Journal of Electroanalytical Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Portable detection of clenbuterol using a smartphone-based electrochemical biosensor with electric field-driven acceleration

Yanzhi Dou [a,b], Zhineng Jiang [b], Wangping Deng [b], Jing Su [b], Shixing Chen [b], Haiyun Song [c], Ali Aldalbahi [d], Xiaolei Zuo [b], Shiping Song [b,*], Jiye Shi [e], Chunhai Fan [b,*]

[a] Key Laboratory for Organic Electronics & Information Displays (KLOEID), Institute of Advanced Materials (IAM), National Syngerstic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts & Telecommunications, 9 Wenyuan Road, Nanjing 210023, China
[b] Division of Physical Biology & Bioimaging Center, Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China
[c] Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of sciences, Shanghai 200031, China
[d] Chemistry Department, King Saud University, Riyadh 11451, Saudi Arabia
[e] Kellogg College, University of Oxford, Oxford OX2 6PN, UK

ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form 7 April 2016
Accepted 12 April 2016
Available online xxxx

Keywords:
Electrochemistry
Portable
Sensor
Smartphone
Electrical field

ABSTRACT

We have developed a fast, highly sensitive and low-cost biosensing system for the detection of clenbuterol (CLB), using a homemade mobile electrochemical device with an electric field-driven acceleration strategy. This system consists of an embedded circuit in smartphone for signal processing and a screen-printed carbon electrode (SPCE) modified with multi-walled carbon nanotubes (MWNTs) and goat anti mouse-immunoglobulin G (IgG) sensing layer (MWNTs-I-layer). CLB monoclonal antibody was assembled through its binding to the surface-confined antibody. Such modified electrodes were used for rapid and sensitive amperometric immunosensing detection of CLB. Horseradish peroxidase-coupled CLB (CLB–HRP) competed with free CLB in the samples to bind the monoclonal antibody. By using this mobile system, we could detect CLB ranging from 0.3 $ng \cdot mL^{-1}$ to 100 $ng \cdot mL^{-1}$ with the detection limit of 0.076 $ng \cdot mL^{-1}$. The whole competitive-type detection process was finished within 6 min. We expect this device can meet the requirements for field detection of various food security-related species.

1. Introduction

Electrochemical analysis provides a vast array of important quantitative methods for detecting analytes, and has been widely demonstrated in many vital fields, including clinical diagnosis, environmental monitoring, homeland security, and food analysis [1–12]. In recent years, electrochemical immunosensing assay (ECIA) has gained considerable interest as an electrochemical bioanalytical method [13–17]. Although useful in a variety of settings, these methods are generally limited to well-resourced laboratories run by skilled personnel. Simultaneously, due to the lack of necessary equipment and devices in much of the developing world, especially in remote and rural areas, it is still a major challenge to communicate the results of testing with others. Thus, simplified and inexpensive versatile biosensing devices could become broadly applicable tools in the hands of healthcare workers, clinicians, farmers, and military personnel who need accurate and quantitative results in field, especially in resource-limited settings. Recently, in order to enable analytical devices to be used and communicated in any setting, the concept of using smart mobile phone has been proposed due to their ubiquitous availability and convenient connection to information networks for improving mobile detection [18,19]. Therefore, some devices are now being explored for point-of care (POC) diagnostics and environmental monitoring, aimed to develop useful mobile detection equipment. Even these devices are trying to use mobile phones for many different fields [20–23], few of them are being developed for detecting and analyzing mobile food security. Especially, many of them only provide qualitative ("yes" or "no") or visual readout, which can be ambiguous and difficult to interpret. However, only quantitative measurements offer an accurate and reproducible means for disease diagnosis and food security monitoring.

To combine smartphone with biosensors to develop a mobile detection system with multi-performance, we have designed a universal mobile and portable electrochemical device, which has both rapid electrochemical biosensing capability and information processing and communication property. Fig. 1 shows the smartphone-based mobile electrochemical device connected to a biosensor chip, electrochemical detection component and electrical circuit frame diagram of the electrochemical device in smartphone. The smartphone-based electrochemical biosensing device has several advantages. First, an electrochemical detector was integrated into a smartphone, and a humanistic application (app) for the sensor is provided. Second, the most common electrochemical

* Corresponding authors.
E-mail addresses: spsong@sinap.ac.cn (S. Song), fchh@sinap.ac.cn (C. Fan).

http://dx.doi.org/10.1016/j.jelechem.2016.04.022
1572-6657/

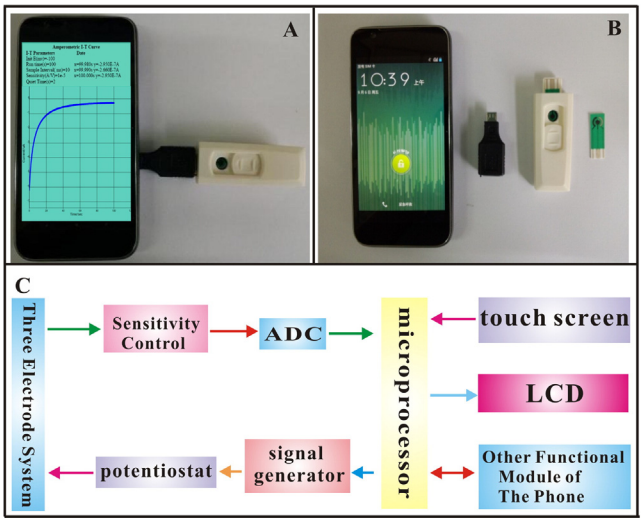

Fig. 1. (A) A photograph of the smartphone-based electrochemical biosensor. (B) A photograph of the smartphone, USB port, chipbox and screen-printed carbon electrode. (C) An electrical circuit frame diagram of the mobile electrochemical device, enables parameter input from the user to the mobile phone and data collection from three electrode system connected with the phone. (ADC: analog to-digital converter. LCD: liquid crystal display.)

methods are available, including various forms of amperometry and voltammetry. Third, the smartphone-based electrochemical biosensor can operate as a modem to convey the results of testing to a remote facility through any available communications network (2G, 3G, WiFi, bluetooth). Forth, it supports extended memory (for instance, the largest 32G/64G MicroSD) for saving data. It is ready for use at any time in our daily life. Especially, the smartphone-based device can also support electric field-driven acceleration for rapid immunosensing analysis.

Rapid immunosensing is highly desired for detection of proteins and drug molecules in POC testing. In order to accelerate the immunoreaction at the solid-liquid interface of electrodes, several techniques have been explored, including magnetic stirring, low-power microwave radiation, the electric field-driven and electrophoresis-assisted immunoassay [24–31]. Among these techniques, the electric field-driven method used to control nucleic acid hybridization, is an excellent enrichment and acceleration strategy for the transport of low-abundant protein and drug molecules. Positive driving potential was applied to accelerate the transport of negatively charged antigens to the electrode surface, while a low negative driving potential was applied to accelerate the transport of positively charged antigens to the electrode surface.

Herein, we apply our device as a smart mobile electrochemical detector by combining screen-printed carbon electrode chip with the electric field-driven acceleration strategy (Fig. 2A). The integrated system can perform extremely easy-to-use, rapid, and portable electrochemical measurements. To demonstrate the availability of this system for POC testing, we designed an electric field-driven acceleration method for detection of clenbuterol (4-amino-[t-butylaminomethyl]-3,5-dichlorobenzyl alcohol hydrochloride, CLB). CLB was found to improve growth rate, reduce fat deposition and increase protein accretion so that it has been illegally used in livestock raising for the economical benefits [32]. However, CLB can easily remain in animal tissues and result in clinical symptom of human such as temporary dizziness and palpitations [33]. Accidents of CLB poisoning were found in some areas, even though it has been a banned feed additive in food-producing animals in most countries [34]. Therefore, the continuous surveillance of CLB abuse becomes seriously necessary for public health and the development of food industry. In our designed experiments, horseradish peroxidase-coupled CLB molecule (CLB–HRP) competed with free CLB molecule in the samples to bind to the monoclonal antibody modified on screen-printed carbon electrode surface by electric field-driven acceleration method (Fig. 2C). Greatly improved sensitivity and shortened detection time are achieved by enzyme amplification and electric forces, demonstrating that the easy-to-use device has great promise for rapid and sensitive detection in other POC testing applications.

2. Experimental

2.1. Reagents and materials

MWNTs (50 nm in diameter, 1–2 μm in length) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Bovine serum albumin (BSA) and Tween-20 were acquired from Sigma. Goat anti-mouse IgG was from Solaribio S&T Co., Ltd.; Anti-CLB monoclonal antibody was purchased from Abcam Co. Ltd. Standard CLB sample was from Sigma. TMB (3,3′,5,5′ tetramethylbenzidine) substrate was purchased from Neogen (Lexington, KY) in the format of a ready-to-use reagent (K-blue custom substrate, H_2O_2 included). PEG2000 was from Sigma.

All solutions were prepared with ultrapure water (18 ΩM cm^{-1} resistivity) from a Millipore Milli-Q water purification system.

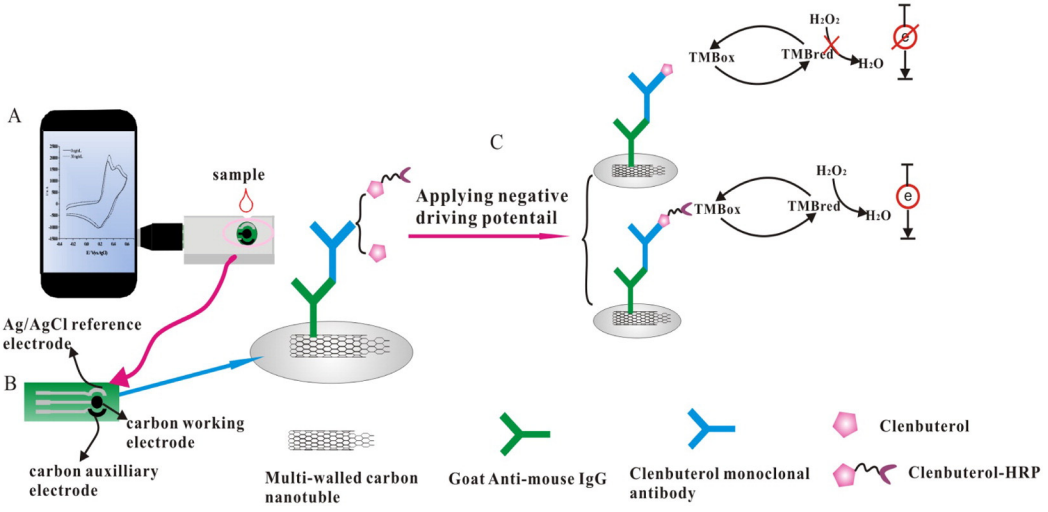

Fig. 2. Design of the electrochemical immunosensor for CLE detection with an electric field-driven incubation process; (A) homemade smartphone-based electrochemical device; (B) screen-printed electrode chip; (C) free clenbuterol competed with CLB-HRP to bind to the limited anti-clenbuterol antibody on the electrode surface. CLB-HRP combined with anti-clenbuterol antibody catalyzed the electrochemical reaction of TMB substrate solution to obtain a detective current signal.

Please cite this article as: Y. Dou, et al., Portable detection of clenbuterol using a smartphone-based electrochemical biosensor with electric field-driven acceleration, Journal of Electroanalytical Chemistry (2016), http://dx.doi.org/10.1016/j.jelechem.2016.04.022