Biosensors and Bioelectronics 129 (2019) 216–223

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Smartphone-based differential pulse amperometry system for real-time monitoring of levodopa with carbon nanotubes and gold nanoparticles modified screen-printing electrodes

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ARTICLE INFO

*Keywords:*
Smartphone
Differential pulse amperometry
Nanocomposites
Screen-printed electrode
Levodopa
Parkinson's disease

ABSTRACT

Parkinson's disease caused by lack of dopamine in brain is a common neurodegenerative disorder. The traditional treatment is to replenish levodopa since it could pass through blood brain barrier and form dopamine. However, its accumulation can cause patients' movement disorders and uncontrollable emotion. Therefore, it is critical to control the levodopa dosage accuracy to improve the curative effect in clinical. In this study, a smartphone-based electrochemical detection system was developed for rapid monitoring of levodopa. The system involved a disposable sensor, a hand-held electrochemical detector, and a smartphone with designed application. Single-wall carbon nanotubes and gold nanoparticles modified screen-printed electrodes were used to convert and amplify the electrochemical current signals upon presence of levodopa molecules. The electrochemical detectors were used to generate electrochemical excitation signals and detect the resultant currents. Smartphone was connected to the detector, which was used to control the detector, calculate data, and plot graph in real-time. The smartphone-based differential pulse amperometry system was demonstrated to monitor levodopa at concentrations as low as 0.5 μM in human serum. Furthermore, it has also been verified to be able to distinguish levodopa from other representative substances in the body. Therefore, its performance was more sensitive and rapid than electrochemical workstation. With these advantages, the system can be used in the field of point-of-care testing (POCT) to detect levodopa and provide the possibility to solve clinical demand for levodopa detection.

## 1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease in the central nervous system, and commonly happened to elder people (Jackowska and Krysinski, 2013; Molaakbari et al., 2014). Its clinical manifestations include static tremor, postural difficulties, rigidity, etc. (Sampson et al., 2016; da Silva et al., 2018). Therefore, it could deeply affect the patient's life quality. Its pathological feature is dopaminergic neurons degeneration in the substantia nigra, reducing the dopamine content with the substantia nigra-striatal pathway (Mosharov et al., 2015; Ferapontova, 2017). Previously most researchers thought that brain secretes dopamine continuously during movement (Lotharius and Brundin, 2002; Ferapontova, 2017). Along with the damage of dopaminergic neurons, dyskinesias usually occurred in Parkinson's disease patients. Therefore, the standard regimen for Parkinson's disease is to replenish levodopa in order to maintain a steady concentration of dopamine in patient's brain (Fahn, 2008; Jackowska and Krysinski, 2013). However, some researchers found that although dopamine was essential for movement, it is just a trigger (Liu et al., 2018). Specifically, dopamine will be excreted in large quantities before movement to promote its happening. Once a person moved, the motion could be maintained even without the presence of dopamine. At the same time,

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https://doi.org/10.1016/j.bios.2018.09.082
Received 1 July 2018; Received in revised form 18 September 2018; Accepted 22 September 2018
Available online 24 September 2018
0956-5663/ 

other researchers discovered that dopaminergic neurons related to movement secreted dopamine with highly temporal and spatial accuracy (da Silva et al., 2018). Obviously, it was reported that the release of dopamine was not sustained, slow or large-scale as we thought, but large quantity of dopamine was rapidly and accurately transmitted to the downstream neurons in incredible and remarkable short period of time. These studies also explained the reason why current Parkinson's disease standard regimen was accompanied with a large number of side effects, probably due to the fact that the traditional therapies replenished excessive dopamine or levodopa for patients, resulting in the activation of wrong neurons (Vasta et al., 2017; Kühn et al., 2017). Therefore, rapid, convenient and real-time levodopa concentration detection in vivo could not only reduce side effects caused by over-replenishment of levodopa, but also provide a reference for doctor's diagnosis, improving the life quality of Parkinson's disease patients.

In recent decades, various methods have been used for levodopa detection, such as chemiluminescence, high-performance liquid chromatography, electrochemistry, and spectroscopy (He et al., 2006; Ajmal et al., 2017; Yue et al., 2017; Belal et al., 2018). Among them, electrochemistry has received widespread attention due to its high stability, simple operation, fast testing speed, simultaneous detection of redox voltage and other advantages (Mazloum-Ardakani et al., 2012; Zhang et al., 2016a; Beitollahi et al., 2016). Electrochemistry could be employed to detect electroactive materials rapidly and conveniently, which were important for health and environment, such as urine acid, thyroid stimulating hormone, hydrazine, and tryptophan (Mazloum-Ardakani et al., 2011; Ji et al., 2018; Beitollahi et al., 2018). Hence, electrochemical method plays an important role in determination of biological and environmental analysis (Beitollahi et al., 2008; Wang et al., 2015, 2017a). In order to improve the sensitivity and detection range of levodopa, conductive polymers, carbon nanomaterials and metal nanomaterials have been used to modify electrodes for improved conductivity and electrocatalytic properties (Kamyabi and Rahmanian, 2015; Babaei and Sohrabi, 2016; Özcan and Topçuoğulları, 2017). For example, nanomaterials modified electrode is an effective method. With excellent electrical, optical, and mechanical properties, carbon nanotube has become the focus of research (Lu et al., 2015; Kato et al., 2017; Qian et al., 2018). With the advantages of large length-to-diameter aspect ratios, fast electron-transfer kinetics, unique tubular structure, and wide electrochemical stability window, the single-wall carbon nanotubes could be used to build nanosensors and to improve the performance of electrode (Chen et al., 2016; Li et al., 2018; Qian et al., 2018). On the other hand, gold nanoparticles have also been widely applied in biosensing due to its good biocompatibility, electrical conductivity, and electrocatalytic properties (Su et al., 2017; Li et al., 2017; Wang et al., 2017b). To heighten the performance of electrodes, gold nanoparticles were combined with the single-wall carbon nanotubes to form nanocomposites (Huang et al., 2010; Wang et al., 2012; Zhang et al., 2016b). The nanocomposites were modified on the electrode to reveal synergistic effect, which generate better performance and higher response than gold nanoparticles or single-wall carbon nanotubes alone (Fu et al., 2015; Song et al., 2016; Afkhami et al., 2016). Hence, immobilizing these two materials on screen-printed electrode could not only improve the sensitivity and detection range of levodopa, but also achieving fast and portable detection with disposable sensors.

Smartphone has become the most popular mobile devices in world (Zhang and Liu, 2016). According to the report of Global System for Mobile Communications Association, smartphone users would reached 5.9 billion by 2025, which is equivalent to 71% of the total global population (GSMA). Smartphone's high-speed computing capabilities could be comparable with that of personal computer or laptop (Guo, 2016; Zhang and Liu, 2016). Moreover, it has smaller volume, wireless communication, and user-friendly interface to enable easy operation for all ages (Wang et al., 2015; Guo, 2017). The Mobile Health (mHealth) is defined as “medical and public health practice supported by mobile devices, such as mobile phones……and other wireless devices.” In recent years, researchers are beginning to focus on the development of mHealth devices to support health services (Martínez-Pérez et al., 2013). Patients could use the related mHealth applications on smartphone to self-monitor their condition, such as hypertension and diabetes (Guo and Ma, 2017; Ji et al., 2017). They can also receive remote health services since their physiological data can be transferred by online mHealth application to doctors in clinical houses or hospitals (Aronoff-Spencer et al., 2016; Xu et al., 2018). Multiple physical sensors on smartphone have been used to do gait analysis and tremor detection for Parkinson's disease, such as acceleration sensors and gyro sensors (Lakshminarayana et al., 2017; de Lima et al., 2017; Schneider and Biglan, 2017),but there has not been sensor for levodopa concentration detection so far. Many researchers combined smartphone and electrochemistry to detect biochemical substances, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) (Zhang et al., 2015; Ji et al., 2017, 2018; Wang et al., 2017a). Differential pulse amperometry was a preeminent electrochemical method, which could be used for real-time monitoring of biochemical substances and drug, such as phenylketonuria and diazepam (Antunović et al., 2018; Moreira et al., 2018). Two potential pulses were applied on the sensors and the subsequent current at the end of each pulse is recorded in differential pulse amperometry. The difference between the two current samples is displayed as a function of time, indicating that differential pulse amperometry could be used to reflect the concentration in real-time. At the same time, the influence of the background current was brought down. Compared with classical amperometric detections, differential pulse amperometry present a forceful analytical signal and it is faintly affected by interferences. With the advantages of smartphone, differential pulse amperometry could be used for continuous monitoring of biochemical substance. The concentration of biochemical substance could be detected in time. Hence, differential pulse amperometry could combine well with smartphone-based system for real-time monitoring of substance in the point-of-care testing.

In this paper, a smartphone-based differential pulse amperometry system was structured and employed to measure the current changes corresponding to levodopa concentration changes on the sensors. Gold nanoparticles, single-wall carbon nanotubes, and chitosan were used to modify the screen-printed electrodes and used as sensors, which showed high sensitivity to levodopa. A hand-held device was used to perform differential pulse amperometry and deliver the electrical signal data. The smartphone with a designed Android application (App) was employed as the core of the system to give command, perform calculation, and real-time data display. The smartphone-based system successfully detected levodopa as low as 0.5 μM in human serum and distinguished levodopa from other representative substances, which usually exist in human body.

## 2. Materials and methods

### 2.1. Design of the detection circuit and App

The detector circuit realized the majority functions of signal measurement. As shown in Fig. 1a, the microcontroller unit (MCU, C8051f005, Silicon Laboratories, USA) controlled the digital analog converter (DAC, DAC8552, Texas Instruments, USA) to generate specific analog signals, which was applied to the electrodes. The analog digital converter (ADC) on the MCU converted the measured voltage signals to digital signals. The transimpedance amplifier (TIA) (OPA2349, Texas Instruments, USA) was utilized to form the potentiostat module to measure the current precisely. The Bluetooth component (HC-06, Guangzhou Huicheng Information Technology Co., Ltd., China) accomplished communication between the detector and smartphone. The App on smartphones served as the interface between

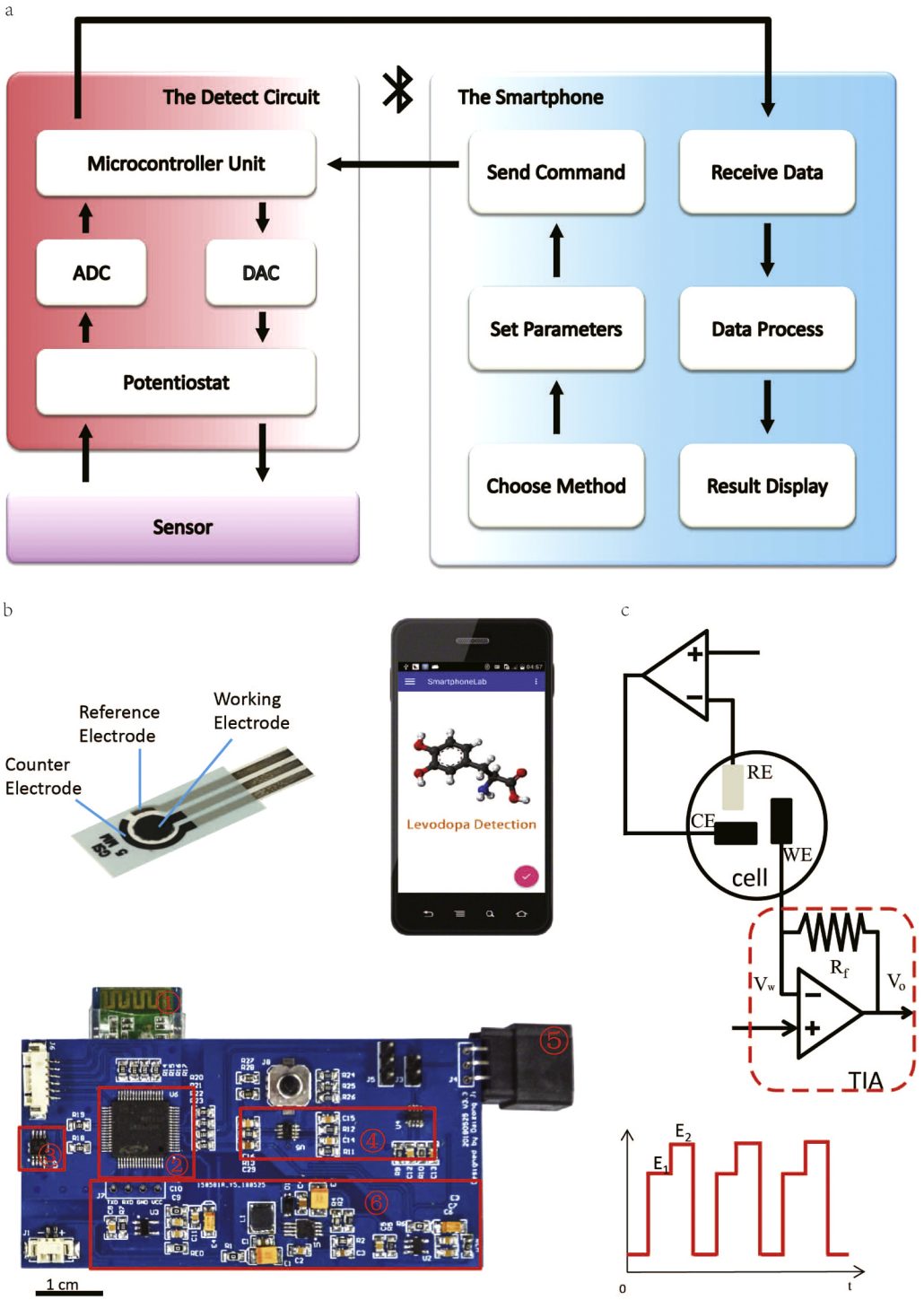


**Fig. 1.** (a) The schematic diagram of the smartphone-based differential pulse amperometry system. (b) The image of system ① Bluetooth module ② microcontroller unit③digital analog converter ④ potentiostat module ⑤ electrode socket ⑥ power management module (c) The schematic diagram of potentiostat and the wave form of differential pulse amperometry.

smartphone and circuit, giving commands to the circuit and receiving digital signal from the circuit. In addition, it processed the received data and plotted the result.

### 2.2. Smartphone-based differential pulse amperometry system

The system was composed of detection circuit, modified screen-printed electrodes and App on the smartphone. After connecting to the App via Bluetooth, the detector circuit began to work. Based on the pulse voltage $E_1$, pulse voltage $E_2$, and duration time, the circuit would general electrical to the sensor. Then, sensor converted the chemical reaction signals into measurable electrical signals. Then the circuit could convert the analog signals to digital signals and then transmit the data to smartphone. The data could be converted to current value through the APP. Ultimately, the current curve could be displayed on the smartphone in real-time.

### 2.3. Modification of screen-printed electrodes with gold nanoparticles / single-wall carbon nanotubes /chitosan

The solution for modification included gold nanoparticles (AuNPs), single-wall carbon nanotubes (SWNTs), and chitosan. Gold nanoparticles were prepared as follows. First, 10 mL of chloroauric acid ($HAuCl_4$, 0.01%) was heated to 80 °C with continuous stirring. Second, 185 μL trisodium citrate ($Na_3C_6H_5O_7$, 1%) was added quickly for the reduction of chloroauric acid at 80 °C. The solution was kept at 80 °C for 60 min with the stir and then cooled down to room temperature with the stir for 15 min. Third, the obtained gold nanoparticles solution was stored at 4 °C in an opaque glass container (Fig. S1, in the supplements materials). Then, single-wall carbon nanotubes were purchased from NanJing Ji Cang Nano Technology Co., Ltd, China. The chitosan solution (0.5%) was obtained via dissolving 50 mg chitosan in 10 mL acetic acid (1%). After the above preparations, the gold nanoparticles /single-wall carbon nanotubes /chitosan solution was obtained by dissolving the centrifugate of 1 mL gold nanoparticles solution and 2 mg single-wall carbon nanotubes in 1 mL chitosan solution with ultrasonication for 2 h.

The screen-printed electrodes used in this study (GSI Technologies, USA) consisted of working electrode (carbon), counter electrode (carbon) and reference electrode (silver /silver chloride). Prior to material modification, the screen-printed electrodes were washed with mixture of deionized water and alcohol. Then, screen-printed electrodes were activated with sodium sulfate solution (NaOH, 0.1 M) using cyclic voltammetry. The scan rate was 0.05 V/s and the voltage range was from −1 to 1 V (Fig. S2, in the supplements materials). After these steps, 6.0 μL gold nanoparticles /single-wall carbon nanotubes /chitosan solution was dropped evenly onto the working electrode and the electrode was air-dried for 30 min. The aqueous solution of ferricyanide/ferrocyanide (1 mL, 10 mM, 1:1) was used as the redox couple. Scanning electron microscopy (SEM) images of bar electrode and modified electrode were taken on HITACHI UHR FE-SEM SU 8010, at an accelerating voltage of 3000 V to observe the morphology of the material-modified SPE.

### 2.4. Electrochemical measurements of levodopa

Levodopa solutions of 0.5, 1, 2.5, 5, 10, 25, 50, 75, 100, 150, and 200 μM were obtained by dissolving levodopa into artificial serum (Huzhou Inno Reagents Co., Ltd., Zhejiang, China). Then chronoamperometry, differential pulse voltammetry and differential pulse amperometry were performed by electrochemical workstation (CHI660e, Shanghai Chenhua Instrument Co., Ltd, Shanghai, China), with artificial serum as blank. Moreover, differential pulse amperometry was performed by the smartphone-based detection system. The stable current of determinant was normalized with blank and calculated into normalized current (NCC).

Calcium chloride, sodium chloride, glucose, ascorbic acid, serotonin, glutamate, cysteine, bovine serum albumin, uric acid, and levodopa were dissolved in artificial serum respectively to obtain 1 mM solutions. Differential pulse amperometry with same parameters was performed with the smartphone-based system and the electrochemical workstation to verify the specificity. All chemicals and reagents employed were of analytical grade and purchased from Sigma-Aldrich Co., LLC as well.

## 3. Results and discussion

### 3.1. The smartphone-based differential pulse amperometry system and its performance

The smartphone-based system was successfully structured to perform differential pulse amperometry. As shown in Fig. 1b, the smartphone-based system contained these main parts: a disposable sensor, a hand-held detector, and a smartphone with a designed application. The screen-printed electrodes were modified with single-wall carbon nanotubes and gold nanoparticles and acted as the sensors for sensitive detection of levodopa. The detector circuit was used to generate excitation voltage, measure the subsequent current on the sensors, and deliver electrical signal data via Bluetooth module. The designed application was employed to calculate the current based on the data and plot current curve for real-time display on smartphone. The parameters of differential pulse amperometry such as pulse voltage $E_1$, pulse voltage $E_2$, and the number of cycle could also be set in the application. Furthermore, a 5-point moving average filter was programmed in the App to reduce the influence of electrochemical and electronic noise for acquisition of accurate current. As shown in Fig. 1c, the potentiostat applied an excitation voltage signal between the working electrode and the reference electrode of the sensor. The subsequent current was measured with a resistive feedback transimpedance amplifier (TIA). Thus, the current in the electrochemical cell was described as followed formula:

$$I = \frac{V_o - V_W}{R_f} \quad (1)$$

where $V_w$ and $V_o$ were the input potentials and output potentials of the amplifier, respectively, while $R_f$ was the value of feedback resistor. In differential pulse amperometry, two kinds of potential pulses were applied and the current at the end of each pulse was recorded. The difference between the two current samples was calculated as a function of time and displayed on the screen of smartphone.

As a point-of-care device, power consumption, power supply, and cost are important parameters that will influence the design. In order to obtain accurate test results of differential pulse amperometry, a normal lithium polymer battery was used as the power source to reduce electronic noise. At the same time, a power management module was also designed on the circuit to provide steady reference voltage for each module. The power consumption of the detector could be demonstrated through total time spent in operation T, which can be calculated by

$$T = \frac{Q_{battery}}{I_{average}} \quad (2)$$

where $Q_{battery}$ is the battery lifetime, $I_{average}$ is the average current consumption. A low resistor was connected in series with the hand-held detector and battery to detect the average current consumption of the system. The current was 30 mA during differential pulse amperometry measurement. With the normal lithium polymer battery, with lifetime of 210 mAh, the detector could work continuously for 7 h. The working hour of the system could satisfy the Parkinson's disease patient's demand for real-time monitoring of levodopa. In order to reduce the cost of the system, there is no external analog digital converter in the circuit, but the 12-bit analog digital converter on the microcontroller unit was directly selected, which is enough for most electrochemical testing. To further reduce the costs of the system, the microcontroller unit selected to be able to satisfy basic function of the circuit, such as controlling digital analog converter to generate specific analog signals, converting voltage signal to digital signal, and transmitting the data to smartphone via Bluetooth. With the advantage of advanced computing capability, smartphone was employed to perform calculation of current value, reckoning of the difference between two current, and data filtering processing. Thus, the hand-held and low cost system could be used for long term monitoring of levodopa.

### 3.2. Characterization of the gold nanoparticles / single-wall carbon nanotubes /chitosan modified on electrodes

In order to detect levodopa, the electrodes were modified with gold nanoparticles and single-wall carbon nanotubes to improve the characteristics. The electrochemical reaction of levodopa on the sensors was illustrated in Fig. 2a. Levodopa reacted with the single-walled carbon

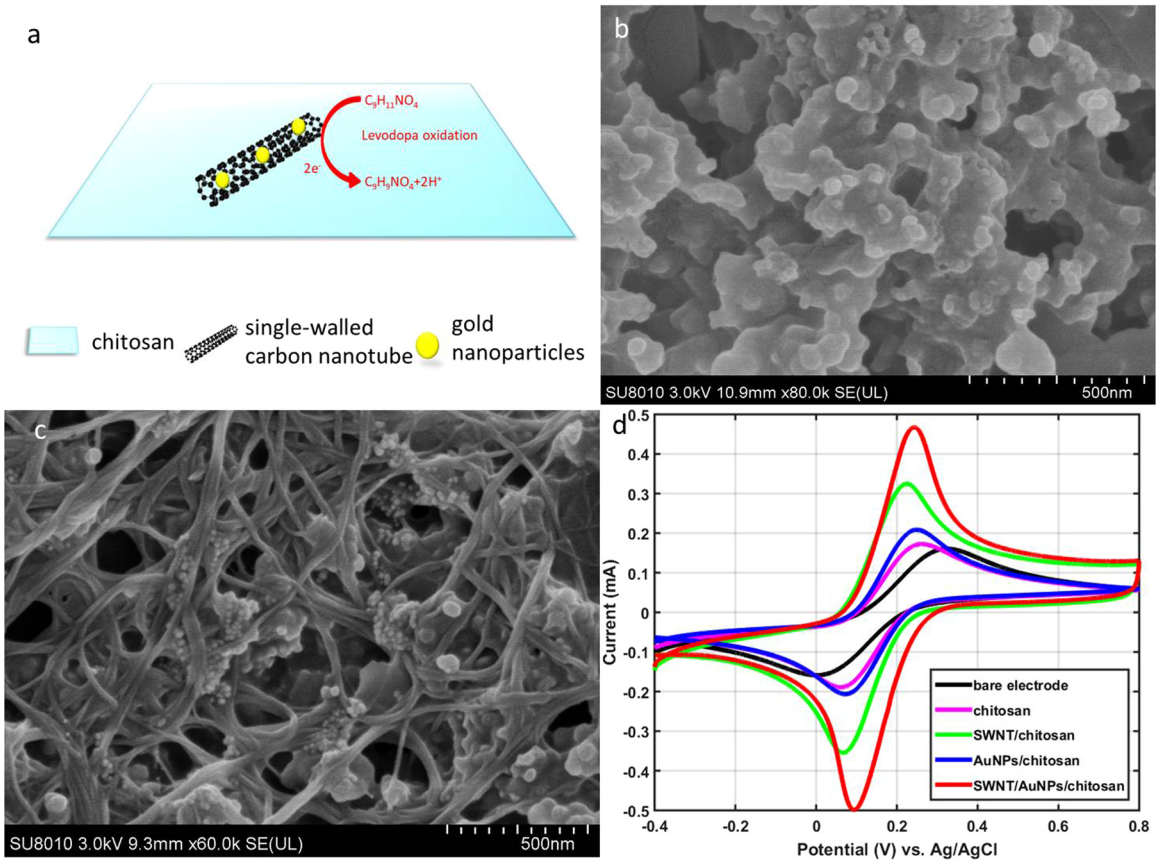


**Fig. 2.** (a) Oxidation of levodopa on the surface of the modified working electrode. (b) The scanning electron microscope image of bare screen-printed electrode. (c) The scanning electron microscope image of gold nanoparticles / single-wall carbon nanotubes /chitosan film on the screen-printed electrode. (d) Cyclic voltammetry of redox couple at bare electrode (black line), chitosan modified electrode (violet line), gold nanoparticles and chitosan modified electrode (blue line), single-wall carbon nanotubes and chitosan modified electrode (green line), and gold nanoparticles / single-wall carbon nanotubes / chitosan modified electrode (red line).

nanotubes and gold nanoparticles on the surface of the modified working electrode, then the product would be oxidized on the electrode and electron would be transferred, synchronously. Fig. 2b and Fig. 2c displayed the scanning electron microscope (SEM) images of the bare screen-printed electrode and the gold nanoparticles / single-wall carbon nanotubes / chitosan modified electrode. As shown in the scanning electron microscope images, the graphite coating on the working electrode was rough and littery, while the gold nanoparticles / single-wall carbon nanotubes /chitosan film was more well-regulated. Single-wall carbon nanotubes / chitosan composite film exhibited a net structure and gold nanoparticles were firmly clung to the single-wall carbon nanotubes. Chitosan was located between the gold nanoparticles and single-wall carbon nanotubes. It favored an effective adhesion which improved the stability of the biosensor. The single-wall carbon nanotubes formed the architecture on the electrode with numerous junctions, which increased the active surface area of the electrodes. The results demonstrated that single-wall carbon nanotubes, gold nanoparticles, and chitosan were immobilized on the electrode by physical adsorption stably. Fig. 2d compared the electrochemical performance of the electrodes with different modification for redox couple detection. In the cyclic voltammogram, bare electrode showed the lowest response. With different kinds of modification, the chitosan modified electrode had a slightly higher response than the bare electrode, the gold nanoparticles / chitosan modified electrode and the single-wall carbon nanotubes / chitosan modified electrode showed higher response than chitosan modified electrode, while the electrode with gold nanoparticles / single-wall carbon nanotubes / chitosan modification showed the highest response. The results demonstrated that single-wall carbon nanotubes and gold nanoparticles could be used to improve the performance of screen-printed electrode, respectively, while the nanocomposites of single-wall carbon nanotubes and gold nanoparticles could further improve the conductivity, electrochemical performance, and electrocatalytic properties of screen-printed electrodes.

Due to the advantages of large length-to-diameter aspect ratios, fast electron-transfer kinetics, and wide electrochemical stability window, single-wall carbon nanotubes were applied in various fields, such as biosensors, photovoltaic applications, and electrolytic water. Thus, single-wall carbon nanotubes could be employed as an excellent nanoscale building block to structure nanosensors. Although chitosan slightly changed the electrode characteristics, it was used to construct sensors due to the excellent film-forming ability, high permeability, good adhesion, nontoxicity, and cheapness. Furthermore, gold nanoparticles were firmly clung to the single-wall carbon nanotubes, which could improve the electrode performance observably. Compared with bare electrodes, the conductivity of modified electrodes was significantly changed at the same conditions. Moreover, the peak-to-peak separation of bare electrodes was larger than the modified electrode. Thus, these results further supported that single-wall carbon nanotubes and gold nanoparticles could be used to improve the characteristic of electrodes.

It is well known that levodopa is an electrochemical active substance with two OH groups of active sites for oxidation of levodopa. Therefore, differential pulse voltammetry could be employed to research the electrochemical reaction of levodopa. The current responses of gold nanoparticles and single-wall carbon nanotubes modified electrode for levodopa could be observed in Fig. 3a. It was found that the oxidation current caused by levodopa had a significant rise at around 0.17 V in the voltage range from 0 V to 0.4 V. Furthermore, a trend was found that the oxidation peak current increased along with the augment

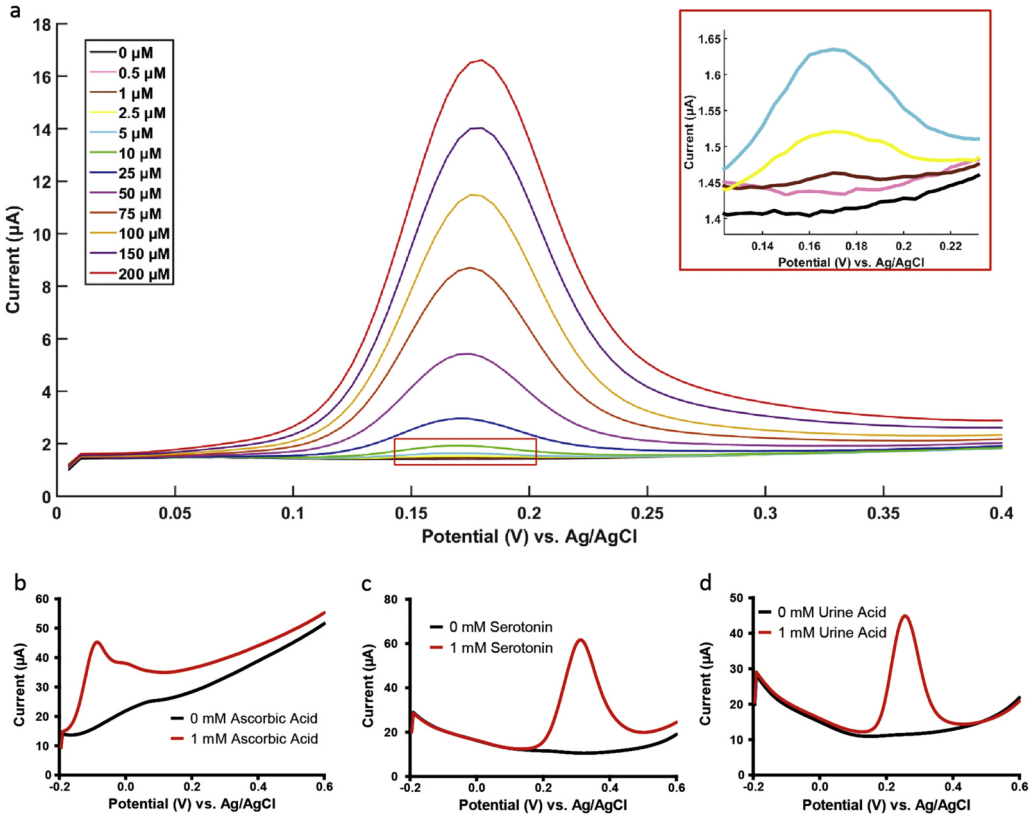


**Fig. 3.** The diagram of differential pulse voltammetry measurement for levodopa at different concentrations.

of the concentration of levodopa. The results proved that the oxidation peak current of levodopa had dose-dependent characteristics by gold nanoparticles and single-wall carbon nanotubes modified screen-printed electrodes. The corresponding potential of the oxidation peak current could be employed as excitation voltage for the differential pulse amperometry of levodopa monitoring. Furthermore, the electrodes were also used to distinguish other electrochemical active substances in human serum, such as ascorbic acid, urine acid, and serotonin. The results of differential pulse voltammetry for ascorbic acid, urine acid, and serotonin were shown in Fig. 3b, Fig. 3c, and Fig. 3d. The corresponding potentials of the oxidation peak current for ascorbic acid, urine acid, and serotonin were located at −0.08 V, 0.25 V, and 0.305 V, respectively. The results demonstrated that the modified screen-printed electrodes could be used to sensitively and efficiently detect levodopa in presence of other electrochemical active substances.

Because of subtle influence for changing current of the background current, differential pulse voltammetry was used to verify the relationship between oxidation peak current and concentration of levodopa. With the modified screen-printed electrodes, the detected linear range was from 0.5 μM to 200 μM and peak current was located at around 0.17 V. Furthermore, other electrochemical active substances, such as serotonin, ascorbic acid, and urine acid, were also detected on the modified electrodes by differential pulse voltammetry. Their peak currents were different from the peak current of levodopa. The results demonstrated that the single-wall carbon nanotubes and gold nanoparticles modified electrodes could be used to not only detect the concentration of levodopa, but also to distinguish levodopa from other electrochemical active substances.

### *3.3. Levodopa detection using the smartphone-based differential pulse amperometry system*

With gold nanoparticles and single-wall carbon nanotubes modified screen-printed electrodes, the smartphone-based differential pulse amperometry system was used to detect levodopa in real-time. The results displayed in Fig. 4a were obtained by the detection of different concentrations of levodopa in serum. When the levodopa reacted on the surface of the electrode, the current values changed obviously compared to the serum without levodopa. The currents of levodopa at stability climbed from 0.1 μA to 9 μA linearly as the concentrations of levodopa increased from 0.5 μM to 200 μM. As shown in Fig. 4b, the dose-dependent curve of levodopa was generated by using levodopa in serum ranging from 0.5 μM to 200 μM normalized with the stable current value of serum without levodopa, fitted into linear relationship as followed equations:

$$NCC_{levodopa}(\mu A) = 25.69C_{AA}(mM) + 0.05$$

In addition, the coefficient of determination ($R^2$) for real-time levodopa monitoring was 0.9875. By several testing (n = 3), the relative error of each concentration was approximately 5% in the measurement. The limit of detection (LOD) of levodopa with the system was estimated at 0.1 μM with 3δ/slope calculation for the dose-dependent fitting curve in serum. Furthermore, for the sake of assessing the detection ability of the smartphone-based system, different concentrations of levodopa were also detected with differential pulse amperometry on the electrochemical workstation. The calibration curves of smartphone-based system and commercial electrochemical workstation were simultaneously and displayed also in Fig. 4b, where we can see obviously that differential pulse amperometry shows the best ability to monitor levodopa. The real-time differential pulse amperometry monitoring plot based on the smartphone was shown in Fig. 4c and the current was displayed on the screen. The biosensor we fabricated was well-performed in levodopa detection, providing a promising approach to monitoring levodopa rapidly and quantitatively.

Different kinds of solutions including neurotransmitter, electrochemical active substances, and amino acids were detected to evaluate the specificity of this biosensor. The detection procedure was the same as levodopa. Serotonin is one type of neurotransmitter distributed in the

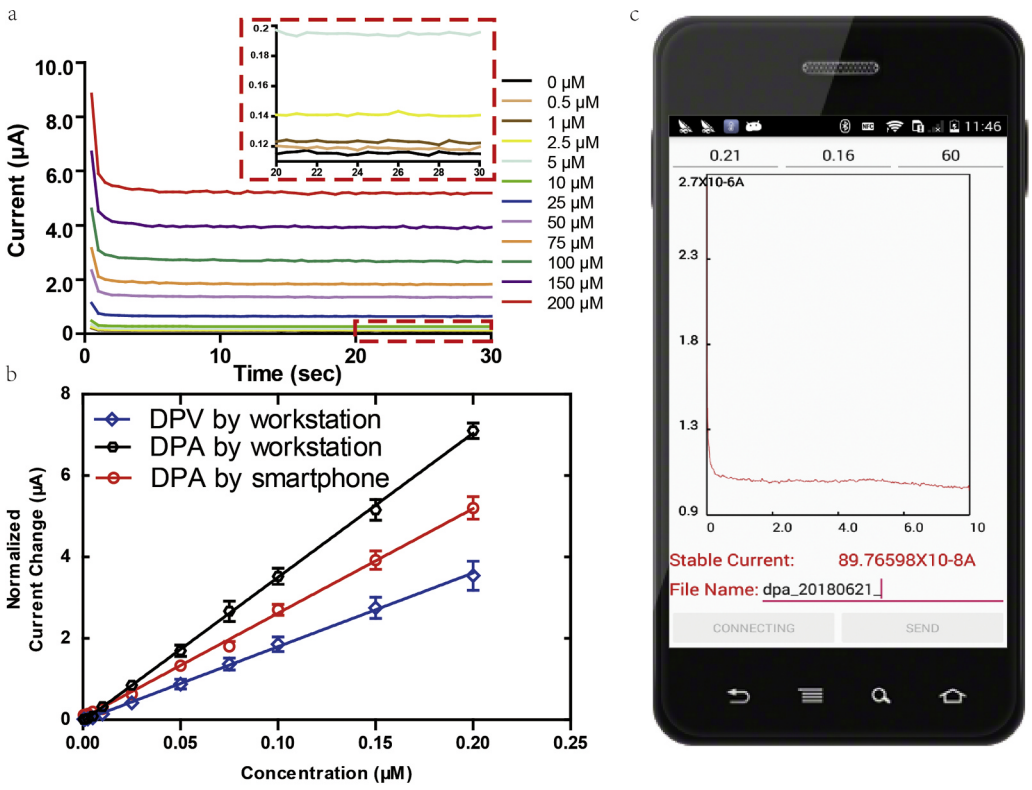


**Fig. 4.** (a) The diagram of differential pulse amperometry measurement using the system for levodopa at different concentrations. Inset: (b) The dose-dependent curve of levodopa using the system and electrochemical workstation with differential pulse amperometry (DPA), differential pulse voltammetry (DPV), and chronoamperometry (CA), n = 3. (c) Real-time differential pulse amperometry detection on the smartphone screen. The top part inputs the parameter of the detection, the middle part plots real-time differential pulse amperometry curve, and the bottom part gives information about currents.

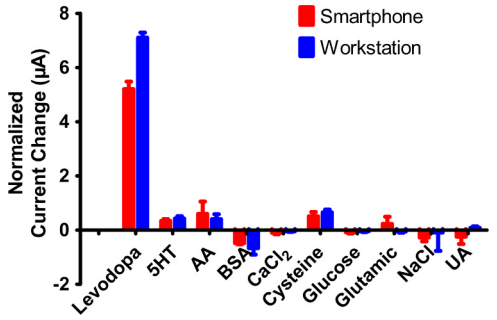


**Fig. 5.** The diagram of specific test of levodopa using smartphone-based system and electrochemical workstation through differential pulse amperometry. The concentration of serotonin, ascorbic acid, bovine serum albumin, calcium chloride, cysteine, glucose, glutamic acid, sodium chloride, and uric acid were all fixed on 1 mM. The concentration of levodopa was 200 μM.

brain similar as dopamine, which could diffuse into the blood. Ascorbic acid and uric acid are electrochemical active materials presented in the blood which may affect the detection of the Levodopa. Bovine serum albumin is a typical bio-macromolecule protein. Calcium chloride and sodium chloride are electrolytes commonly exist in the blood. Cysteine and Glutamic acid are two kinds of amino acids. Fig. 5 showed the normalized current change of these solutions at 1 mM concentration in human serum using differential pulse amperometry by the smartphone-based system and the workstation, respectively. According to the data acquired by the smartphone-based system, it clearly illustrated that the current of levodopa was the largest, while currents of other solutions were significantly smaller than levodopa's. Among these solutions other than levodopa, the current of ascorbic acid reached the largest level. But it was still smaller than one fifth of levodopa's at 200 μM concentration, which meant this detection method has a high Signal to Noise Ratio (SNR). The currents of the smartphone-based system correspond with those of the workstation. This result proved that the smartphone-based system had a high specificity towards levodopa. Thus, levodopa can be specifically detected, when it was mixed with several solutions.

The sensitivity of differential pulse amperometry was similar with that of differential pulse voltammetry, because of the similar detection principle of the two electrochemical methods. In addition, the currents of differential pulse amperometry were a function of time. That meant the response time of differential pulse amperometry was short and the current could reflect the concentrations of levodopa in time (Table S1). The concentration of levodopa in human serum is about 5 μM, while the smartphone-based system showed good linear property in the range of 0.5 μM and 200 μM, which proved the feasibility of the system to detect levodopa in human serum. The smartphone-based system also showed high specificity for the common substances in human serum, such as electrochemical active substances, protein, electrolyte, and amino acid. Furthermore, compared with traditional electrochemical workstation, the system possessed the advantages of portability and lower cost. Thus, the smartphone-based differential pulse amperometry system showed great potential for real-time and continuous monitoring of levodopa.

## 4. Conclusion

In this paper, a smartphone-based differential pulse amperometry system was developed for real-time monitoring of levodopa. The screen-printed electrode was modified with single-wall carbon nanotubes and gold nanoparticles, which was connected with the detector for the detection. The detector could be used to apply excitation signals and detect the resultant currents, which was connected to the smartphone via Bluetooth. Smartphone could be used to transmit commands, calculate data, and plot the curve. The results showed that this system could distinguish levodopa as low as 0.5 μM. All these results indicated the high sensitive and stability of the system. Hence, the system showed great potential in the field of POCT to help control the clinical dosage of levodopa.

## Acknowledgment

This work was supported by the National Natural Science Foundation of China, China (Grant No. 31671007), the Zhejiang Provincial Natural Science Foundation, China (Grant No. LZ18C100001) and the Collaborative Innovation Center of Traditional Chinese Medicine Health Management of Fujian province of China.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.082.

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