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Sensitive Electrochemical Detection of Creatinine at Disposable Screen-Printed Carbon Electrode Mixed with Ferrocenemethanol

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A rapid, simple and sensitive electrochemical assay of creatinine based on disposable screen-printed carbon electrode mixed with ferrocenemethanol (Fc-SPCE) was developed. The Fc-SPCE was covered with the enzyme ink containing creatinine amidinohydrolase, creatine amidinohydrolase, sarcosine oxidase and horseradish peroxidase to form creatinine biosensor. Then the electrocatalytic behavior of creatinine at creatinine biosensor was investigated employed electrochemical methods. Under optimized conditions, the catalytic current at creatinine biosensor exhibited a linear relationship with creatinine in the range from 5 to 1000 uM with a detection limit of 2.4 uM. Furthermore, the creatinine biosensor showed excellent storage stability for more than 6 months when kept dry at room temperature.

Keywords: Creatinine; biosensor; ferrocenemethanol; sensitive; stability

1. INTRODUCTION

Following the demand of the point-of-care testing and on-spot monitoring in clinical, environmental and industrial analysis, the development of simple analytic methods become more and more important[1-3]. Since Clark and Lyons proposed the first glucose enzyme electrode in 1962[4], there has been a lot of attention paid to developing novel biosensors for the fast, stable, reproducible, sensitive and selective quantification of glucose, especially for disposable electrochemical sensors

based on screen-printed carbon electrode (SPCE) technology[5-8]. Such sensors are usually made by screen-printed electrode with suitable conducting pastes (e.g., carbon, Ag/AgCl, etc.) on a substrate and then immobilizing a bioreagent layer on the electrode surface[9,10]. Compared to the common electrode, the screen-printed electrode, low cost, easy to prepare and high reproducibility, have attracted extensive interest in various field[11-14].

Creatinine, a breakdown product of creatine phosphate inmuscle, is the most important for the diagnosis of renal function, thyroid and muscle dysfunctions[15,16]. In clinical analysis, creatinine concentration is detected using automatic biochemical analyzer based on colorimetric determination of Jaffe reaction[17,18], the method is simple, however, the specificity of this reaction is not very well and it is interfered by many substances such as glucose, pyruvate and ascorbic acid[19,20]. Although methods like pre-treatment of the samples or using kinetic measurement were investigated, the detected accurate for creatinine detection remains a problem[21-23].

The aim of this work is introducing of a rapid, simple and sensitive creatinine biosensor based on disposable screen-printed carbon electrode mixed with ferrocenemethanol (Fc-SPCE). The enzyme ink containing creatinine amidinohydrolase, creatine amidinohydrolase, sarcosine oxidase and horseradish peroxidase was printed at the Fc-SPCE to form creatinine biosensor. According to investigate the electrochemical behaviors of the ceatinine at biosensor, the concentration of creatinine can be determined. Finally, storage of biosensor was also investigated.

2. EXPERIMENTAL

2.1 Materials

Carbon ink and insulation ink were acquired from JUJO (Tokyo,Janpan). The hydrophilic film and double sided adhesive tape were from 3M China Co., Ltd(Shenzhen, China) $CaCl_2$, polyethylene glycol(PEG-400), Cabosil M5 amorphous untreated fumed silica powder and hydroxyethyl cellulose(HEC), sarcosine, uric acid, ascorbic acid, ferrocenemethanol, creatinine and creatinine amidinohydrolase, creatine amidinohydrolase, sarcosine oxidase, horseradish peroxidase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aqueous solutions were prepared using Millipore water (Simplicity Model, Billerica, MA, USA).

2.2 Electrode Preparation

5 g ferrocenemethanol was dissolved in The carbon ink, and deposited onto polyethylene terephthalate(PET) which contained a working electrode and a reference/counted electrode. The react area was constructed according to printed the insulation ink at working electrodes and the counter electrode strips. For preparation of the enzyme ink, 10 mM $CaCl_2$, 1 wt% PEG-400, 4 wt% Cabosil M5 amorphous untreated fumed silica powder and 2.5 wt% hydroxyethyl celluose(HEC) added into citrate buffer solution (pH=7.4) hydated 6 h, then 400 units creatinine amidinohydrolase, 350 units creatine amidinohydrolase, 300 units sarcosine oxidase, and 400 units horseradish peroxidase were added to

each milligram of reagent ink. The reagent ink was mixed 15minutes to produce the 8500 cps enzyme ink. Then the enzyme ink was printed at reacted area formulation the enzyme ink. For fabrication of the stable sample reaction cell, the hydrophilic film and double sided adhesive tape was covered the react area.

2.3 Detection of creatinine at creatinine biosensor

All electrochemical measurements were carried out with a model CHI832C Electrochemical Workstation (CH Instruments, Austin, TX, USA). The supporting electrolyte was 0.1 M NaCl. For amperometric detection of creatinine at electrochemical creatinine biosensor, the applied potential was set at 0.1 V. The value of the current was acquired at 10 s at amperometric I-t curve. After amperometric measurements of creatinine in PBS solution at intervals, the electrodes were kept dry at room temperature. All experiments were carried out at room temperature unless otherwise stated.

2.4 Real sample preparation and analysis

Aliquots (1.0 mL) of blood samples were accessed to the heparin anticoagulation tube. For measurement of the creatinine concentration in blood sample, the results were compared to those determined with automatic biochemical analyzer(Beckman Instruments, Inc., California, USA). Creatinine was dissolved in PBS solution to prepare 100 uM and 400 uM creatinine stored solution. Then the stored solution were added into the same volume of blood sample without any treatment.

For the stability of electrodes, we chose a bath of electrodes containing 50 strips to a sealed packaging. After each measurement, the rest of electrodes were maintained in sealed packaging at room temperature to keep clean and dry. In order to investigate the recovery of the proposed screen-printed electrode, all samples were accessed from healthy donor without any treatment, the stock solutions (0.1 mM and 0.4 mM) were added into the same volume of blood samples immediately.

3. RESULTS AND DISCUSSION

3.1 Electrochemical analysis of Fc-SPCE

Cyclic voltammetry (CV) was employed to determine the electrochemical properties of screen-printed carbon electrode (SPCE) mixed with various concentration of ferrocenemethanol (Fc-OH). The oxidized and reduced current became more obvious with the increasing the concentration of Fc-OH. As can be seen from curve b in figure 1, a pair of well-defined voltammetric peaks, with the cathodic (Epc) and anodic peak potentials (Epa) at 0.11 and 0.22 V (vs. Ag/AgCl), respectively, were observed for PBS solution at screen-printed carbon electrode mixed with 5% Fc-OH. The ratio of oxidative to reductive peak currents is 1.21 and the peak currents were found to be proportional to the square root of scan rate (data not shown), suggesting that Fc-OH undergoes a virtually reversible electron transfer reaction in PBS solution and the Fc-OH can take the electron transfer at SPCE.

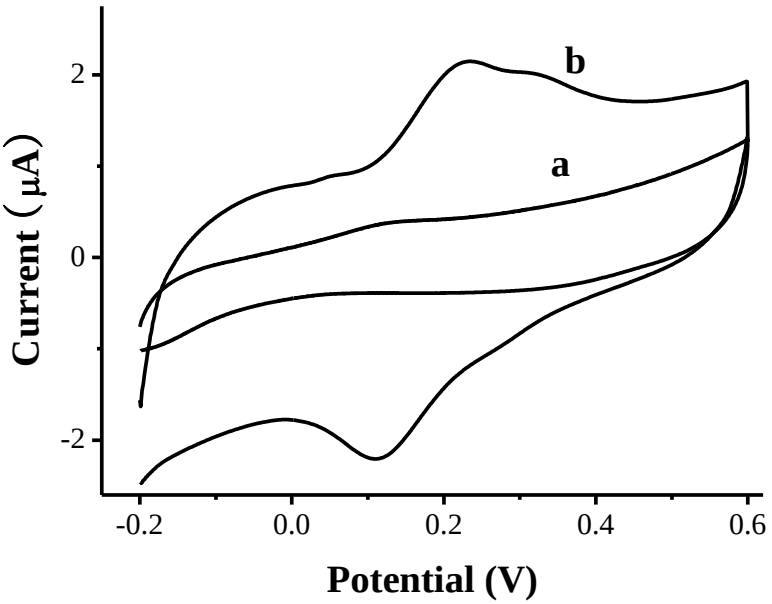

Figure 1. Cyclic voltammograms of PBS solution at screen printed carbon electrode mixed 2%(a) or 5% (b) Fc-OH. The scan rate was 100 mV/s.

3.2 Detection of creatinine at creatinine biosensor

The Fc-SPCE was covered with the enzyme ink containing creatinine amidinohydrolase, creatine amidinohydrolase, sarcosine oxidase and horseradish peroxidase to form the creatinine biosensor.

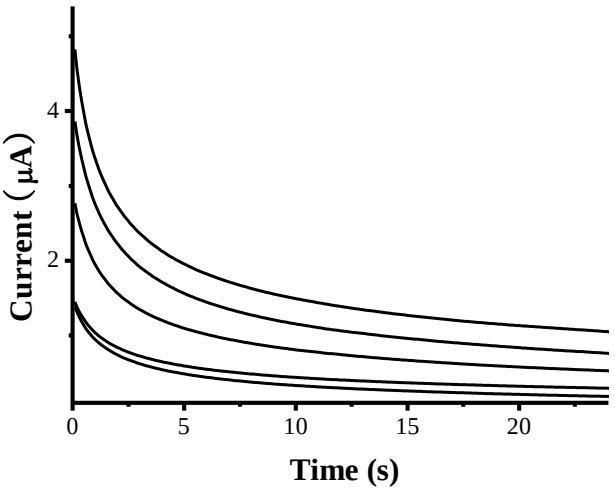

Figure 2. The I-t curve of various concentration of 0, 100, 400, 800, 1200 uM (from bottom to top) for creatinine at creatinine biosensor with the potential of 0.1 V.

Creatinine was hydrolyzed to creatine by creatinine amidinohydrolase, and the formed creatine was converted to sarcosine in the presence of creatine amidinohydrolase. The sarcosine was oxidized

to glycine, formaldehyde and hydrogen peroxide (H_2O_2) by sarcosine oxidase. Then H_2O_2 can be catalytic reduced by Fc-OH in the present of horseradish peroxidase. Thus the reduced current at creatinine biosensor increased with addition of creatinine as shown in figure 2 and can be detected using electrochemical method. Figure 3 depicts the plot of the electrocatalytic current at the enzyme screen-printed electrodes with creatinine concentration in the range from 5 uM to 1000 uM. The value of the current were acquired at 10s at amperometric I-t curve when the potential was set at 0.1V. The reduced peak current at the creatinine biosensor exhibits a linear relationship with the creatinine concentration, and the linear equation is I(uA) =0.38[Creatinine](uM) + 9.34×10^{-4}. The limits of detection were calculated on the basis of three times of the background noise and the value was found to be 2.4 uM. Chen et al. employed screen-printed carbon electrode to measure creatinine in human urine[24]. The electrode and the active methylene group in creatinine formed a stable and selective carbon carbon bond in the presence of chloride ions[25]. Creatinine was measured by square-wave voltammetry in phosphate buffer saline at pH 6.7 and with the detection window of 0.37-3.6 mM which was higher than our method. Meanwhile, this approach is simple and screen-printed electrodes are disposable, it offers the possibility for clinical systems to measure creatinine.

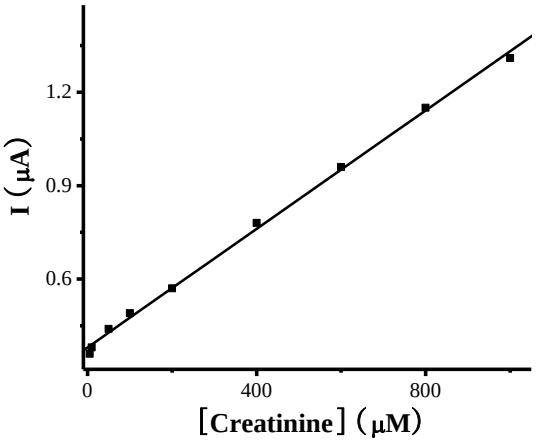

Figure 3. The calibration curve of creatinine concentration from 5-1000 uM with reduced current of I-t curve at 10 s.

3.3 Selectivity and specificity of the creatinine biosensor

It is necessary to study the selectivity and specificity of the creatinine biosensor. As shown in figure 4, the uric acid, ascorbic acid, acetaminophen and sarcosine did not change the creatinine response in the presence of these interference species at Fc-SPCE. The results demonstrated the creatinine biosensor has good selectivity and specificity. Madaras employed inner and outer membranes to immobilize the enzyme at the electrode[26]. Although the procedure was quoted and significantly reduced sensitivities, the combination of an inner permselective layer and an outer diffusional barrier gives the sensor excellent selectivity over all relevant interferences (ascorbic acid,

acetaminophen and creatine)[27]. Whether this system can retain its properties when it is miniaturized is not known. So the creatinine biosensor in our method can be used in real sample measurement.

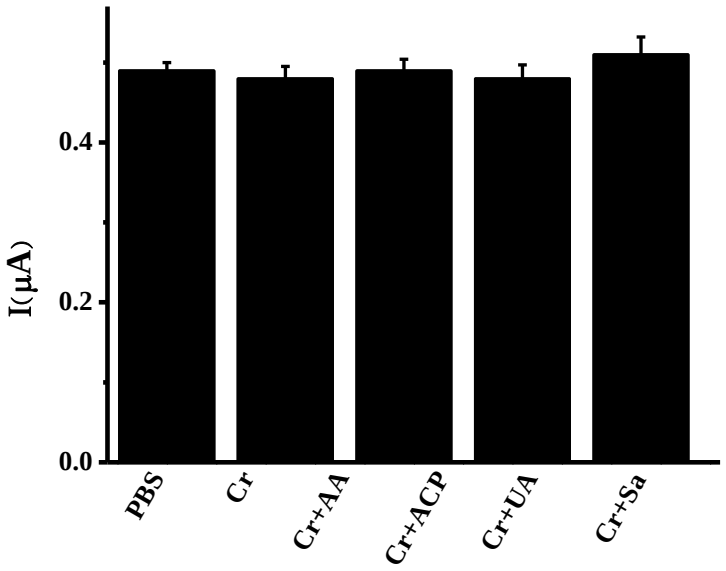

Figure 4. Selectivity of the creatinine biosensor for detection of creatinine(Cr) in the absence or presence of redox-active species (AA, ACP, UA and sarcosine(Sa)). Error bars represent the RSD values (n = 3).

3.4 Stability and reproducibility of the creatinine biosensor

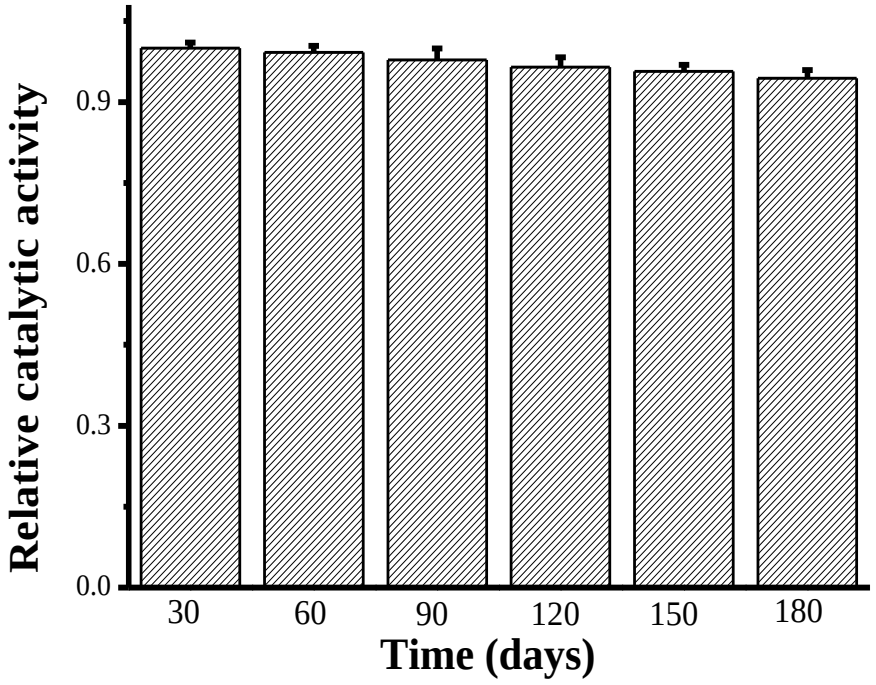

Figure 5. Stability of the creatinine biosensor. Error bars represent the RSD values (n = 3).

The stability of the creatinine biosensor was investigated by determined the response current of 100 uM creatinine. As shown in figure 5, the catalytic current at screen-printed electrode kept stable after three months, which demonstrates that the proposed electrode can be used as the commercial electrode in real application. Over 6-month storage period, the electrode still retained 94.4% of the initial current. Compared to amperometric three-enzyme creatinine biosensor, Jason immobilized the enzyme with polyurethane polymers, the native enzyme quickly lost activity with a half-life of six days, the immobilized enzyme creatinine biosensor improved the activity, but it exhibited lower than 80% activity retention after 3-month[28]. These results revealed that the enzymatic activity maintained almost intact in our method which contributed to the enzyme could retain activity according incorporated by hydroxyethyl cellulose in enzyme ink.

The reproducibility of the creatinine biosensor towards creatinine detection was estimated by assaying the response of 100 uM creatinine in PBS solution. After 20 times continuous measurements for the same bath of electrochemical strips, the results revealed the intra-assay and inter-assay coefficient of variation of the electrode was 1.8% and 4.2%.

3.5 Recovery of creatinine biosensor

To investigate the accuracy of the creatinine biosensor, the recovery of creatinine was considered. Preparation of 100 uM and 400 uM creatinine as the stored solution, and added the stored solution into the same volume of blood sample without any treatment, and the good recovery between 99.0% and 104.8% is obtained(cf. Table 1). the difference can be attributed to the slight variation of the electrocatalytic currents of the creatinine at creatinine biosensor, revealing that the proposed screen-printed method is feasible for the detection of creatinine without the interference of other redox-active species in blood samples.

Table 1. Recovery of creatinine in blood sample.

Samples	Add Creatinine (uM)	Found (uM)	Recovery (%)	RSD (%)
1	0	104±6		1.42
2	100	101±8	99.0	2.19
3	400	264±15	104.8	4.37

3.6 Real sample measurement

At least three replicate measurements were conducted for these blood samples (Table 2) and the results are also compared to those acquired with automatic biochemical analyzer(Beckman Instruments, Inc., California, USA). Samples 3 and 4 were collected from renal patients whose measured creatinine concentrations are indeed greater than the normal level (35-140 uM[29]) as well as those from healthy donors (Samples 1 and 2). As shown in Table 1, the results based on our method are in excellent agreement with those obtained with the commercial automatic biochemical analyzer.

Compared to the commercial automatic biochemical analyzer, there is no significant difference of the accuracy, but the creatinine biosensor is more facile and spends only 10 s to acquire the creatinine concentration in whole blood sample.

Table 2. Creatinine concentrations determined from healthy and renal patients donors.

Samples	Automatic Biochemical Analyzer (uM)	This Method (uM)
1	54±3	55±2
2	104±6	101±9
3	220±18	232±14
4	680±23	657±28

4. CONCLUSIONS

In this paper, we designed a new method of disposable amperometric creatinine sensor successfully. There is good relationship between the electrocatalytic current and the creatinine concentration. Furthermore, the electrochemical strips exhibit the good reproducibility and the intra-assay and inter-assay coefficient of variation of creatinine biosensor. In addition, this method was also successfully employed for creatinine detection in blood samples. We therefore conclude that the rapid and sensitive creatinine integrated to portable devices can apply in the field of clinical analysis.

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