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Differential coulometry based on dual screen-printed strips for high accuracy levodopa determination towards Parkinson's disease management

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ABSTRACT

As a vital therapeutic agent for Parkinson's disease, dosage control of levodopa has always been a major obstacle in ensuring efficacy and curbing side effects. Simple and fast electrochemical detection methods are the main force in this field. Here, we presented a differential dual-strip method based on the coulometry for high accuracy determination of levodopa. The difference between the two strip signals with or without the tyrosinase extracted the levodopa signal from the samples. The Prussian Blue modified carbon screen-printed electrode was used to convert and amplify the electrochemical signal upon the presence of levodopa. The system exhibited a linear behavior in the 0–10 μM concentration range and a detection limit of 0.25 μM. Furthermore, it was proved to be stable in effectively distinguishing levodopa from complex samples through anti-interference experiments and serum tests. We demonstrated the superiority of dual-strip differential coulometry for the determination of levodopa towards Parkinson's disease clinical management.

1. Introduction

Levodopa (L-3,4-dihydroxyphenylalanine), the immediate precursor of dopamine, has been the most effective symptomatic therapy for the treatment of Parkinson's disease (PD) since the 1960s [1,2]. A major cause of Parkinson's disease is the lack of dopamine, which cannot cross the blood-brain barrier to reach the brain when taken directly [3]. Therefore, patients often need to take levodopa as an alternative therapy, which can be reduced into dopamine under the action of dopamine decarboxylase in the brain [4].

Levodopa can effectively relieve various symptoms caused by Parkinson's disease, but the dosage problem still confuses medical workers [5]. Underdosage is unable to stop the disease effectively, while overdosing can cause a host of side effects, including dyspraxia [6], myospasm [7], mental disorder [8], etc. What's more, the differences in drug effect and drug metabolism between individuals increase the difficulties of drug dose control [9]. Therefore, during the treatment of PD, the detection of levodopa in human serum is of great importance for drug safety and health management.

Methods of biological, food, pharmacological, and environmental analysis have long been a front-burner topic of concern due to the direct relevance to human security [10,11]. Among multitudinous analytical technologies, electrochemical sensors and biosensors have been extensively applied over the others for the advantages such as low cost, simple manufacturing, high sensitivity, selectivity, etc [12,13]. Different inspection methods can extract the vital characteristic values of samples containing target signals, each of which possesses superiority depending on the nature of the object. In recent years, several electrochemical biosensors have been developed for the detection of levodopa in body fluids [9,14–17]. Sweat non-invasive monitoring band based on gold dendritic nanostructures improved sensitivity [16]. The smartphone platform was combined with the differential pulse voltammetry to realize the handheld efficient detection [9]. Based on the simple chronoamperometry, a carbon nanotubes-modified screen-printed electrode was described for the determination of levodopa in undiluted human serum [17]. However, the extremely low therapeutic concentration of levodopa [18] and the interference caused by other

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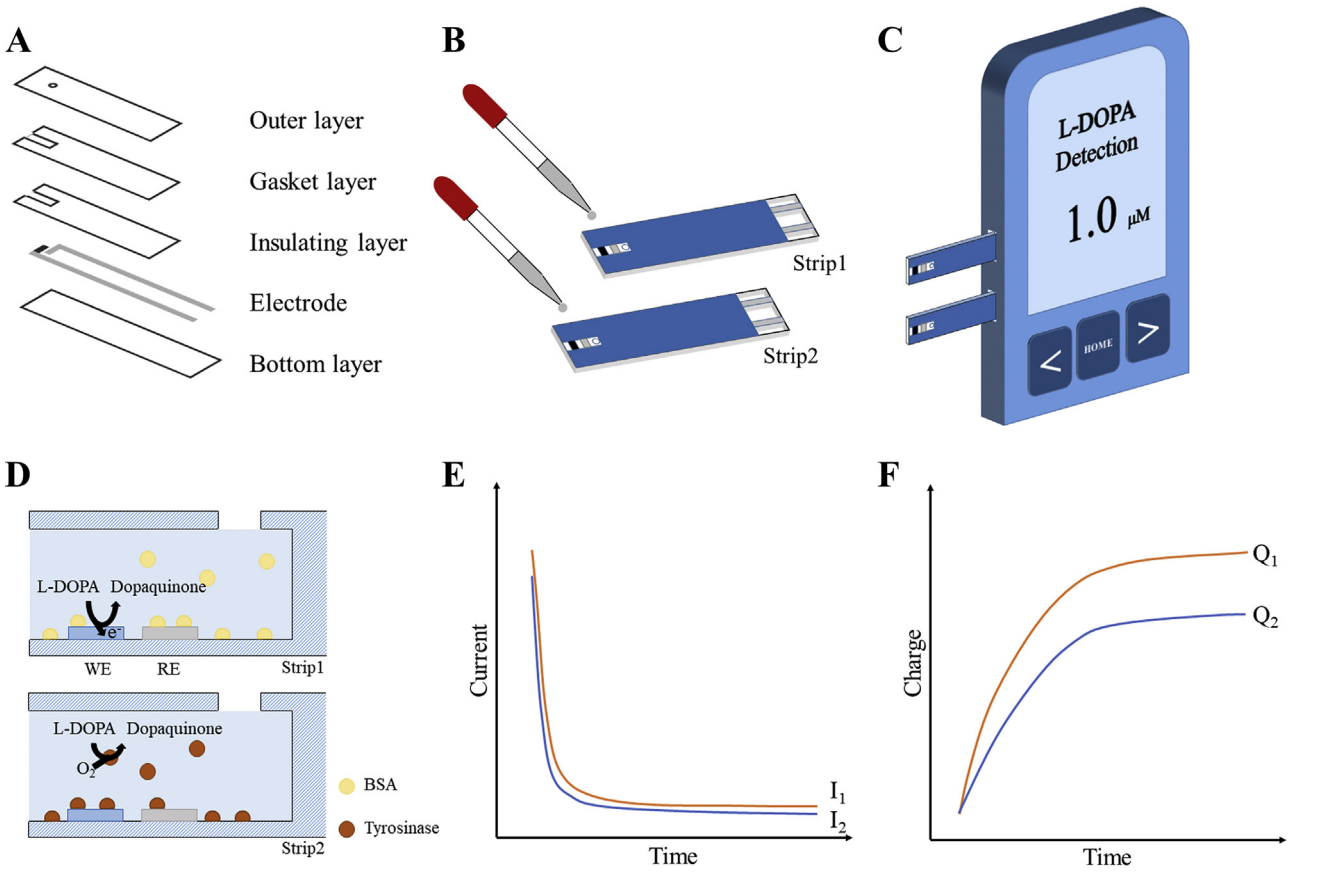

Fig. 1. Scheme of the measurement process: (A) exploded view of the strip; (B) preloading tyrosianase and BSA in the dual strips; (C) image of the sensor; (D) reaction process of levodopa at dual strips; (E) chronamperometric response; (F) coulometric response.

active substances [19] bring difficulties in obtaining accurate determination of levodopa in human fluids. Moreover, most methods require a variety of complex electrode modifications to promote the sensitivity, or even multistep processes, which is not conducive to the low-cost batch manufacturing of sensors.

Screen printing is considered to be one of the most important technologies for fast, cheap and mass production of biosensors, which has been widely used in immune, enzymatic and other biosensors [20]. Similarly, screen printing technology has also shown remarkable advantages in the field of levodopa detection [1,19]. Modifying materials with catalytic activity and nanostructures on the screen-printed electrodes can efficiently improve detection sensitivity and reduce the limit of detection (LOD). Prussian Blue (PB) is a kind of common electrode trimmer with good catalytic properties and biocompatibility [21]. Here our method constructed the sensor with a screen-printed electrode modified with PB (Fig. 1A).

Based on the calculation principle of coulomb quantity corresponding to electron transfer in an electrode redox reaction, coulometry is a simple, anti-interference and stable electrochemical detection method [22,23]. Coulometry can effectively improve the sensitivity and reduce the detection limit by processing and analyzing the chronoamperometric current, which is suitable for trace detection [24]. Therefore, the combination of coulometry and the screen-printed electrode may realize accurate detection of levodopa content in body fluid and mass production of the biosensor. In this work, we proposed a dual-strip differential coulometry to detect levodopa. Fig. 1B shows that a single measurement is made using two strips that fixed equal amounts of tyrosinase and bovine serum albumin (BSA) in the work area. Then, the strips could be tested on a double-channel detector such as Fig. 1C. As shown in Fig. 1D, levodopa in the one strip sample is catalyzed by tyrosinase to generate dopoquinone, which cannot participate in the electrode reaction, obtaining the base current I_2 (Fig. 1E) including charge current and the current caused by the interference. While in the other strip sample, levodopa is oxidized directly at the work electrode under the applied positive potential with the generation of the current I_1 containing the signal of levodopa [25]. The corresponding charge quantities are calculated as shown in Fig. 1F. The responding charge of levodopa can be gained through the difference of two above quantities, then which is converted to the levodopa content in the sample. The proposed study exhibited superior sensitivity and selectivity in both pure and serum samples, more preeminent than most current researches (Table S1). Our detection strategy focuses on ingenious method conception rather than expensive and complicated surface structure design of electrodes, in favor of the massive and frequent determination of levodopa towards Parkinson's disease clinical management.

2. Experimental

2.1. Reagents and materials

PB carbon paste (C2070424P2) and Ag/AgCl paste (C2130809D5) were purchased from Gwent (UK). Levodopa, tyrosinase (≥500 units/mg dry weight, from mushroom), urine acid (UA), ascorbic acid (AA) were purchased from Aladdin Biochemical Technology (Shanghai, China). Glucose and BSA were purchased from Sinopharm Chemical Reagent (China). Fetal bovine serum (FBS) was provided by CellMax (China). PBS solution (1×10^{-2} M, pH = 7.4) was purchased from Sangon Biotech (Shanghai, China). All aque-

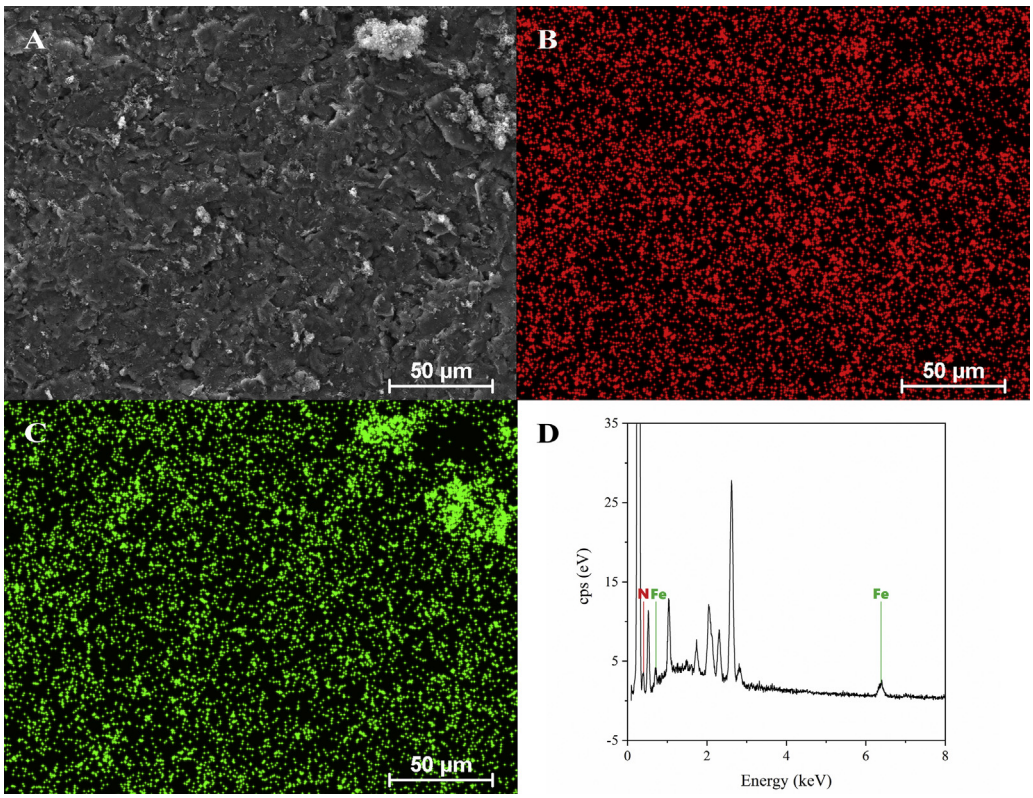

Fig. 2. (A) SEM image, (B) EDS map with iron labeled red, (C) EDS map with nitrogen labeled green and (D) EDS pattern of the PB modified carbon screen-printed electrode.

ous solutions were prepared with deionized water (DI, 18 MΩ, ELGA-PURELAB).

2.2. Fabrication of screen-printed electrode strip

Fig. 1A shows the exploded view of the strip we proposed. Firstly, the PB carbon paste and the Ag/AgCl paste were printed on the bottom layer by screen-printed technology to realize the dual-electrode system. Then, the insulating layer and gasket layer were covered above, and the latter had a certain thickness to form a small chamber (around 2 μL) at the front of the strip. The outer layer completed the formation of the reaction chamber and left a small hole for gas flow. The strip drew the sample through capillary action.

A single strip was directly adopted for the determination of levodopa in PBS, while double strips in the process of anti-interference study and serum sample experiment. Before testing, equal amounts of tyrosinase and BSA were injected into the chambers of a pair of strips and filled. Then, dry naturally and store at 4°C.

2.3. Surface morphology and composition analysis

The surface morphology of the PB modified carbon screen-printed electrode was characterized by a scanning electron microscope coupled with an energy dispersive X-ray spectroscope (FESEM, SU-70, Hitachi, Japan).

2.4. Electrochemical characteristics and measurement

All electrochemical experiments were performed in an electrochemical workstation (μAutolab III, Metrohm, Switzerland). The fundamental electrochemical studies of levodopa on the PB modified carbon electrode and carbon electrode were carried out using cyclic voltammetry (CV) at a scan rate of 50 mV/s with a potential range of - 1.0 V to + 1.0 V. The sample was 50 μM levodopa dissolved in PBS. As a comparison, CV curves of PB modified carbon electrode in PBS and levodopa solution treated with tyrosinase were also tested. Chronoamperometry was adopted for the determination of levodopa with the increasing concentrations (0, 1, 3, 5 and 10 μM) in PBS. This was done by injecting samples into the chambers of strips and applying a constant potential of 0.35 V. Chronoamperogram for different levodopa concentrations was performed by recording the current for 150 s. The charge was calculated by integrating the current and the standard fitting line of the charge vs. concentration was drawn.

2.5. Anti-interference test and serum sample detection

5 μM levodopa solutions respectively containing 200 μM glucose, 50 μM UA or 50 μM AA were prepared. Each sample was injected into the dual-strips in turn to record the current signal at a potential of 0.35 V. Then the charges converted during the detection in dual-strips and the difference between them were calculated. Levodopa solutions of 1, 3, 5, 10 μM were obtained by dissolving levodopa into FBS. In accordance with the above method, the differential coulometric quantities were measured and the standard curve was drawn.

3. Results and discussion

3.1. Structural properties and identification

The SEM microphotograph shown in Fig. 2A illustrates rough amorphous grain PB clusters with even distribution on the relatively smooth carbon flakes. The rough surface structure of PB provided more reaction area and catalytic sites for electrochemical reactions. According to the EDS examination (Fig. 2B-D) of this area, the elements of Fe and N were evenly distributed on the elec-

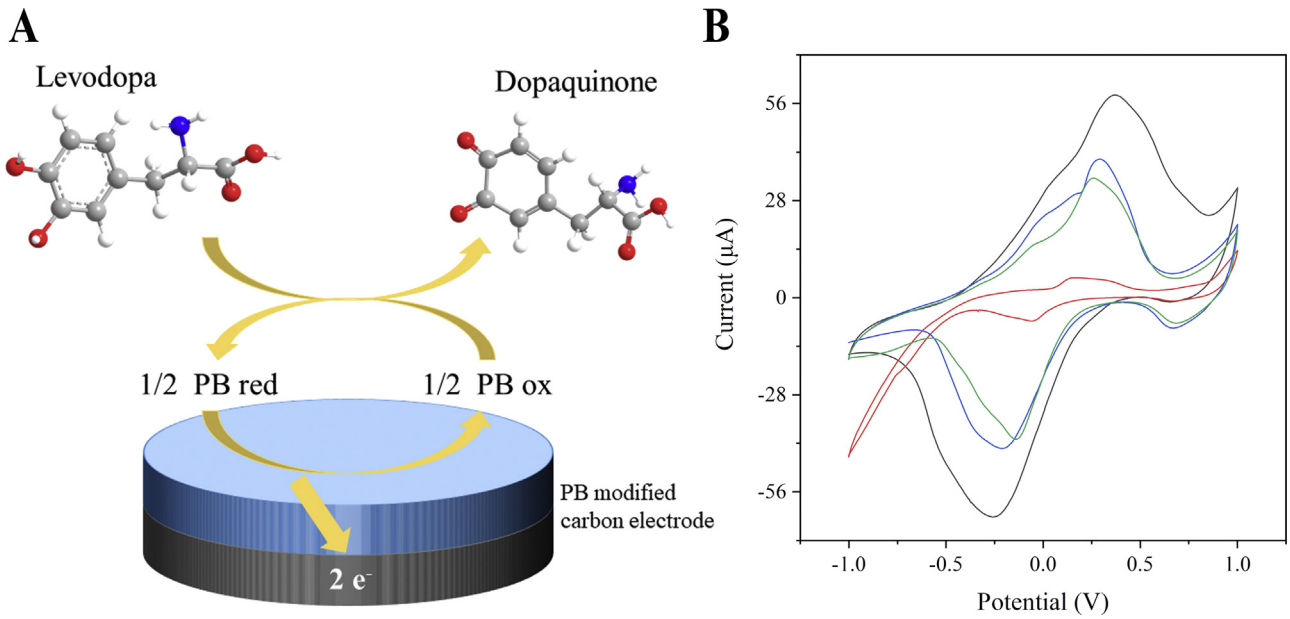

Fig. 3. (A) Schematic diagram of levodopa redox reaction at PB modified carbon electrode. (B) CV curve of PB modified carbon electrode in 50 μM levodopa solution (black line), PBS (blue line) and levodopa solution treated with tyrosinase (green line) and carbon electrode in 50 μM levodopa solution (red line).

trode. The results verified that PB was widely distributed on the electrode.

3.2. Electrochemical detection of levodopa in PBS

PB exhibits excellent catalysis in many electrochemical reactions, as does the oxidation of levodopa [26]. PB has been verified to play an electron transfer role in the oxidation of catechol which has the same functional group as levodopa in the reaction [27]. Therefore, PB was selected as a mediator to enhance the electron transfer rate between substrates and electrodes. The schematic diagram of the reaction principle is shown in Fig. 3A.

The electrochemical behavior of levodopa was investigated by cyclic voltammetry at PB modified carbon screen-printed electrode. As shown in Fig. 3B, levodopa exhibits well-defined and sharp peaks, whose oxidation peak and reduction peak are around 0.35 V and - 0.25 V, analogous to previous literature findings [28]. Therefore, the potential of 0.35 V was applied to the electrode in all subsequent detection processes in this paper to achieve the optimal catalytic effect of the oxidation reaction. Also, levodopa almost had no redox peaks on the carbon electrode, indicating that PB played an excellent catalytic role. There was no significant difference between the CV curve of detection in PBS and levodopa solution treated with tyrosinase, and whose redox peaks were lower than that of detection in levodopa solution. The electrode reaction of levodopa can produce an observable current signal, which tyrosinase can eliminate. It means that the differential strategy can extract the signal of levodopa.

Levodopa with the increasing concentrations was catalyzed at the work electrode, whose oxidation gave rise to a current. As shown in Fig. 4A, the recorded current surged at the moment the potential was applied and then gradually decreased back to the baseline. As a consequence, the current value selected at 70 s was directly proportional to the levodopa concentration. Fig. 4B shows the responding current value of the sensor at various levodopa concentrations and the optimal linear fitting result. The correlation coefficient R^2 (0.963) was not good enough, which might be caused by fluctuations and noise in current detection (Fig. 4A). Furthermore, the sensitivity of the strip using chronoamperometry was only 1.23 nA/μM. The small current responding signals would bring great difficulties to the detection of levodopa in the complex real samples. Therefore, coulometry was employed to improve the sensitivity.

To reduce the impact of charging current and shorten the detection time, the current between 20 s and 120 s was selected for integral processing to obtain the charge amount. Fig. 4C shows the curve of the integrated charges vs. time over increasing levodopa concentration (0–10 μM). The corresponding electric charge exhibited a significant proportional relationship against the concentration of levodopa, as shown in Fig. 4D. The levodopa detection sensitivity of the system using coulometry was 143.06 nC/μM. The response degree and stability of the system output to levodopa had been remarkably improved, and the interference of high-frequency noise spikes was effectively avoided. According to the calculation formula: LOD = 3 × δ / slope (where δ is the standard deviation of blank signals) [29], the detection limit of the system was 0.25 μM.

3.3. Anti-interference test and serum sample detection

Although there is a definite proportional relationship between the electric charge detected by a single strip and the concentration of levodopa, the method has no anti-interference ability. It means that other substances in clinical test samples (such as blood, urine, sweat, etc.) which are much higher than levodopa will interfere with responding charge level and even affect the test results. Thus, the responses of levodopa in complex samples were obtained using the dual-strips differential coulometry.

Fig. 5A shows the responding electric charge of the system in the presence of 5 μM levodopa and its potential disruptors, such as glucose (200 μM), UA (50 μM) and AA (50 μM). The integrated charges detected by the sensor exposed to various kinds of interferences varied greatly, but the electric charge detected by the strip fixed with tyrosinase was always lower than that of the corresponding control strip fixed with BSA. The differential quantities of charge were stable at around 715 nC. The relative error of multiple tests (n = 3) for each sample did not exceed 5%. According to the sensitivity of the system gained above, the electric charge of 5 μM levodopa was almost consistent with this value. The results indicated that the levodopa detection strategy could effectively identify the levodopa coexisting with the distractors.

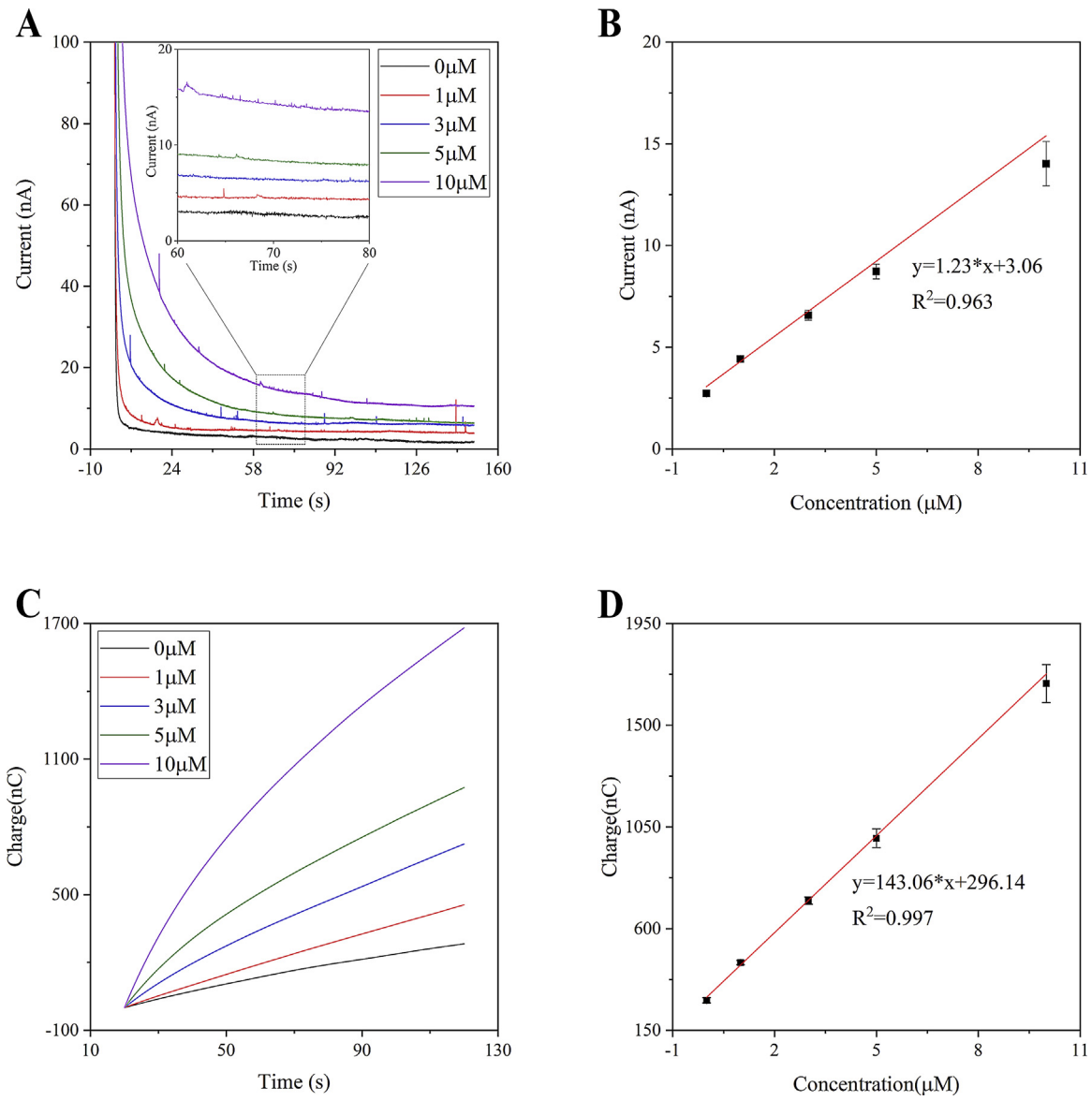

Fig. 4. Characterization of the system in PBS containing levodopa at various concentrations (0, 1, 3, 5, 10 μM): (A) chronamperometric response; (B) calibration curve of current vs concentration; (C) coulometric response; (D) calibration curve of charge vs concentration.

On behalf of further investigating the possibility of applying this strategy to human body fluids, serum test was needed. The levodopa detection result over the concentration range from 1 to 10 μM in FBS was shown in Fig. 5B. With the increase of levodopa concentration, the electric charge of the BSA strip increased, while that of the tyrosinase strip remained stable. It was in accordance with the expectation that the basic quantity of electric charge obtained in serum after the enzyme-catalyzed oxidation of levodopa at all concentrations were extremely close. The functional relationship between levodopa concentration and differential charge and ideal values of fitting were shown in Fig. 5C. According to the detection principle, the fitted line was forced to pass through zero. It was obvious that the input and output had a superior direct proportional relationship, and the relative error of the measurement was less than 5% (n = 3). Notably, the sensitivity of the system was 143.72 nC/μM in serum, which was the same as that in PBS. The experimental findings illustrated that the system was capable of extracting levodopa signals from complex samples stably. The serum test also proved the stability and anti-interference ability of the system, as well as the practical possibility in human sample detection. Especially based on the responding electric charge (sensitivity) of levodopa obtained in this paper, this detection strategy can be directly applied to the analysis of complex real samples without correction. Therefore, we believed that this system can offer a new idea for the clinical detection of levodopa in body fluids.

4. Conclusion

In this paper, the differential coulometric dual-strips system based on screen-printed electrode technology was developed for the detection of levodopa. The critical point of this strategy is the difference between the basic electric charge of the strip fixed with tyrosinase which eliminates levodopa signals and that of the other strip without enzyme, which effectively extracts the coulometric response signal of levodopa from the complex system. With the characteristics of high sensitivity, low detection limit, high stability, low cost and simple manufacturing, the proposed technology has the great potential of clinical application in the levodopa dosing control for patients with Parkinson's disease.

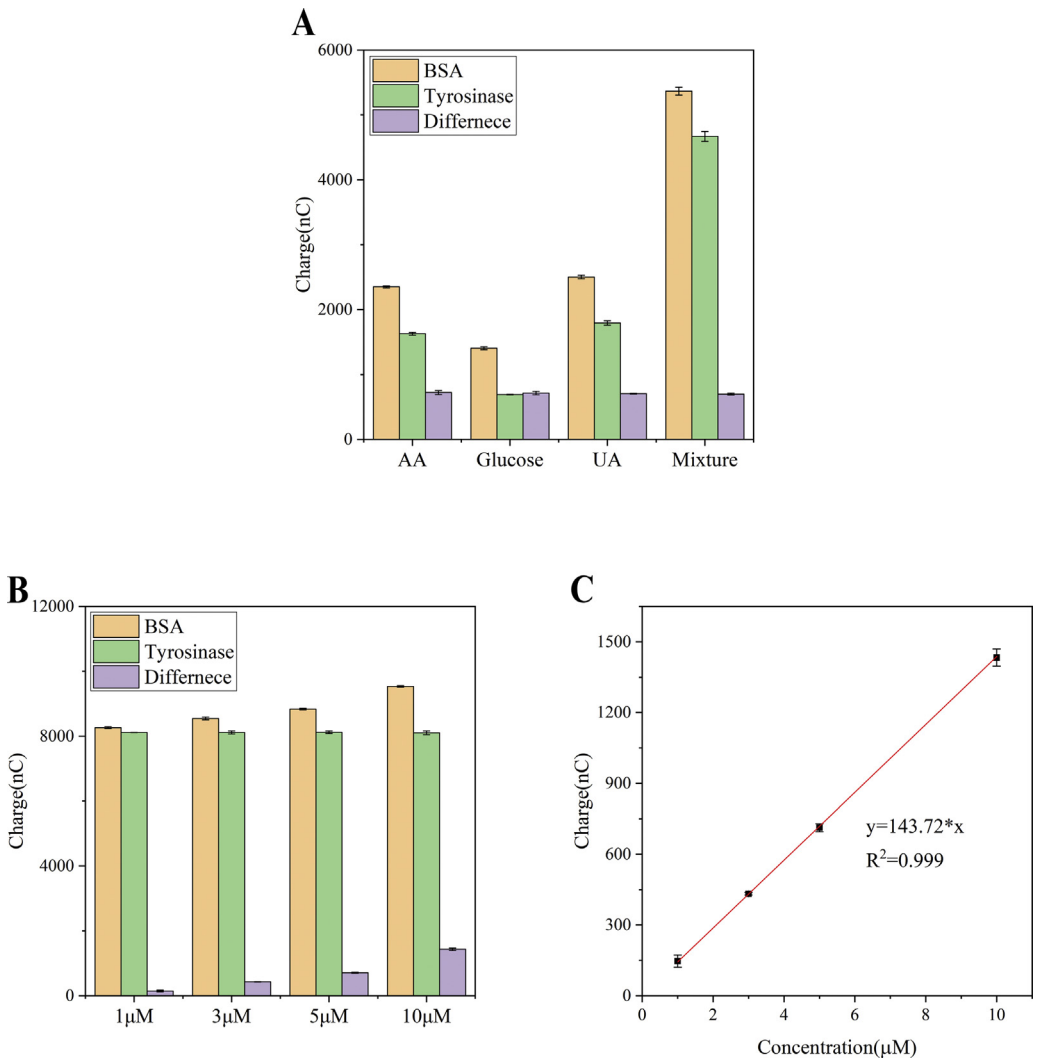

Fig. 5. (A) Responding electric charge of the system in the presence of 5 μM levodopa and its disruptors, including 200 μM glucose, 50 μM UA and 50 μM AA. (B) Coulometric response of the system exposed to FBS sample spiked with increasing levodopa concentrations (1, 3, 5, 10 μM). (C) Corresponding calibration curve.

CRediT authorship contribution statement

Congcong Yu: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft. **Qingpeng Cao:** Conceptualization, Methodology, Validation, Writing - review & editing. **Tingting Tu:** Methodology. **Yu Cai:** Validation. **Lu Fang:** Validation, Funding acquisition. **Xuesong Ye:** Writing - review & editing, Resources, Supervision, Project administration, Funding acquisition. **Bo Liang:** Conceptualization, Validation, Investigation, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2020.113498.

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