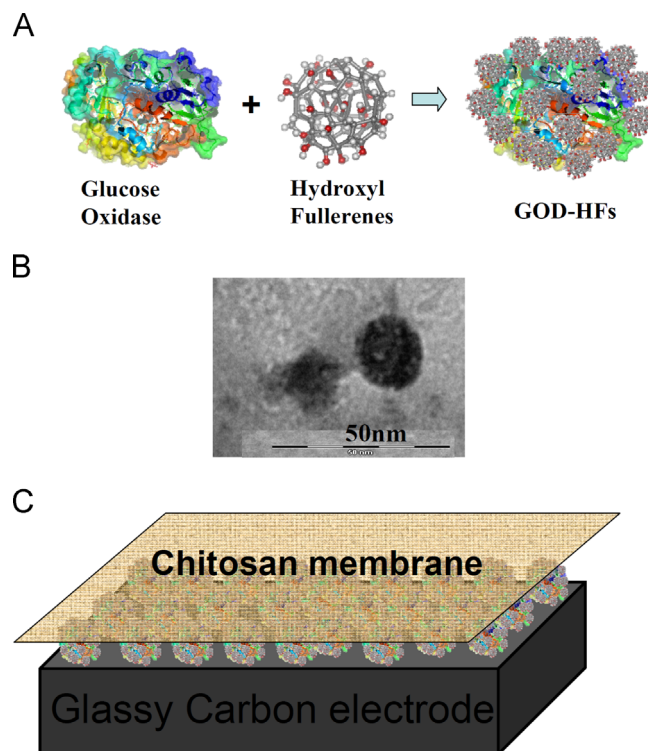


Fig. 1. (A) Possible structure of GOD-HFs, (B) transmission electron microscopy (TEM) image of Chit/GOD-HFs and (C) schematic structure of the Chit/GOD-HFs modified glassy carbon (GC) electrode.

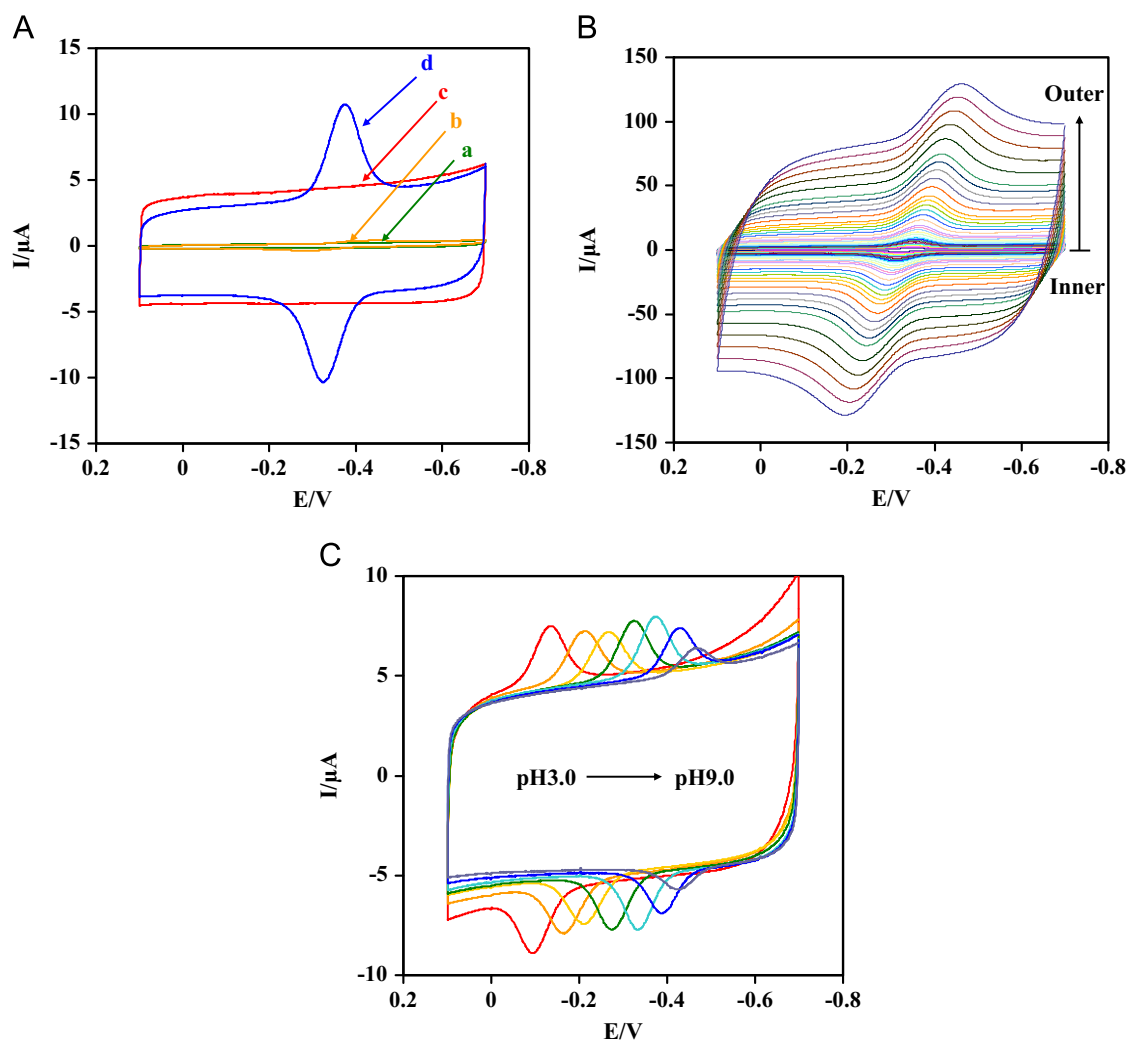


Fig. 2. (A) The cyclic voltammograms (CVs) of different modified electrodes: (a) bare GC, (b) Chit/GOD/GC; (c) Chit/HFs/GC; (d) Chit/GOD-HFs/GC. The experiments were carried out in 50 mM PBS (pH 7.0) at a scan rate of 0.05 V/s. (B) CVs of the Chit/GOD-HFs/GC electrode in 50 mM PBS (pH 7.0) at different scan rates (from inner to outer): 0.02, 0.03, 0.04, 0.05, ...2.0 V/s, respectively. (C) CVs of Chit/GOD-HFs/GC electrode in 50 mM PBS at different pH values (from left to right): 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively, at a scan rate of 0.05 V/s.

3.2. Electrochemical investigations

Fig. 2A shows the cyclic voltammograms (CVs) of different modified electrodes. No redox peak was observed at the bare GC electrode (curve a), Chit/GOD/GC electrode (curve b) and Chit/HFs/GC electrode (curve c). However, a pair of well-defined redox peaks was observed at the Chit/GOD-HFs modified GC electrode. The formal potential (E°) of the electrode was determined to be -353 ± 2 mV versus Ag/AgCl. This value was greater than the most reported results, as shown in Table S1. Furthermore, the positive potential shift for the electrode facilitated the electrode reaction and led to a more efficient bio-catalytic reduction (Hong et al., 2007).

The CVs of Chit/GOD-HFs modified GC electrode in 50 mM PBS (pH 7.0) at various scan rates are shown in Fig. 2B. Peak current was increased linearly with increasing scan rate (ν) in the range of 0.02–0.5 V/s (Fig. S2A). The CVs remained essentially unchanged on consecutive potential cycle indicating that the Chit/GOD-HFs was stably confined on the GC electrode (Hong et al., 2007). The cathodic peak potential (E_{pc}) in the range of 0.25–0.6 V/s changed linearly versus $\ln \nu$ (Fig. S2B). The n and α could be evaluated to be 2 and 0.47 ± 0.2 , respectively (Laviron, 1974; Ma et al., 2000). The value of apparent heterogeneous electron transfer rate constant (k_s) was calculated to be 2.7 ± 0.2 s $^{-1}$ (Laviron, 1979).

The average surface concentration (Γ) of electro active sites (FAD groups) of GOD on the surface of GC electrode was estimated to be $(8.3 \pm 0.4) \times 10^{-11}$ mol cm $^{-2}$, based on the slope of peak current I_p versus scan rate ν (Fig. S2B) (Hong et al., 2013), this value is smaller than the theoretical Γ value of 1.7×10^{-10} mol cm $^{-2}$ based on PDB data of GOD (PDB ID: 1cf3, about 10 nm diameter), and larger than the Γ value 1.0×10^{-11} mol cm $^{-2}$ based on the TEM image (about 20 nm diameter of GOD-HFs) (Fig. 1B). For a globular protein, the theoretical Γ value can be easily evaluated based on the following equation:

$$\Gamma = \frac{10^{14}}{N_A D^2} (\text{mol cm}^{-2}) \quad (1)$$

where, N_A and D are Avogadro constant and diameter value (nm) of a globular protein.

Fig. 2C shows the CVs of the modified electrode in 50 mM PBS at various pH values. The formal potential (E°) of the electrode was pH dependent with a slope of 55.1 ± 0.4 mV/pH (Fig. S2C). This value was close to the Nernst's value of 59.2 mV (Hong et al., 2013).

3.3. Electro catalytic investigations

Fig. S3A shows the linear sweep voltammograms (LSVs) of Chit/GOD-HF modified GC electrode. The optimized potential for

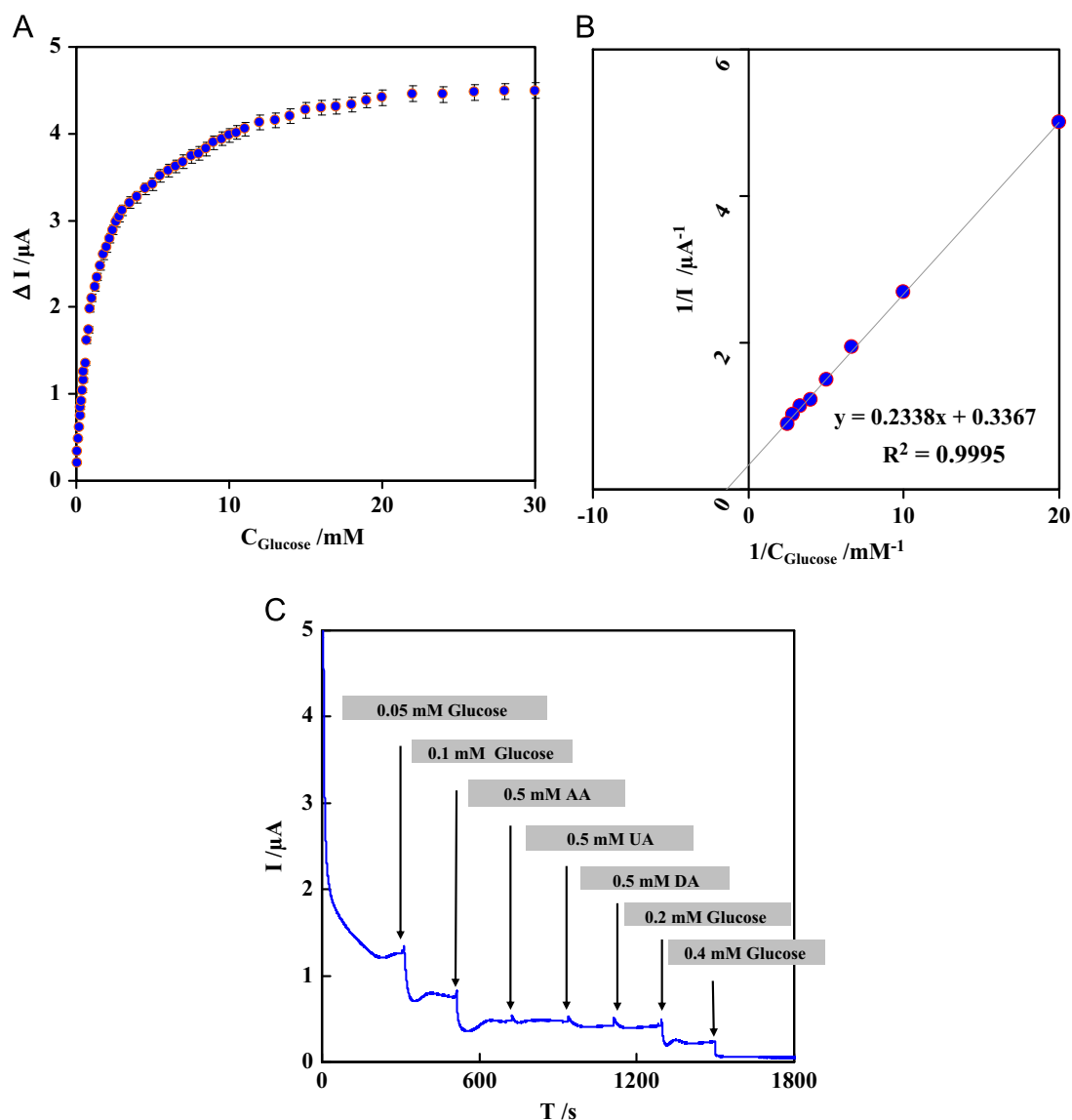


Fig. 3. (A) Plot of response current (ΔI) versus concentration of glucose ($C_{Glucose}$), (B) Lineweaver-Burk plot for K_m^{app} determination, and (C) Amperometric responses of Chit/GOD-HFs/GC electrode upon addition of glucose (0.05 or 0.1 mM), AA(0.5 mM), UA(0.5 mM), DA(0.5 mM), glucose(0.2 or 0.4 mM). The experiment was carried out in 50 mM PBS (pH 7.0) at a potential of -0.35 V versus Ag/AgCl.

amperometric detection of glucose was -0.35 V versus Ag/AgCl. The electrocatalytic process of Chit/GOD-HFs/GC electrode was expressed by the literature (Liu et al., 2007).

The modified electrode responded to glucose at concentrations ranging from 0.05 to 30 mM (Fig. 3A) (linear range: 0.05–1.0 mM in Fig. S3B and 3–10 mM in Fig. S3C), with a detection limit of $5 \pm 1 \mu M$. The apparent Michaelis-Menten constant (K_m^{app}) was calculated to be $694 \pm 8 \mu M$ from the electrochemical version of the Lineweaver-Burk (Fig. 3B) (Hong et al., 2007).

K_m^{app} value was lower than that of most recent modified electrodes (Table S1). A low K_m^{app} value indicates a strong substrate binding and demonstrates a higher affinity of glucose for the modified electrode. HF's may help to reduce the bridge length between electro active center (FAD group) of GOD and the electrode (Hong et al., 2007).

Anti-interference ability of the modified electrode was investigated by introducing electro active species such as AA, UA and DA. The influence of AA (0.5 mM), UA (0.5 mM) and DA (0.5 mM) on the detection of glucose at the modified electrode is shown in Fig. 3C when these species were consecutively added into a

continuously stirred 50 mM PBS at the applied potential of -0.35 V versus Ag/AgCl. These electro active species could cause negligible effect on the glucose detection.

Though, Chit/GOD-HFs modified GC electrode has excellent properties of the glucose in serum samples, it is strongly suggested that the detection is completed after dilution of a real blood sample (say 50 times):

- I. The modification could maintain the conformational structure of GOD, and the modified GOD had the similar catalytic properties as those of the native GOD. As an enzyme, GOD could work perfectly with good sensitivity and selectivity for glucose.
- II. In a real sample, concentrations of carbonic acid, bicarbonate, hemoglobin, plasma proteins, and phosphate are high. Some of these will cover the surface of electrode, block channel of Chit membrane, and seriously affect detection results. However, dilution will significantly reduce the mentioned adverse effects and detection errors, improve accuracy, and extend the service life of a biosensor.

III. At low concentrations (0.05–1.0 mM) or diluted real samples, the detection results are similar to those gained with hospital's blood analyzer. However, at high concentration or real samples without dilution, not only the results will vary with increasing concentration and operating time, but also the deviations will become worse.

4. Conclusions

A Chit/HFs-GOD was immobilized on a GC electrode and was used as a third generation biosensor for determination of glucose with good sensitivity and selectivity. Chit/HFs could preserve the conformational structure of GOD and leads to high sensitivity and selectivity of the Biosensors for glucose. Dilution of blood samples could be helpful in improving accuracy and reducing detection errors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2014.04.005>.

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