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Electrochemical Redox Cycling Realized by Chromatography Paper-based Sensor

Kenichi Fukayama*[1], Sou Yamamoto[2], Shigeyasu Uno[3]
Departement of Electrical and Electronic Engineering, Ritsumeikan University,
1-1-1 Noji-Higashi, Kusatsu, Shiga, 525-8577, Japan. telp/fax: +81-77-599-4278
Corresponding author, e-mail: ro0024hx@ed.ritsumei.ac.jp*[1], suno@fc.ritsumei.ac.jp[3]

Abstract

In this work, we demonstrated that enhancement of electrochemical current due to redox cycling could be accomplished by paper-based biosensor without any expensive micro-fabrication process. The paper-based sensor had layered structure to generate higher current than a conventional one. We took advantage of the fact that the paper thickness was micrometer-sized (180um), and it defined the distance between two electrochemical electrodes on both sides of the paper. Experimental results showed signatures of the redox cycling, where the electrochemical current from low concentration molecules could be arbitrarily increased by decreasing the distance between electrodes. Such a structure was advantageous for detecting target molecules at very low concentration, proposing a low-cost highly-sensitive biochemcal sensor.

Keywords*: low-cost, chromatography paper, paper-based sensor, redox cycling, chronoamperometry*

1. Introduction

The number of patients with chronic diseases such as diabetes and high blood pressure is increasing in developed countries because of the unhealthy lifestyle. The monitoring devices to supervise these health conditions needs to be portable and their running cost should be low. So far various compact blood glucose sensors have been developed and are commercially available as point-of-care devices. However, easing out the manufacturing process along with the cost reduction remains one of the main challenges so that it can be used in developing countries. In order to meet these demands, chromatography papers have gathered attention as a material of a low-cost disposable biosensor chip. Such a paper sensor is refered to as paper-based biochemical sensor (PBBS) and it is made of cellulose fiber widely known as the chromatography paper (ChrPr) [1-3]. The most aarent advantage of PBBS is its chemical stability and low cost. Futhermore, PBBS manufacturing process can also be simplified by using graphite powder as an electrode and wax inks to define diffusion regions [4-6]. However minimum achievable distance between two electrodes is of the order of mm as it is difficult to cut or place a piece of paper in micrometer range. Meanwhile, interdigitated array electrode has been proven to show enhanced electrochemical current, and the distance between two electrodes in such cases is of μm-order [7]. During the measurements, so-called redox cycling occurs, and the current can be enhanced by reducing the distance between two electrodes [8-10]. As a result, low concentration of analyte can be easily detected by means of the enhanced current because the current is inversely propotional to the distance between two electrodes. We proposed that the redox cycling can also be realized in PBBS by using electrodes on both sides of the ChrPr, because the distance between the two electrodes can be as small as the thickness of the paper (~100μm).

In this study, we demonstrate that the redox cycling can be observed on ChrPr while using paper thickness as the inter-electrode distance. We perform and figure out electrochemical characteristics from cyclic tammetry (CV) measurements, and the effectiveness of a new structure is tested through chronoamperometry (CA) measurement.

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2. Research Method

We used two types of measurement techniques, namely Cyclic tammetry and Chronoamperometry. In CV, the working electrode (WE) potential is cyclically varied and current plot with respect to varying tage is obtained commonly known as tammogram. The CV current is attributed to electrode reactions, and the current is determined by the charge transfer process and diffusion process. Charge transfer process is caused by potential difference between electrode and solution near electrode surface. The current is defined by the following Butler-mer's equation:

$$I = -FAk^{0}\left\{C_{\mathrm{OX}}(0,t)\exp\left[-\frac{\alpha F}{RT}\left(E-E^{0'}\right)\right]-C_{\mathrm{red}}(0,t)\exp\left[\frac{(1-\alpha)F}{RT}\left(E-E^{0'}\right)\right]\right\}, \quad (1)$$

Where I is the current through the CE to WE, F is the Faraday constant, A is the cross-sectional area of ion conduction, k^0 is the reaction rate constant, C_{OX} is the concentration of the oxidant, α is the transfer coefficient, R is the gas constant, T is the absolute temperature, E is the actual tage across WE and electrolyte, $E^{0,}$ is the formal potential, and C_{red} is the concentration of the reductant. According to Equation (1), the CV current is related to electrode kinetics. As the electrode reaction continues the concentration difference of measuring subject between electrode surface region and bulk solution changes. The measuring subject thus travels from a region of high concentration to low concentration as defined by Fick's diffusion law and such current is named as diffusion current.

$$J = -D\left(\frac{dC}{dx}\right) \quad (2)$$

Where J is the flow rate, D is the diffusion coefficient, and dC/dx is the concentration gradient. Furthermore, the concentration gradient changes as time proceeds, and the diffusion current is defined by the following Cottrell's equation:

$$I = nFAC_{\mathrm{red}}\sqrt{\frac{D_{red}}{\pi t}}\ , \quad (3)$$

Where t is measurement time, the current varies inversely proportional to the square root of the time.

Second measurement technique we use is chronoamperometry in which a step potential is alied at working electrode and the current is measured as a function of time. At the chronoamperometric measurements, diffusion process dominates, and the current is defined by the Cottrell's equation. Change in concentration gradient is caused by diffusion layer thickness spread, and the thickness is given by the following equation:

$$d = \sqrt{\pi D_{\mathrm{red}} t}. \quad (4)$$

When the distance between two electrodes is of mm-order, the thickness of diffusion layer would keep expanding during measurement and thus leading to continuous reduction in current. However, in case, when inter-electrode distance is of μm-order, the diffusion layer reaches the other electrode and diffusion stops therefore making current constant. In this case suly of measuring subject matches with its demand, such current is given by:

$$I = nFAC_{red}\frac{D_{red}}{L}, \quad (5)$$

Where L is the distance between two electrodes, this current is independent of time and depends inversely to the distance between two electrodes.

The fabrication process of PBBS is described below. As shown in Figure 1 (a), hydrophobic pattern is printed on the ChrPr (Whatman, 1CHR) by wax printer (Xerox, ColorQube8580). The printed ChrPr is then annealed in the oven at 120^{o}C for 60 seconds to soak and spread wax-ink throughout the thickness of ChrPr. Gold plates (Nilaco, 173380) cut in 5mm x 10mm are used as both Working (WE) and Counter (CE) Electrodes, and both plates are placed on the ChrPr using vinyl tape before measurement, as in Figure 1 (c) or (d).

The solution is prepared with potassium ferrocyanide (wako, 152560) and phosphate buffered saline (PBS) (wako, 164-18541) solution is droed by 11uL prior to the measurements. It was made sure that the solution spreads thoroughly in the hydrophilic part of the paper and finally using a clip, the suly is given, as in Figure 1 (e). All of the electrochemical measurements were performed with ALS/CH Instruments Electrochemical Analyzer Model 6081E (BAS, CAT No. 013046) at room temperature.

For comparison, we prepared two different structures of PBBS, namely, planar and layered structures. The planar one has the WE and CE on the same side of the ChrPr as shown in Figure 2 (a), while the layered one has two electrodes on both sides of the ChrPr as shown in Figure 2 (b). As shown in Figure 2 (a), (b), the major difference is the distance between the two electrodes, as well as the cross-sectional area of ion conduction.

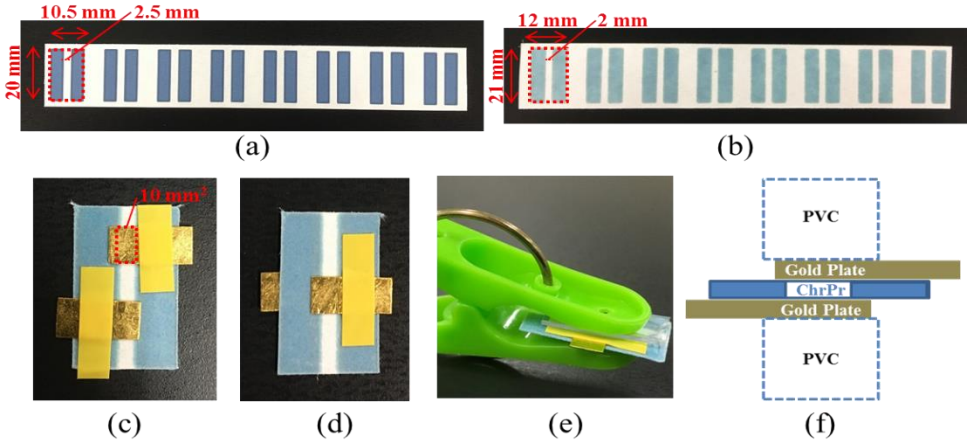

Figure 1. Photographs of fabricating PBBS, (a) printed ChrPr, (b) annealed ChrPr, (c) planar structure BS, (d) layered structure PBBS, (e) before starting each measurement, (f) cross sectional views of layered structure

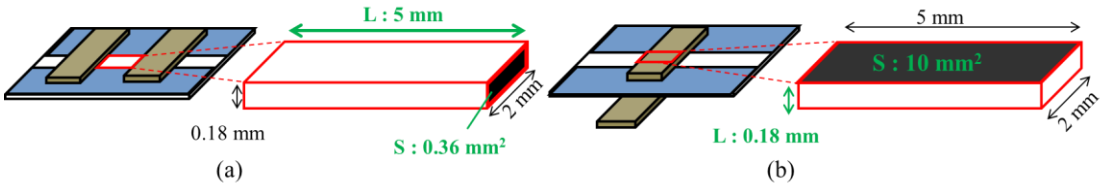

Figure 2. The schematic image and cross sectional views of two paper-based biochemical sensors, (a) planar structure, (b) layered structure

3. Results and Analysis

3.1. CV Measurement

Figure 3 (a) and (b) shows representative results of the CV measurements continuously at 100mV/s sweep rate with 50mM potassium ferrocyanide in a PBS, droed onto PBBS with planar and layered structures. In Figure 3, the vertical axis is the working electrode current, and the horizontal axis is cyclically varying potential. The purpose of this measurement is to characterize the gold plates for two-electrode measurements. Positive and negative peaks of the current can be seen and the value of peak current is same in all sweep iterations from as shown in Figure 3 (a) and (b). Hence gold can be used as electrodes in the two-electrodes formats discussed earlier. Figure 3 (c) and (d) shows representative results of the CV measurement at 1mV/s sweep rate with 50mM potassium ferrocyanide in a PBS, droed onto PBBS with both structures. The purpose of this measurement is to observe the diffusion process for a long time. The current magnitude in planar structure in Figure 3 (c) decreases after the current peak is reached which indicates diffusion layer is still expanding and hence the current

is decreasing as illustrated by Cottrell's equation. However, the CV plots of layered structure in Figure 3 (d) are different from planar structure one, and the most important difference is that, in case of layered structure, there is stabilization of current which indicates that diffusion process has almost stoed. Same phenomeno is observed when interdigitated electrodes are used [10]. Hence redox cycling using a layered structure is confirmed as indicated by experimental data. Layered structure is less affected by diffusion process because the distance between two electrodes is short. Further, we take CA measurements to experimentally calculate the time after which the diffusion in layered structure stops and to further confirm the redox cycling process in proposed PBBS.

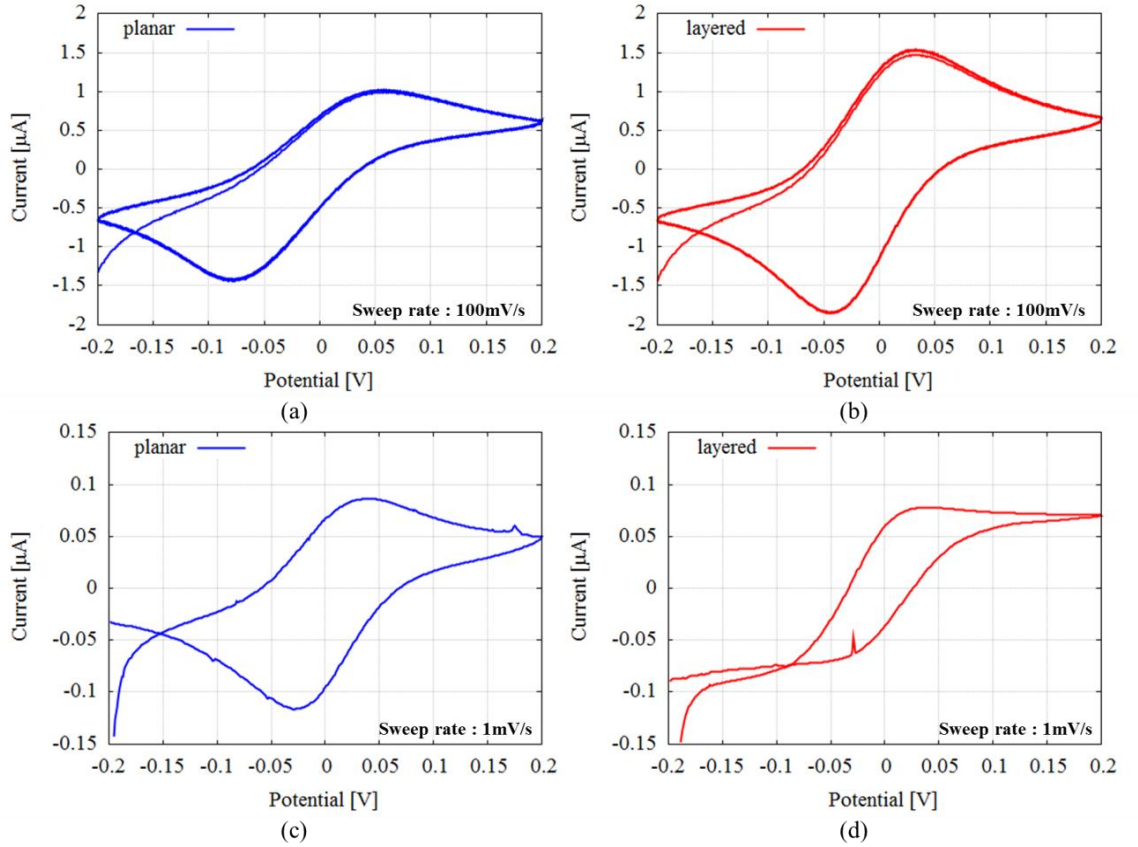

Figure 3. Cyclic tammograms of two paper-based biochemical sensor structures measuring $K_4[Fe(CN)_6]$: 50mM, (a) planar structure and sweep rate = 100mV/s, (b) layered structure and sweep rate = 100mV/s, (c) planar structure and sweep rate = 1mV/s, (d) layered structure and sweep rate = 1mV/s

3.2. CA Measurement

Figure 4(a) shows the results of the CA measurement with 50mM potassium ferrocyanide in a PBS, droed onto PBBS with both structures. In Figure 4 (a), the vertical axis is working electrode current, and the horizontal axis is measurement time. As shown in Figure 4(a), in planar structure, the current continuously decreases with time, as indicated by Equation (3). On the other hand, in layered structure, current becomes almost constant after certain amount of time. This behavior is exclusively seen when redox cycling occurs.

Figure 4(b) shows the logarithmic plot of Figure 4(a). It is clear from the graph that in planar structure the slope of the curve is aroximatly -0.5 which is due to Cottrell's behavior. But, in layered structure, there is a change in slope from -0.5 to zero at around 10 - 20 s which indicates that diffusion has stoed and current has become constant. These results are testimony to the efficiency of our design which aims for redox cycling.

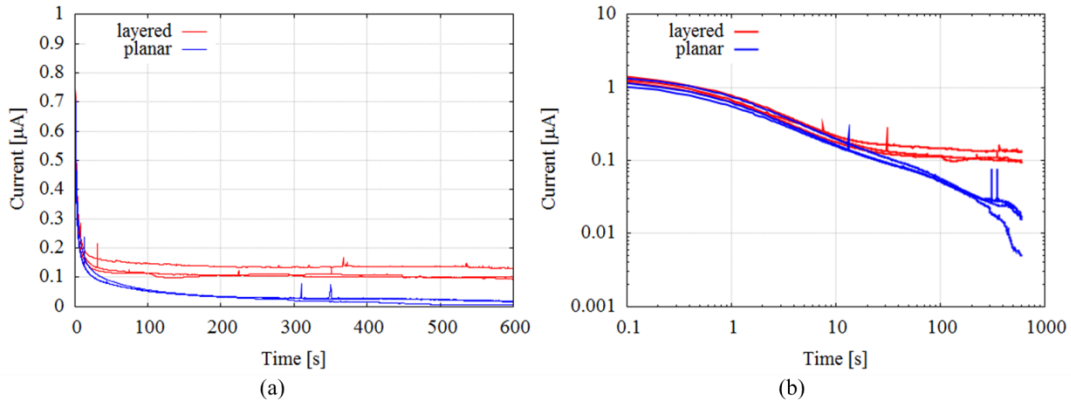

Figure 4. Chronoamperometry of two paper-based biochemical sensor structures measuring $K_4[Fe(CN)_6]$: 50mM (a) : planar structure and layered structure, (b) double logarithmic chart of Figure 4 (a)

4. Conclusion

For the first time, the current enhancement due to redox cycling in a paper-based biosensor has been demonstrated without any expensive micro-fabrication processes. The current observed after 10-20 s seconds in layered structure is larger than that in conventional planar structure, and it is almost constant in time. Thanks to these redox cycling properties, the paper-based sensor with layered structure exhibits two distinctive advantages: higher sensitivity to the redox molecule concentration, and time-independent current-time relation. Looking forward, we can also use graphite powder electrodes instead of gold plates to further curtail the cost and thereby accomplishing our goal of a low cost, highly sensitive disposable biosensor.

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