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Development of New Electrochemical Sensor based on Kudzu Vine Biochar Modified Flexible Carbon Electrode for Portable Wireless Intelligent Analysis of Clenbuterol

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Kudzu vine biochar (KVB) modified flexible carbon electrode (FCE) for the portable wireless intelligent sensing of clenbuterol (CLB) in bovine serum using cheap miniaturized analyzer composed of both smartphone and electrochemical mini-workstation was developed. Kudzu vine derived from abundant biomass residues of important traditional Chinese medicinal vine plants was selected for the preparation of KVB produced by pyrolyzing fine powders of kudzu vine at high temperature under oxygen-limited conditions. The morphology of KVB was characterized by scanning electron microscopy. FCE as flexible substrate electrode was prepared by the laser direct writing technique. The KVB modified FCE displayed excellent electrocatalytic response towards CLB in wide liner ranges from 0.95 to 14.31 μM with a low limit of detection of 0.75 μM. Practical application with satisfactory results will provide new opportunity for the comprehensive utilization of biomass residues of traditional Chinese medicinal plants as low-cost biochar for advanced electrode materials in portable wireless intelligent sensing platform based on cheap miniaturized analyzer composed of both smartphone and electrochemical mini-workstation for harmful residues in the quality safety of agro-products and food.

Keywords: Kudzu vine biochar, Flexible carbon electrode, Laser direct writing, Wireless electrochemical sensor, smartphone, Clenbuterol

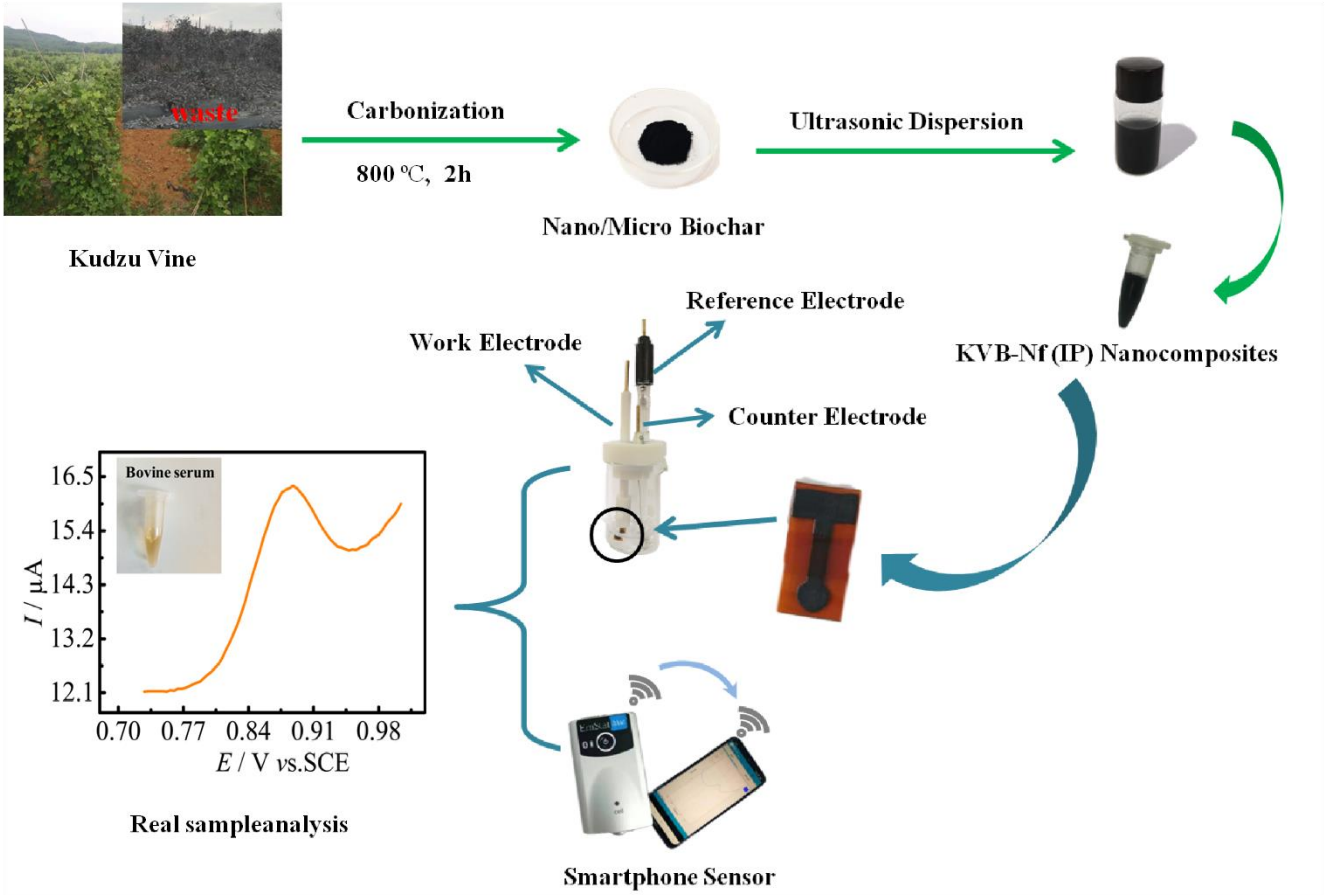
1. INTRODUCTION

Kudzu (*Pueraria lobata*) is one of the earliest medicinal vine plants used in traditional Chinese medicine. Its root has the function of medicine and health care, which can prevent and treat a variety of diseases, kudzu root has also edible function, which can produce more than 1000 kinds of functional food through deep processing [1]. However, its vine is abundant biomass residues, its chemical composition is similar to that of other natural fibers, including cellulose, hemicellulose, lignin, pectin, and other ingredients. The comprehensive utilization of large quantities of kudzu vines in China remains a challenge. Biochar is one of the ways of comprehensive utilization of biomass wastes, which have been employed for environmental remediation, climate change, renewable fuels, and fertilizers [2-5]. Biochar is also developed as new advanced materials for different applications. For example, Biochar as advanced electrode materials are being applied in electrochemical devices such as sensors, batteries, fuel cells, supercapacitors [6,7]

Electrochemical sensors as a new method/technique for modern analysis has been developed due to the advantages of simple operation, high sensitivity, wide application range, less sample consumption, low instrument cost, suitable for rapid on-site detection and efficient real-time monitoring of various objects in the fields of environmental monitoring, medical diagnosis, food supervision and agricultural safety [8,9]. The traditional electrochemical sensors are on the basis of large-scale instruments, which are far from meeting the demand of low-cost, portable and intelligent rapid detection of both agricultural products and food safety in the new era of high-speed development of both social economy and science and technology. With the dual improvement of people's living standard and economic income, people put forward higher requirements for food safety and their own health. and people are more eager to monitor food safety and their own health anytime and anywhere using cheap portable devices for on-site intelligent rapid detection [10-12]. The advent of mini devices will promote the development of modern inexpensive micro devices. Smartphone has become the most common mobile smart device in daily life due to its convenience, wireless transmission and intelligent operation, and has gradually replaced the computer to develop rapidly into modern control equipment for data reading of sensors [13-16]. New technologies such as screen printing, laser direct writing and 3D printing promote the use of flexible electrodes instead of traditional electrodes for portable electrochemical sensing platforms and flexible wearable electronic devices[17-19]. Wireless communication technologies such as bluetooth and WiFi further separate the detection end from the control end, which is expected to solve the problems caused by the wired transmission on mobile living body, field environment and non-contact on-site detection/monitoring [11,14,20].

Herein, inspired by these considerations and our previous work [21-25], A new strategy was proposed for kudzu vine biochar (KVB) onto the homemade flexible carbon electrode (FCE) for portable wireless intelligent sensing of clenbuterol (CLB) in bovine serum. KVB was prepared by pyrolyzing fine powders of kudzu vine at high temperature under oxygen-limited conditions and was characterized by scanning electron microscopy (SEM), FCE as flexible substrate electrode was prepared by the laser direct writing (LDW) technique, which was successfully applied for the portable wireless detection of CLB in bovine serum sample using cheap miniaturized analyzer composed of both smartphone and

electrochemical mini-workstation. The preparation of KVB and the portable wireless detection of CLB are vividly shown in Scheme 1.



Scheme 1. Wireless bluetooth sensing platform based on KVB/FCE for portable intelligent analysis of CLB in bovine serum sample using cheap miniaturized analyzer composed of both smartphone and electrochemical mini-workstation.

2. MATERIALS AND METHOD

2.1 Reagents

CLB was purchased from Shanghai Aladdin biochemical technology Co. Ltd (Shanghai, China, www.aladdin-e.com). 99.7 wt.% IP (isopropanol) was obtained from Xilong Chemical Co. Ltd (Guangdong, China, www.pvc123.com). 5 wt.% Nafion (Nf) was purchased from Tianjin Alfa Aesar chemical co. Ltd (Tianjin, China, www.alfachina.cn). $ZnCl_2$ was bought from Shanghai Vita Chemical Reagent Co., Ltd (Shanghai, China, www.shanghai-vita.com). 1144 μL acetic acid (99%) and 133.9 μL phosphoric acid (85%) were accurately piped into a 50 mL beaker with a pipette, and 1.24 g boric acid was placed in the beaker. The three mixed acids were dissolved to prepare a 0.04 M Britton–Robinson buffer (B-R) with a constant volume of 500 mL. Sodium hydroxide solution with 0.1 M is used to adjust buffer solution with different pH. All other reagents were analytical grade and were used without further purification. Deionized water was used in all experiments.

2.2 Apparatus

Electrochemical measurements were performed at the EmStat blue (PalmSens, Red Matrix China Limited, www.palmsenscorrosion.com) using the traditional three-electrode system. SEM was conducted with a Quanta 250 microscope (FEI Company, United States, www.fei.com). Electric blast drying oven was used for drying treatment (Zhongyi Guoke Beijing Technology Co., Ltd, www.zy-lab.com). Kudzu vine was carbonized by Jingyi jinggong series electric furnace (Yixing Jingyi Electric Furnace Co., Ltd, www.yxjydl.com).

2.3 Preparation of KVB

The dried kudzu vine was crushed and screened (10 μm ≤ diameter ≤ 0.074 mm), and kudzu vine powders were collected. The fine kudzu vine powders were dissolved into 3 mol L^{-1} $ZnCl_2$ solution allowing to stand overnight at less than 10 ºC. Subsequently, the centrifugation was performed to collect the precipitate, and carbonization treatment was applied after drying. The carbonization process was as follows: the carbonization furnace was pretreated with nitrogen for about 20 min, and then put into the homemade carbonized container containing fine kudzu vine powders. The temperature was increased by 5 ºC per minute to 800 ºC, maintaining for 2 h at the temperature, and then cooled for about 30 min. Took out the ark to collect KVB for grinding. After that, it was repeatedly washed with 75% alcohol six times and then dried, and the powder was collected as a KVB material. In the subsequent experiments, KVB material was dissolved in double distilled water to prepare the electrode modified material with a concentration of 1mg mL^{-1}. The preparation of KVB was similar with our previous reports on the preparation of biochar [23-25].

2.3 Preparation of FCE and KVB-Nf (IP)/FCE

The FCE was achieved by controlling the laser direct writing instrument to score the PI film with a thickness of about 70 μm. The laser wavelength of the laser is 450 nm, which has a higher focus. Its direct writing accuracy is 10 μm, and the maximum laser power is 5.5 W. The operation parameters of the laser instrument were optimized. Then, the laser instrument was used to score PI film with the control of these parameters (the power = 5.23 W, the speed = 5.5 mm s^{-1}). FCE is a working electrode with a fixed diameter of 3 mm, and it has the advantages of disposables, flexibility, avoiding the pollution, performance degradation caused by electrode reuse, and greatly saving time. KVB material, Nf and IP are prepared into a uniform mixed solution with the proportion of v : v : v = 20 : 1 : 4. 5 μL mixed solution was dropped onto the circular area surface of FCE using pipette, and then places them under the infrared lamp for drying to prepare KVB-Nf (IP)/FCE. Nf (IP) is defined as the nafion (Nf) solution with isopropanol (IP), Nf is defined as the Nf solution without IP. Nf is selected to enhance the adhesion and electrode stability of KVB, and the introduction of IP can improve electrochemical response of Nf towards CLB [22, 26].

3. RESULTS AND DISCUSSION

3.1 Structure and composition

The surface morphology of the prepared KVB is characterized by SEM (Fig. 1A). Fine microparticles with pore structures are important reasons for the strong adsorption capacity of biochar, which makes it play an important role for the adsorption of analytes. For electrochemistry, the effective specific surface area of the modified electrode is significantly increased, and the electron transfer capacity is accelerated. The obvious protrusion is caused by salt ions, and cracks is the unique morphology of Nf (IP) (Fig. 1B). both fine microparticles and protuberances are simultaneously observed in Fig. 3C, indicating that both KVB and Nf (IP) are successfully integrated, which is very important to play a synergistic role in enhancing the sensing performance of the electrode.

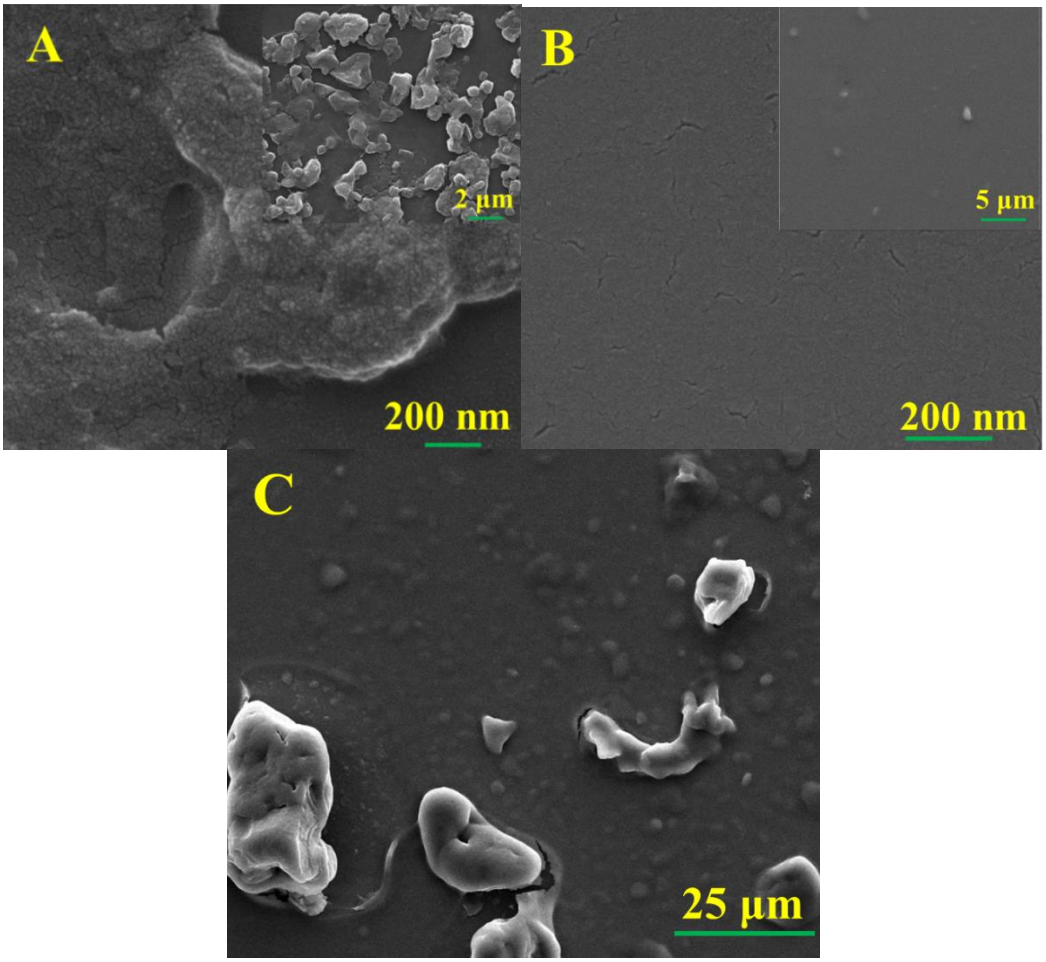


Figure 1. SEM images of KVB film (A, magnification, 20,000×and inset magnification, 160,000×), Nf (IP) film (B, magnification, 160,000× and inset magnification, 5000×), KVB-Nf (IP) film (C, magnification, 2000×).

3.2 Electrochemical behaviors of CLB

Fig. 2 shows the electrocatalytic oxidation ability of different modified FCEs for 0.19 µM CLB. In comparison with the bare FCE, there is a more obvious oxidation peak after the FCE is modified by

the KVB material that is treated by Nf. The oxidation peak current obviously increases when the KVB material is treated by Nf(IP). The electrocatalytic performance of the modified electrode for CLB is improved due to the decrease of the overpotential, relatively sharp peak shape, and high peak height, which is in accordance with our previous reports [22].

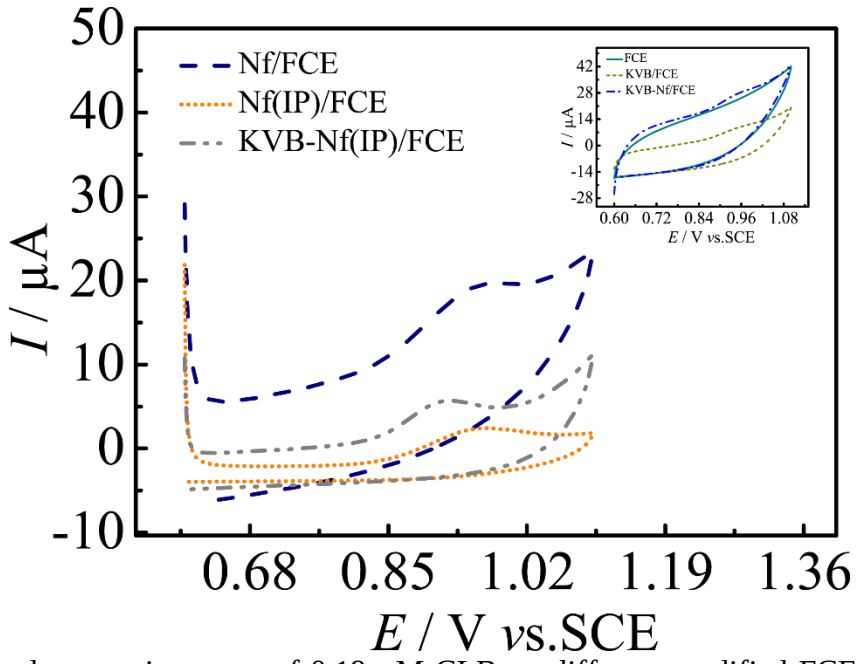


Figure 2. Cyclic voltammetric curves of 0.19 μM CLB on different modified FCEs in B-R (pH 2.6) buffer. Potential scan rates: 50 mV s^{-1}.

3.3 Optimization of pH

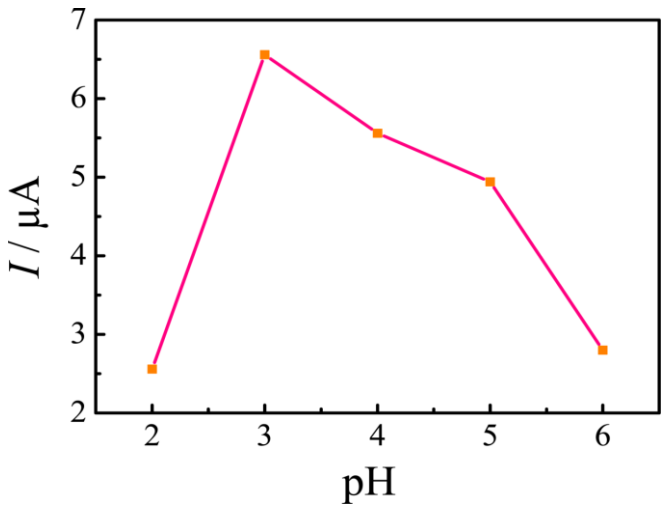


Figure 3. The effect of pH on oxidation peak currents of 0.19 μM CLB at KVB-Nf (IP)/FCE. Scan rate: 50 mV s^{-1}.

The effect of pH on the voltammetric response of CLB is shown in Fig. 3. The CLB peak current values increase with increasing pH, and the maximum value is obtained at pH 3.0. Subsequently, the

peak current values gradually decreased. Therefore, pH 3.0 is used as the optimal solution condition for subsequent experiments.

3.6 Voltammetric sensing of CLB

DPV was used for the voltammetric detection of CLB using the cheap wireless intelligent portable sensor based on KVB-Nf(IP)/FCE (Fig. 4). The peak current values were positively correlated with CLB concentrations in a wide linear range of 0.954 - 14.31 µM, and the equation was $I = 0.148C + 0.443$ ($R^2 = 0.991$). The limit of detection (LOD) was calculated, and its equation is LOD = 3 σ/m, where σ is the standard deviation of the blank and m is the slope of the calibration plot. The calculated LOD was 0.75 µM. The wide linear range and low LOD show that the cheap wireless intelligent portable sensor based on KVB-Nf(IP)/FCE can be employed for practical applications.

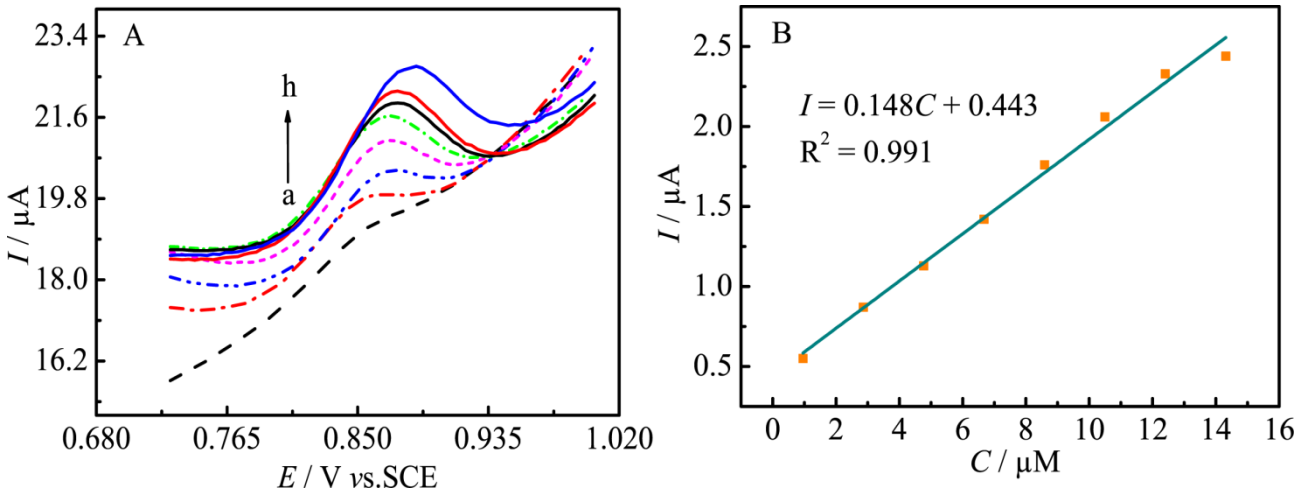


Figure 4. (A) DPVs of KVB-Nf (IP)/FCE in B-R (pH 3) buffer with various CLB concentrations: 0.954 – 14.31µM. (B) Plots of both peak heights and CLB concentrations of 0.954, 2.862, 4.77, 6.678, 8.586, 10.494, 12.4, and 14.31 µM.

Table 1. The comparison analysis of electrochemical sensors based on previously reported different modified electrodes for the determination of CLB.

Modified electrodes	Methods	Linear ranges (M)	LOD (M)	Samples	Ref
Nafion-modified multilayer-shaped CeO_2	DPV	3.9×10^{-7}–2.79×10^{-6}.	8×10^{-8}	Pig urine	[27]
MoS_2-Au-PEI-hemin	DPV	$3.1 \times 10^{-7} – 6.39 \times 10^{-6}$	6.13×10^{-6}	Pork	[28]
IP-Nf (BP)	DPV	$6 \times 10^{-8} – 2.4 \times 10^{-5}$	3.7×10^{-9}	Bovine meat and bovine serum	[22]

DNA/RGO-Nafion	DPV	5.0×10^{-7} - 4×10^{-6}	3.2×10^{-7}	-	[29]
ZnSQD@PANI	EIS	3.19×10^{-11} – 3.19×10^{-8}	1.75×10^{-11}	Pig urine	[30]
CA-AuNPs	colorimetry	5×10^{-8} – 1×10^{-6}	5×10^{-8}	Blood	[31]
KVB-Nf (IP)/FCE	DPV	9.5×10^{-7} - 14.31×10^{-6}	7.5×10^{-7}	Bovine serum	This work

The comparison analysis of electrochemical sensors based on previously reported different modified electrodes for the determination of CLB listed in Table 1,the results indicated that the developed electrochemical sensor based on KVB-Nf (IP)/FCE for the determination of VC was better due to a wider linear range and a lower LOD in some cases. Moreover, new electrochemical sensor based on homemade KVB-Nf (IP)/FCE can realize the determination of VC using cheap miniaturized analyzer composed of both smartphone and electrochemical mini-workstation.

3.4 Repeatability and reproducibility

The repeatability and reproducibility of the modified electrode are very important for the portable sensing platform. As shown in Fig. 4, the repeatability and reproducibility of the disposable KVB-Nf (IP)/FCE are explored. The KVB-Nf (IP)/FCE is consecutively detected for 10 repetitive measurements in 0.19 μM CLB to evaluate the repeatability. The relative standard deviation (RSD) is calculated to be 0.52% (Fig. 5A), satisfactory result indicated that this homemade KVB-Nf (IP)/FCE can also reuse. In addition, 0.19 μM CLB is monitored by five KVB-Nf (IP)/FCEs with the same treatment. The results are used to evaluate the electrode reproducibility (Fig. 5B). The RSD with 1.82% further confirmed that the KVB-Nf (IP)/FCE with satisfactory reproducibility prepared by this strategy has bright future.

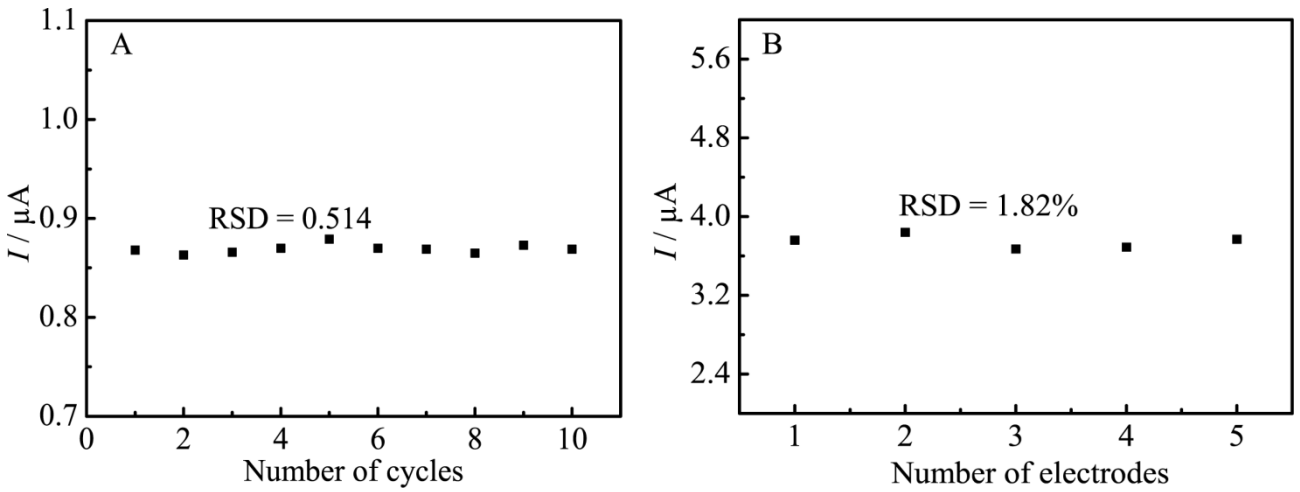


Figure 5. The repeatability (A) and reproducibility (B) of KVB-Nf (IP)/FCE in the B-R (pH 3) buffer containing 0.19 μmol L^{-1} CLB. Scan rate: 50 mV s^{-1}.

3.5 Interference studies

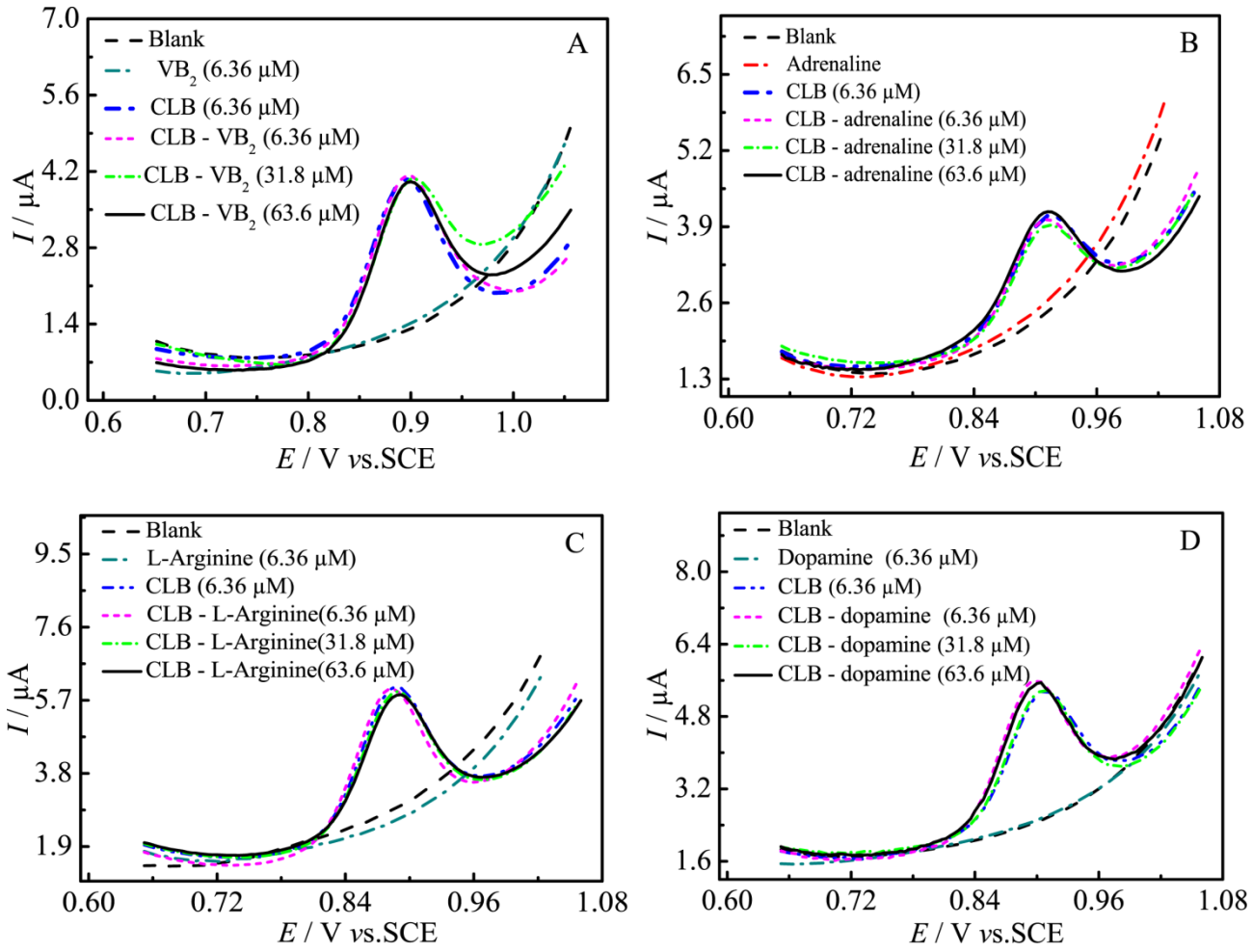


Figure 6. Interferent studies of CLB containing various substances with different concentrations in B-R (pH 3) buffer, and effects of VB_2 (A), adrenaline (B), L-arginine (C) and dopamine (D) on DPV responses of CLB.

On the basis of our previous studies [22], VB_2 (Fig. 6A), adrenaline (Fig. 6B), L-arginine (Fig. 6C), and dopamine (Fig. 6D) are selected as four kinds of crop interferents to investigate their influence on the anti-interference performance of the KVB-Nf (IP)/FCE. The results indicated that no noticeable interferences with CLB are caused by these four substances. Thus, the modified FCE has better anti-interference performance.

3.7 Real sample analysis

Bovine serum as the real sample of this experiment is obtained from the laboratory in College of animal Science, Jiangxi Agricultural University. The blood is collected using the centrifuge tube, and the upper layer is taken as the bovine serum sample after centrifugation. The recoveries of CLB are measured by standard addition method. Results as shown in Table 1, adding CLB standards with different concentrations, the calculated recoveries are in the range of 91.8% - 99.5%, RSD values are in

the range of 0.26% - 1.42%. Satisfactory results show that the KVB-Nf (IP)/FCE for the portable wireless intelligent sensing of CLB in real sample is feasible.

Table 2. The portable wireless intelligent sensing of CLB in bovine serum using KVB-Nf (IP)/FCE in 0.04 M B-R (pH 3, n = 3).

Sample	Added (mol L^{-1})	Found (mol L^{-1})	$\bar{x}$ ± SD	Recovery (%)	RSD (%)
Bovien serum	3	2.91	2.92 ± 0.042	97.3	1.42
		2.89			
		2.97			
	6	5.97	5.97 ± 0.015	99.5	0.26
		5.96			
		5.99			
	9	8.24	8.27 ± 0.031	91.8	0.37
		8.3			
		8.28			

4. CONCLUSION

KVB modified FCE for the portable wireless intelligent sensing of CLB in bovine serum using low-cost miniaturized analyzer composed of both smartphone and electrochemical mini-workstation was developed. Kudzu vine was pyrolyzed to produce KVB at high temperature under oxygen-limited conditions. The FCE as flexible substrate electrode was successfully prepared by the LDW technique. This sensor based on KVB-Nf(IP)/FCE displayed excellent electrocatalytic response towards CLB in wide liner ranges from 0.95 to 14.31 μM with a low limit of detection of 0.75 μM. Practical application with satisfactory result will provide new opportunity for the comprehensive utilization of biomass residues of traditional Chinese medicinal plants as low-cost biochar and its advanced electrode material and portable wireless intelligent sensing platform based on cheap miniaturized analyzer composed of both smartphone and electrochemical mini-workstation for application in the quality safety fields of agricultural products and food.

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