Portable, low cost smartphone-based potentiostat system for the salivary α-amylase detection in stress paradigm *

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Abstract—**The aim of this study was to explore a novel, low-cost smartphone-based biosensor system and establish an efficient analysis method for the detection of potential stress biomarker, salivary α-amylase(sAA). This system consists of Smartphone with sAA-detection, Screen-printed electrodes(SPEs) and potentiostat module. The presented technique that using a small perturbation to obtain a linearized response could affect the composition of the sample as to cyclic voltammetry which repeatedly reduces and oxidizes the sample. For application, 6 healthy graduate students took a 5 minutes Trier Social Stress Test(TSST). Results show that this method could detect sAA activities conveniently and accurately. Calibration curve of sAA whose quantitative range is from 50 and 1200 U mL−1 with a limit of detection(LOD) of 1.6 U mL-1, is Y=-52.324X+295.63, R^2=0.9933. After psychological stress, sAA secretion was significant verified. It is concluded that this smartphone-based sAA detection system is reliable for determination of Salivary α-amylase and useful in the assessment of Psychological stress.**

I. Introduction

Stress has significant influence on health and working efficiency, and it has become one of important international problems. Recent data suggests that the majority of non-invasive biomarkers, such as salivary cortisol or α-amylase (sAA), show modest correlation with stress . So, the evaluation of sAA has an significant meaning. Accurate, fast and convenient analytical methods are needed for stress detection. Different methods have been proposed for the measurement of sAA, which are mainly based on flow injection spectrophotometric analysis (FIA)[1,2], electroph- oretic assay[3,4], and immunoassay[5,6]. Electrophoretic and immunoassay are time-consuming while the flow injection spectrophotometric assay needs big and heavy instruments. By contrast, it is of great interest to use Point-of-care testing (POCT) techniques due to its high efficiency, portability and inexpensiveness[7]. POCT has been defined as an efficient method in respect of biomedical diagnosis worldwidely with the rapid development of medical technology.

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With the market of Android system continues to enlarge, mobile intelligence technics bring new opportunities to the technology of POCT. The development of Android-based medical mobile phone applications (APP) has become a top priority, in order to better serve the health care workers.

In addition, it is necessary to be compatible with different versions of the SDK. In this study, a novel smartphone-based transportable, handheld sAA potentiometric system was developed. In order to test the applicability of the current method in psychology research, a laboratory protocol consisting of a TSST was used to induce stress and detect sAA activities. This strategy will be helpful for the development of real-time methods and techniques with high sensitivity to detect sAA concentration lever with high efficiency.

II. Materials and Methods

A. Apparatus and reagents

All reagents which were used in this work are analytical grade, α-amylase standard, Soluble starch, $K_3[Fe(CN)_6]$ and NaOH phosphate buffered saline (PBS, pH 7.4) were purchased from Jie Ke Sai Si Biology, Co, Ltd. (Nanjing, China).

B. Instruments

The whole sensing system was comprised of three components: potentiostat, SPEs and smartphone with sAA-detection. The Voltage amplification module of potentiostat and APP was supported and provided by Xenstat, Mxense Bio-Tech Co., Ltd. (Shanghai, China); Microprocessor module was built on Arduino Mega 2560 using Arduino Uno platform (JKSS Co., Ltd.); Flow injection system (Xi'an Remex Analytical Instruments Co. Ltd, China); Ultraviolet-visible spectrophotometer (Agilent 8453, Agilent) was used for sAA detection based on previous method[2]. The SPEs used in this study was a kind of novel, disposable electro-analytical electrode(Xenstat, Ningbo Mxense Bio-Tech Co, Ltd.). This kind of electro-analytical electrode includes a Polyethylene terephthalate (PET, width 0.5cm, length 1.5 cm and height 0.08 mm) and a integrated three-electrode system consisted of carbon working, counter electrode and Ag/AgCl reference electrode.

C. Potentiostat Design and Test

The whole system was developed with integration of previous open-source methods[8, 9]. The system voltage amplification and microprocessor module could amplify the analog signal and converted to a digital signal when analog potential signal came through SPEs interface, and then the digital signal was sent out to smartphone by Bluetooth module to be picked (Fig. 1). Our Android application has been developed in Android Studio 3.3.1 which allows to open the serial port connection

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through the Bluetooth and USB port. Java language was used for logical design while xml language for page design. Through the Android coming with lightweight SQL database, a memorandum of data storage and extraction was achieved. The application has three operation modes: Differential Pulse Voltammetry (DVP), Square Wave Voltammetry (SWV), and Cyclic Voltammetry (CV). The CV mode is defined by the scan rate and the peak-to-peak VPOT parameters as previous method[10]. When operated in CV mode, scan-rates can be set between 1 and 600 mV/s.

Frist, the potentiostat signal was sent to the smartphone sAA detection APP by Bluetooth module. Second, human saliva sample was accquared through a centrifuge tube and then dissolved and hydrolyzed the starch to produce maltose, which could reduced the $Fe(CN)_6^{3-}$ conversion to $Fe(CN)_6^{4-}$ under special PH condition (Fig. 1A). APP provides backend services by Bomb backend clouds. The information about user, message, voltage and current was created as variable and uploaded to the database of the Bomb cloud platform. Once the user pushed the acquisition button, the data could be loaded from the smartphone memory or cloud. After 300 seconds, the potential shift data was transmited to the APP via the Bluetooth module, and almost at the same time, the result could be analysed by a analytical chemistry algorithm while all data was uploaded from smartphone to cloud platform.

D. Mental Stress Test

In order to evaluate the applicability of the proposed method in real sample, the method was applied to determine the human saliva samples through spiking human saliva and carrying out an TSST experiment.

Subjects: Participants included in the study were 12 healthy students (mean age 21.7 ± 1.5 years), which were divided into two group (control group and stress group). In this study, during which sAA concentration were measured as index of stress responses, TSST as previous method was used for stress test, and presented to all the subjects[11]. Stress responses to the stimulation conditions were compared between different stages. To assess pre and post stress levels corresponding to Salivary α-amylase concentrations, all participants provided four salivary samples (ten minutes before the stress test, pre and after TSST and ten minutes after the second sample). All sessions lasted for about 10 minutes. Written informed consent was submitted from all participants after they received a detailed description of the study. Somatic characteristics of the study group are reported in Table 1. There were no significant inter-group differences in age, Weight, Height and BMI.

Neuroendocrine Assessment: All saliva samples were collected by the Salivette sampling device (salivette, Sarstedt, Rommelsdorf, Germany). The concentration of sAA was performed by means of flow injection spectrophotometry, as described previously method[2]. The lower detection limit was 0.16 μg/mL for a 50 μL saliva sample. The inter- and intra-assay coefficients of variability were 9.4% and 4.6%. Saliva samples from student subjects were analyzed for Saliva α-Amylase through the proposed method and Flow Injection Analysis(FIA) respectively, and results were compared with each other.

Statistical Analysis: All data were processed by SPSS18.0. Demographic data of sAA profiles were compared between the different group and stage by using Student's t-test, and mono-factor analysis of variance was used for analysis.

III. RESULTS AND DISSCUTION

A. Optimization of detection conditions

The main influence factors on the potential shift of redox reaction during the sAA detection, such as $Fe(CN)_6^{3-}$ concentration, PH, Reaction time have been studied(Fig. 2). First, the tests were performed respectively, when $Fe(CN)_6^{3-}$ concentration ranged from 10 to 200 mM mixed up with 2.5 μL of 0.01M PBS solution containing 0.02g mL^{-1} starch and 100mM NaOH (Fig. 2A),when NaOH concentration ranged from 10 to 120 mM mixed up with 2.5 μL of 0.01M PBS solution containing 0.02g mL^{-1} starch and 100mM $Fe(CN)6^3$ (Fig. 2B), and when reaction time ranged from 10 to 400s with 2.5 μL of 0.01M PBS solution (pH 12) containing 0.02g mL^{-1} starch, 100mM $Fe(CN)6^{3-}$ and 50mM NaOH (Fig. 2C), by using the orthogonal experiment design. Then 2.5 μL of sample containing 250 U mL^{-1} sAA was introduced onto the SPEs. Starch is not necessary to generate sufficient and reproducible potential shift for the sAA test[12].

The results show that 300s incubation time allowed the complete reactions (Fig. 2C). Following that, the magnitude of potential shift increased with increasing concentration of $Fe(CN)_6^{3-}$, while it negatively with the passage time or the concentration of the NaOH. In this study, maltose was oxidized by $Fe(CN)_6^{3-}$, the SPEs containing sAA at 250 U mL^{-1}. After reacting on the surface of SPEs for 300s, the potential shift was tested. 300s was chosen to be optimal reaction time for all further experiments. Within 20±1°C, the potential was rather stable and water evaporation was ignored in the study considering that the test could complete within a very short time[12].

B. Analytical performance

All of the orthogonal and real sample tests were accomplished at a room temperature of 20 ± 1 °C. A calibration curve (Y=-0.109X+0.8476) is created by preparing a set of standard solutions with sAA concentrations from 1.5 to 3.0 log[CsAA(U mL^{-1})]. The magnitude of potential change was linearly correlated to the logarithm of sAA concentration. Calibration curve of sAA is Y=-52.324X+295.63, R^2=0.9933, and its quantitative range is from 50 and 1200 U mL^{-1} (Fig. 2D). The limit of detection (LOD) for sAA using the proposed method is 1.6 U mL^{-1}. Compared with the reported methods, the obtained results confirmed the applicability and accuracy of the method.

C. Determination of sAA in real saliva samples

Statistical analysis showed that there is no significant difference between the results of the two methods (Table 2). The accuracy of the proposed method was evaluated with recovery experiments for spiked saliva samples. The results for human saliva samples and spiked human saliva are summarized in Table 3. According to the results of psychophysiology research, the mean sAA concentrations significantly increased after TSST task. 5 minutes After the TSST task, the sAA concentrations significantly declined[13].

IV. CONCLUSION

In this study, a portable smartphone-based biosensor, and a rapid and sensitive method for sAA detection were developed. This detection system have potential for determination of Salivary α-amylase, and useful in the assessment of Psychological stress.

TABLE I. SOMATIC CHARACTERISTICS OF THE SBUJECTS

Group	N	Age (years)	BMI (kg/m^2)	Weight (kg)	Height (cm)
Control	6	23.24 ± 1.26	23.26 ± 5.18	68.31 ± 14.12	172.23 ± 8.54
Stress	6	24.51 ± 1.85	23.37 ± 4.34	69.10 ± 12.46	170.11 ± 7.63

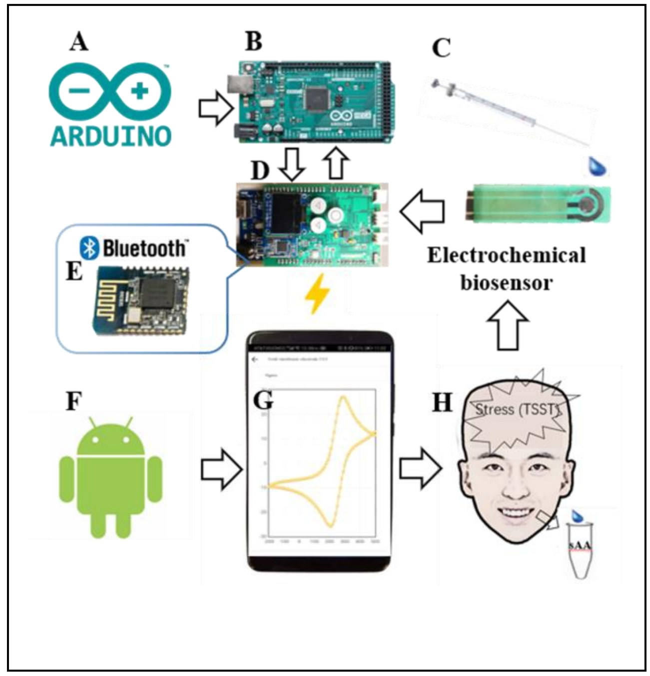
TABLE II. DETECTION OF SAA IN SALIVA SAMPLES.

Sam-ple	Content (U mL^{-1})					Mean (U mL^{-1})	RSD (%)	FIA (U mL^{-1})
No.1	218.6	201.5	234.1	213.0	251.2	223.7±19.4	8.7	228.2
No.2	245.7	289.1	258.3	255.2	274.1	264.5±17.2	6.5	273.6
No.3	358.1	365.4	360.6	345.9	362.5	358.5±7.5	2.1	216.1
No.4	492.6	470.2	453.7	480.4	462.0	471.8±15.3	3.2	481.6
No.5	730.1	769.8	718.5	727.0	755.6	740.2±21.6	2.9	755.8
No.6	542.5	573.9	588.4	581.6	557.4	568.7±18.7	3.3	576.9

TABLE III. RESULTS FOR THE DETERMINATION OF SAA IN SPIKED SALIVA SAMPLES.

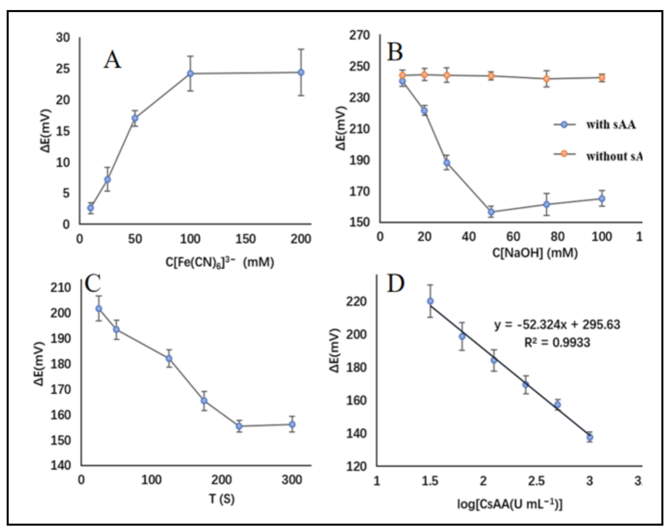
Sample	Added sAA (U mL^{-1})	Found (U mL^{-1})	Recovery (U mL^{-1})
Saliva sample	0	568.7±18.7	–
No. 6	100	672.7±16.3	98.8±12.5
	200	769.5±17.7	97.8±8.9
	300	877.8±12.3	101.3±4.1

Figure 1. Experimental scheme



(A) Arduino Uno; (B) Arduino Mega 2560 (C) SPEs; (D) Potentiostat Voltage amplification module; (E) Bluetooth module; (F) Android studio software; (G) smartphone with sAA-detection APP; (H) salivary α-amylase(sAA) sample

Figure 2. Normalized coordinate description of the stimulation sites

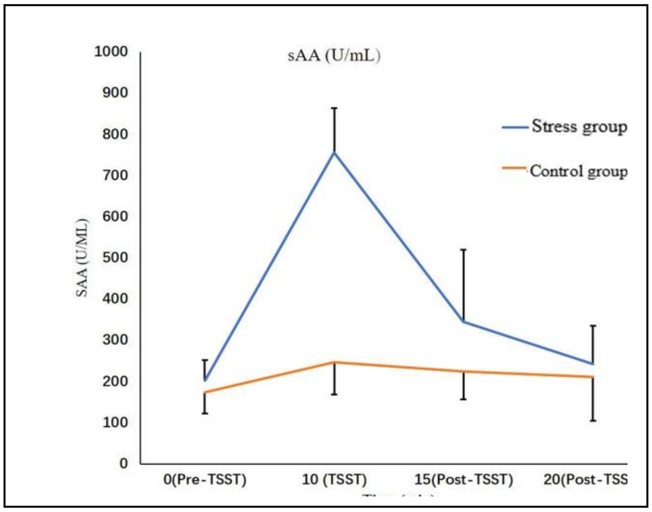


As shown in the Fig. 2, (A)Potential shift (ΔE) as a function of $Fe(CN)6^{3-}$ concentration; (B) Potential as a function of NaOH concentration; (C) Potential as a function of the reaction time after introduction of 2.5µL of sample onto the SPEs; (D) Calibration curve of sAA: as a function of the logarithm of sAA concentration.

The potential shift value was obtained 300s after introducing of 2.5µL of sample at different concentrations.

The sample solution used in study containing 2.5μL of 0.01M PBS (pH 12), 0.02g mL^{-1} starch, 100 mM $Fe(CN)6^{3-}$ and 50 mM NaOH.

Fig. 3 The sAA testing results of control and stress group



As shown in the Fig. 3, the sAA testing results of control and stress group obtained using our proposed method were presented on the following plot.

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