

Portable Electrochemical Gas Sensing System with a Paper-Based Enzyme Electrode

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Abstract

An unconventional portable electrochemical gas sensor composed of a smartphone, a finger-sized sensing chip and a single use paper-based enzyme electrode was proposed to detect a particular target gaseous inclusion for self-breath-analysis with ease. This attempt allowed us to monitor our physical status immediately and continuously regardless of a time, place or person due to the improved convenience, immediacy, and affordability. The custom CMOS chip with the capability of performing an amperometric determination when the power voltage supplied from the earphone jack of a smartphone was designed as an analytical device. A disposable enzyme electrode was prepared simply from a chromatography paper and a commercial carbon pencil instead of the conventional indisposable material and complex manufacturing process. The quantification of ethanol in gaseous samples was demonstrated in range from 50 to 500ppm (V/V) in accord with concentrations in exhaled breath. The response current increased linearly with increasing vapor ethanol concentration.

Keywords: bio-sniffer, ethanol, electrochemical detection, CMOS integrated device, paper-based enzyme electrode

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1. Introduction

Lifestyle diseases linked to lifestyle choices such as diabetes, obesity, hypertension and so forth has raised a common issue for all humanity. Most of cases are reached a fairly advanced stage without any subjective symptoms, therefore the early and regularly diagnosis is really vital for preventing the progression. Nowadays, preventive medicine referred to as personal health care by serial self-checkup using small inspections has been gradually expanding. In the personal health care, the needs of a breath analysis have drawn noteworthy attention. It has been confirmed the existence of various biomarkers which indicate the presence or progress of diseases in exhaled breath [1-3]. This method is non-invasive and not limited in a place or a time when sampling the medical specimen, so can realize as a secure and flexible medical evaluation.

In fact, compact gas sensors for health care have been developed. These sensors often read a change in electrical resistance of a semiconductor film by gas adsorption and have features to enable repeated measurements with high sensitivity. The semiconductor-based detection principle, however, tends to provide a false result due to a poor selectivity, and have an adverse effect on detection accuracy because of repeated measurements [4-7].

Today, the studies of electrochemical gas sensing systems using an enzyme characterized by its high substrate specificity which enables to detect specific target substance for breath analysis have been advanced. The detection of a substance reads electron transfer derived from an enzyme-catalyzed reaction as a current value. In electrochemical method, an enzyme electrode which supports an enzyme and in contact with aqueous phase on the surface is often used as a detection area. The immobilization method of enzyme is vitally significant in the fabrication of an enzyme electrode, a current detection area, and various methods have been reported [8-10]. Additionally, another process is required for the preparation of the aqueous phase [11]. These conventional complex production steps, however, take a lot of time, efforts and costs due to optical, chemical and other complicated processes.

In our attempts, we propose to use a chromatography paper (ChrPr) consisting of cellulosic fiber as an electrode material. Paper has been extensively utilized for the development of cost-effective and portable electrochemical paper-based analytical devices [12- 14]. The porosity and biocompatibility of paper allows reagents to be stored or immobilized [15], so it enables to keep an enzyme and provide a water supporting layer simply. Furthermore, an enzyme electrode is easily fabricated by designing electrode pattern with a carbon pencil on ChrPr [16, 17]. The low cost, bio-degradability and combustibility of the paper-based electrodes make it suitable for disposable and safe single-use sensor [15].

In addition, a small and portable CMOS integrated circuit chip was designed to carry out the electrochemical measurement with the connection to the smartphone. The recent report proposed the sensing devices connected to the earphone jack of a smartphone with low-cost and small-size [18-20]. The sensing devices are allowed to work by the electric power from a smartphone without the external power supply and send back the measured data to the smartphone using analog and digital signals. The CMOS integrated circuit operates at low power and enables to incorporate various circuits in a minute space, so it is suitable for combination with a smartphone, and it is excellent in miniaturizing the sensing system.

This paper proposes the novel approach of electrochemical gas sensor which is made connection with an iPod touch, the custom CMOS chip and the paper-based enzyme electrode, and the usability is tested through amperometric analysis of ethanol vapor.

2. Research Method

The detection of ethanol via the enzyme-catalyzed reaction was realized by the alcohol oxidase (AOD: 70units/mL, CAS RN 9073-63-6, From Picha Pastris, SIGMA ALDRICH), the peroxidase (HRP: 70units/mL, No:9003-99-0, From horseradish, Wako) and the electron mediator ferrocyanide ion $[\text{Fe}(\text{CN})_6]^{4-}$ (Ferro: 10mmol/L, Wako). The reactions in the sensing mechanism are described as follows:



The enzymatic reaction is schematically illustrated in Figure 1. Ethanol ($\text{C}_2\text{H}_5\text{OH}$) is oxidized to the acetaldehyde (CH_3CHO) by dissolved oxygen (O_2) in the AOD catalytic reaction process as the initial step, where the hydrogen peroxide (H_2O_2) is also generated. The hydrogen peroxide is then oxidized to H_2O by Ferro as electron acceptor, ferricyanide ion $[\text{Fe}(\text{CN})_6]^{3-}$ (Ferri) is also generated. The electron transfer from the electrode to Ferri finally occurs. This sensing scheme gives rise to electrical signal generated by the chemical reaction owing to the presence of enzymes derived from adding ethanol as above.

The electron detection method is the chronoamperometry in which the current occurring at the electrode caused by the potential step is monitored as a function of time. The current value, $I(t)$ [A], detected by this measurement method is represented by the Cottrell's equation as shown Equation 4.:

$$I(t) = nFAC\sqrt{\frac{D}{\pi t}}, \quad (4)$$

where n is the number of electrons, F is the Faraday constant [C/mol], A is the area of surface electrode [m^2], C is the initial concentration of reacting substances [mol/cm^3], D is the diffusion coefficient of reacting substances on the electrode [cm^2/s], and t is the time [s]. The current is directly proportional to the concentration of reacting substances on the electrode, and inversely proportional to the square root of time.

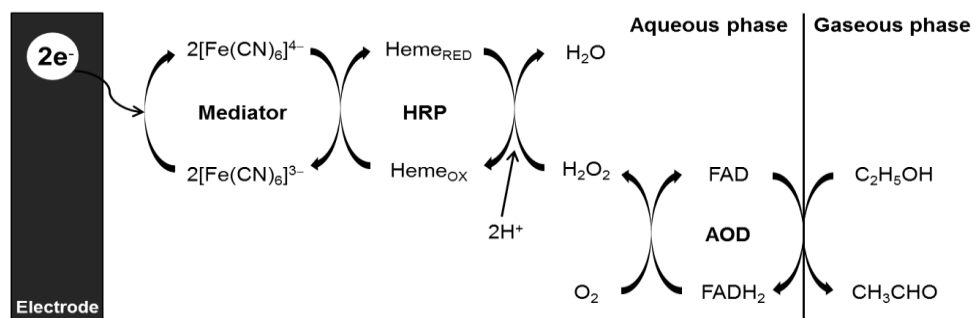


Figure 1. Schematic illustration showing the principle of operation of the biosensor on the electrode for ethanol measurement by the enzymatic reaction

The experimental setup is shown in Figure 2. The connection is established among an iPod touch (MDA714J/A, iOS 7.1.1, Apple Inc.), the custom CMOS chip, a USB connector and the paper-based enzyme electrode. The fabrication process of the paper-based enzyme electrode is shown in Figure 3. The three-electrode system consisting of a working electrode (WE), a reference electrode (RE), and a counter electrode (CE), was mainly made from ChrPr (No.3001-861, Whatman) and commercial 6B grade pencils (StaedtlerMars). The fabrication process is as follows: the two-dimensional pattern was printed on ChrPr with wax printer (ColorQube8580, Xerox), baking into constant temperature dryer (ONW-300S, As One, 120°C) for 1 minute, forming a hydrophobic area impregnated with ink on the reverse side to immobilize enzyme and Ferro (Figure 3. (a)). After the paper was cooled down at room temperature, WE, CE on the enzyme area and wiring lines were directly drawn by carbon pencils by hand. The RE is coated with Ag/AgCl ink (BAS) characterized by high potential stability, drying for 24 hours at room temperature, thereby paper-based electrode were accomplished (Figure 3. (b)). The design of the electrode is determined so that a larger current can be detected with a minute space. The Ferro solution 60 μ L and AOD/HRP enzyme solution 60 μ L is based on phosphate buffer solution (PBS, 100mmol/L pH = 7.0) prepared by mixing K₂HPO₄ and KH₂PO₄ as a solvent dropped on each area (Figure 3. (c)). The PBS has the effect of stabilizing enzyme activity due to the constant pH. After that, the paper-based electrode was baked in a constant temperature dryer (40°C) for 30 minutes to turn into immobilized substances in the ChrPr. These paper-based enzyme electrodes are folded at the boundary of each region for connection to the custom CMOS chip by using a USB connector.

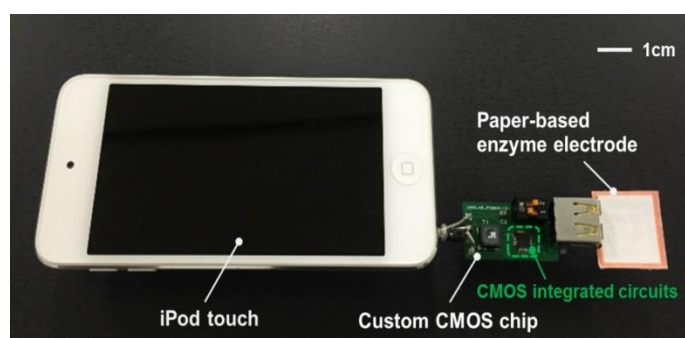


Figure 2. Photograph showing the experimental setup with the connection of an iPod touch, the custom CMOS chip and the paper-based enzyme electrode

The schematic block diagram of the custom CMOS chip in this study is illustrated in Figure 4. The chip is composed of CMOS integrated circuit with a power supply circuit, a potentiostat and a $\Delta\Sigma$ modulator. It takes chronoamperometry at the potential -0.2V vs. Ag/AgCl (RE) when it is connected to the earphone jack of the iPod touch. The power supply circuit with

chip capacitor performs AC/DC conversion on the boosted sinusoidal voltage via the transformer from the right channel, and supplies DC power to the entire circuit. In the potentiostat, -0.2V input voltage is applied to WE with respect to RE and the output current between WE and CE was monitored. The $\Delta\Sigma$ modulator which is clocked by sinusoidal voltage via AC coupler from the left channel transfers the read-out analog voltage data to the iPod touch after analog/digital conversion by the pulse density modulation (PDM) at the sampling rate: 2.5kHz . The transferred data is finally displayed by an in-house application that is installed on iPod touch. The resistance $2\text{k}\Omega$ connected to the $\Delta\Sigma$ modulator in parallel plays an equivalent role to the internal resistance of the earphone and makes it possible to perceive the input to the smartphone.

The sample vapor consisting of ethanol gases (0, 50, 100, 200, 500ppm (V/V)) are prepared by volatilizing ethanol solution (99.5%, Wako) in air, assuming the reference value of car driving under the influence of alcohol [21].

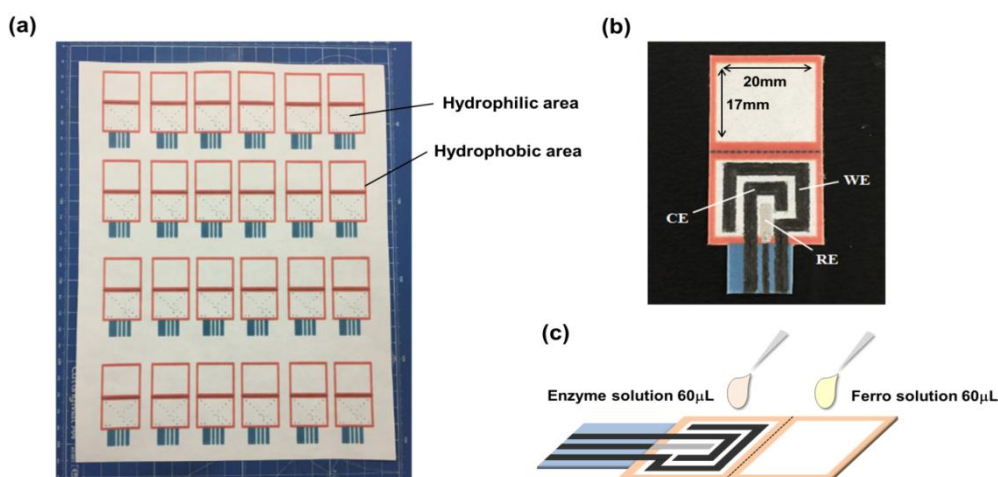


Figure 3. The fabrication process of the paper-based enzyme electrode. (a) Patterns printed on A4 ChrPr and dried at 120°C . (b) Three-electrode system was drawn on the WE, CE and wiring lines using a graphite pencil, RE using Ag/AgCl ink. (c) The adhesion of Ferro and AOD/HRP enzyme to its respective hydrophilic area.

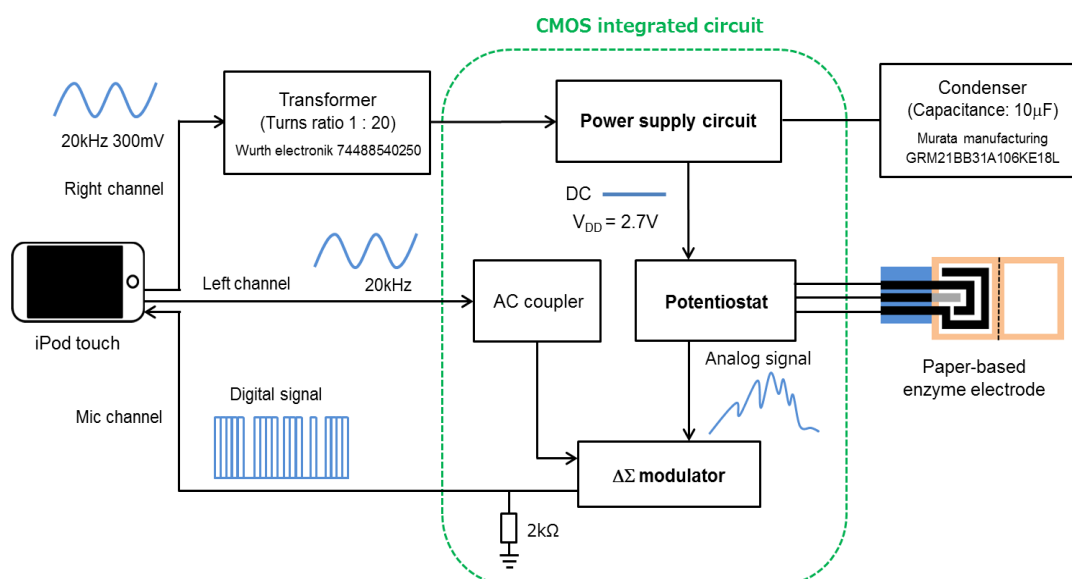


Figure 4. Block diagram of custom CMOS chip

3. Results and Analysis

Typical measurements were started after dropping 120 μ L phosphate buffer solution (PBS, 100mmol/L pH = 7.0) in hydrophilic areas so as to ensure an aqueous phase. In 20s, sample gases by 30mL was blown to the aqueous phase using a syringe simulating human breathing, and performed measurements until 200s. After each measurement, the paper-based enzyme electrode was replaced with a new one in five repeated measurements.

Enlarged screenshots of the measurement results in the iOS application are shown in Figure 5, which shows representative results from five measurements for each concentration. The horizontal line is the measurement time [t] (sampling period: 0.4096s), and the vertical line indicates the output current value [μ A] of each specimen vapor.

The background current which flows immediately after the start of the measurement without blowing samples was detected in every case. It is surely considered to be mainly attributed to the reduction current of Ferri produced due to some undesirable reasons such as oxidative action of O₂. This current decreases with time in accord with the Cottrell equation (4) as stated in the previous section.

After blowing 0ppm vapor at 20s, the increase in the current value with the time was not identified. In contrast, results of other vapor containing ethanol show increase in a current value corresponding to each concentration with time. This is attributed to the generation of Ferri via enzyme-catalyzed reaction in accord with the theory as stated in the previous section. In short, it means that a generated current due to enzyme-catalyzed reaction is added to the background current. From the above, the capability of this sensing system to detect ethanol in gas as a current indirectly and the achievement of the detection limit of 50ppm have been proven.

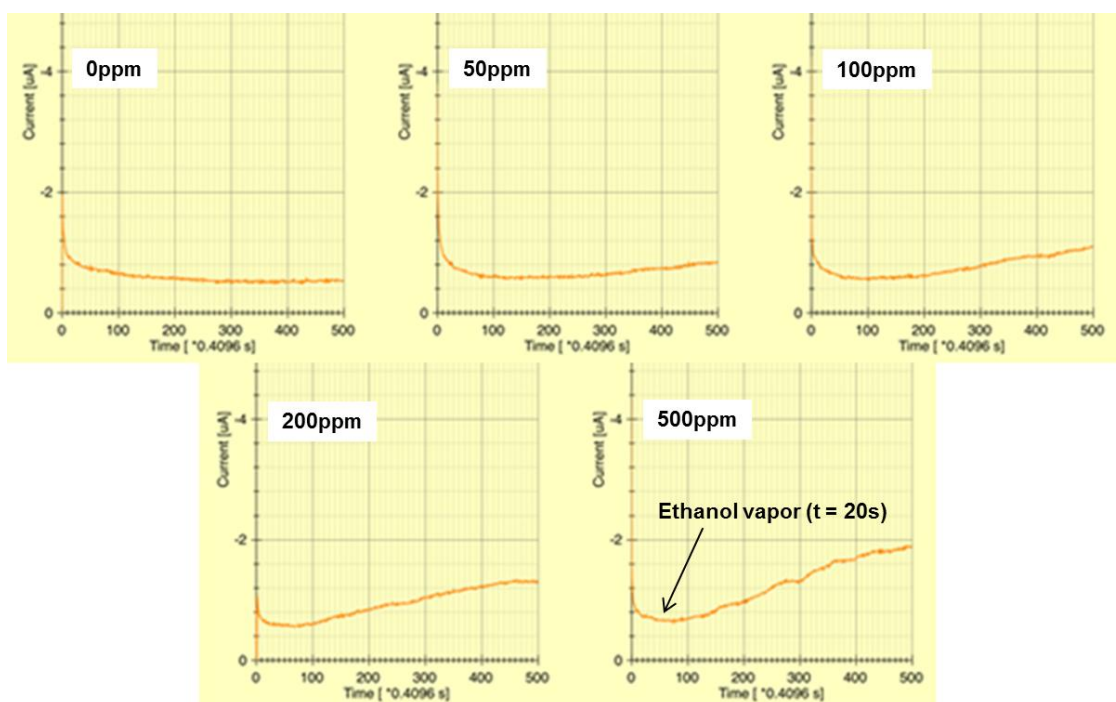


Figure 5. Typical current responses of modified paper-based enzyme electrode for several ethanol vapor concentrations: 0, 50, 100, 200, 500ppm (V/V)

The output current data at 200s obtained from each measurement and the average current value of each ethanol concentration correlated by them are listed in Table 1, and Figure 6 indicates results of ethanol concentration dependence of the output current value, calibration curve based on the average current and error bars of 5 independent examinations at 200s. The amperometric response current increased linearly with ethanol concentrations

between 0 and 500ppm (V/V). The regression equation of calibration curve was estimated from the least square method as follows:

$$I_s = 0.0027S + 0.7761, \quad (5)$$

where I_s is the output current [mA], S is the ethanol concentration [ppm (V/V)]. The slope which indicates the sensitivity 0.0027 and the intercept which indicates the undesirable background current 0.7761 suggest evaluation indexes of a sensitivity and a undesired amperage. The coefficient of determination $R^2 = 0.9620$ calculated from the average current of individual concentration shows the strong correlation between current and ethanol concentration in vapor ranging from 0 to 500ppm (V/V). Moreover, coefficients of variation (CV) value 11.34 correlated in independent concentration was obtained on average, and indicated a satisfactory reproducibility as a biosensor. It is considered that the main cause of this variation is the difference of the blowing ethanol amount, the design of the electrode part by hand labor, and the individual specificity of the ChrPr. If these human errors in the fabrication and measurement process can be avoided, R^2 and CV value are certainly expected to be improved.

Table 1. Results of ethanol detection in gas samples in 200s

Ethanol Con. [ppm (V/V)]	Output current value data of each measurement [μ A]					Average current [μ A]
0	0.5535	0.5497	0.6972	0.6636	0.6254	0.6179
50	0.8692	1.1353	1.0527	0.9832	0.8364	0.9754
100	1.3808	0.9380	1.0711	0.9182	1.1613	1.0939
200	1.5298	1.5649	1.3088	1.1613	1.5183	1.4166
500	2.2071	2.3371	1.8172	2.0710	1.8929	2.0651

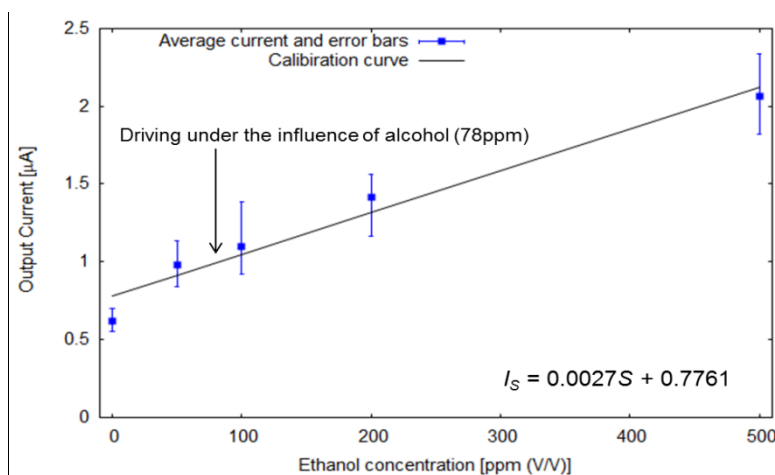


Figure 6. The calibration curve of the reduction current taken at $t = 200$ s with the linear range at 0 - 500ppm (V/V) of ethanol gas. (I_s : Output current [μ A], S : Ethanol concentration [ppm (V/V)])

4. Conclusion

In this work, a novel type of selective, portable and disposable electrochemical gas sensor based on a smartphone, the analytical CMOS integrated device chip and the paper-based enzyme electrode was fabricated. Assessment of the performance by electrochemical quantitative analysis of ethanol vapor within the range of 0ppm to 500ppm (V/V) including the detection limit of 50ppm was carried out, and the ability was verified to apply for gaseous analysis by self-checking.

Further work for diverse applications in this sensing system will be supposed to detect novel biomarkers for lifestyle diseases using dissimilar enzyme. For instance, acetone concentration in exhaled breath varies from 0.3-0.9 ppm for healthy people and more than 1.8ppm can be detected for diabetics [22]. Therefore, we will aim at higher sensitivity and lower abnormal current by devising the enzyme immobilization method or the representative electrode arrangement, and optimizing the amount of use of each reagent and so on.

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