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The design of a novel complementary metal oxide semiconductor detection system for biochemical luminescence

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Abstract

We designed a complementary metal oxide semiconductor (CMOS) chip with accompanied accessories as a system for the detections and quantifications of biochemical luminescence. This is the first of such instruments that has been reported. The semiconductor chip was manufactured through a 0.25 μm CMOS standard process. A current mirror was designed in integrated circuit (IC) to amplify the signal current that was induced by chemiluminescence. Horseradish peroxidase (HRP)–luminol–H_2O_2 system was used as an example to constitute a useful platform for coupling to chemiluminescence reactions which produce H_2O_2. Glucose–glucose oxidase (GOD) reaction was coupled with HRP–luminol–H_2O_2 reaction to demonstrate the ability of the novel CMOS base instrument for quantifying the biological luminescence of a variety of valuable clinical assays. Our results illustrated that the combination of the specifically designed CMOS IC and commercially available electronic devices established a simple and useful bioanalytical tool.
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1. Introduction

Following the progress of modern technology, especially in biotechnology and microelectronics, a worldwide medical revolution is expected. There is a general trend toward more decentralized and immediate diagnostics (Hofmann et al., 2002; Kwakye and Baeumner, 2003; Askari et al., 2001). Thus, the development of an accurate, portable, relatively inexpensive and easy-to-use biosensor has become the most important issue in the healthcare industry (Baeumner et al., 2003; Choi and Gu, 2002; DeBusschere and Kovacs, 2001). The area of micro total analysis systems, also called "lab on a chip" or miniaturized analysis systems is growing rapidly (Reyes et al., 2002; Jain, 2003; Weigl et al., 2003). The current development of semiconductor chips for biosensor may overcome traditional problems and satisfy today and future's requirements (Yang et al., 2002).

1.1. Luminescence assay

Bio- and chemi-luminescence are powerful tools for assaying a variety of important biological molecules. Modern electronic instruments have made it possible to measure light emission precisely. Thus, ordinary chemicals or enzyme-catalyzed reactions coupled with light emitting systems can be used to determine a variety of important biological molecules (Deluca, 1978; Kurittu et al., 2000; Karatani and Konaka, 2000). The horseradish peroxidase (HRP) with its substrate luminol and H_2O_2 reaction system is one of the most popular chemiluminescence enzyme assay systems (Kricka and Thorpe, 1990). This reaction could be a platform reaction to quantify many reactions that produce H_2O_2 (Kricka et al., 2000; Nozaki et al., 1996; Nozaki et al., 1999). In this report, we designed the CMOS photodiodes array IC and constructed a novel instrument for the quantification of luminescence produced by biological reactions.

1.2. COMS photodiodes as chemiluminescence sensor

In the past, a photomultiplier tube (PMT) has been widely used as the sensor of a luminescence reaction. The

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characters of cost, bulk appearance, and high power consumption limited its application to personalized or field used instruments. CMOS photodiode is a semiconductor light sensor that can be produced by standard industrial semiconductor procedures. Recently, the applications of charge-coupled device imager, CMOS camera and photodiode as array biosensors have been compared (Golden and Ligler, 2002) for biological imaging. We have demonstrated, utilizing the sophisticated HP4145 instrument, that CMOS photodiodes could apply to biochemical analysis (Lu et al., 2003). In this report, the novel design takes advantages of a standard CMOS processed IC and a common commercial multimeter to detect and quantify biological luminescence. The development of this technique is a stepping stone toward a hand held, cheap, and convenient home care instruments. This presents a good indication to the potential of the combination of biotechnology and IC design in microelectronics technology.

2. Materials and method

2.1. Electronic apparatus

The commercial instruments used in this report were E3646A power supply (Agilent), 34401A multimeter (Agilent), General Purpose Interface Bus (GPIB) card (National Instruments) and connection wire (National Instruments), and a common personal computer (PC). The whole structure of the novel luminescence detection system reported in this report is shown in Fig. 1.
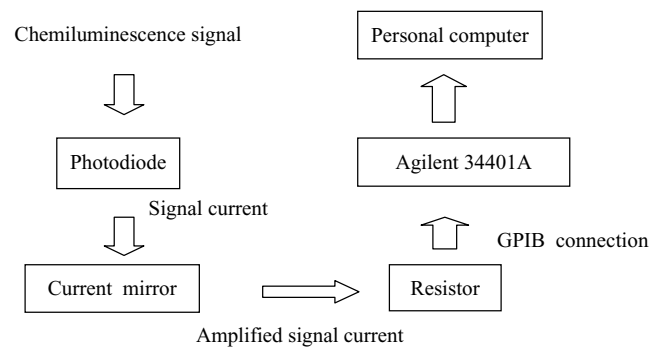

Fig. 1. The flow chart of a CMOS base luminescence detecting system. While exposing to the chemiluminescence, the CMOS photodiodes generated the signal current. The signal current was amplified by the current mirrors and translated into voltage signal consequently by a resistor. Agilent 34401A collected the data and transferred it to the personal computer for further processing.

2.2. The design of current mirror in CMOS chip

Fig. 2 shows the electric circuit design of the CMOS chip. The mathematics model of the current mirror while the FET is working under saturation region is simply described as following (Sedra and Smith, 1998).

$$i_D = \frac{1}{2}kn'\frac{W}{L}(V_{GS} - V_t)^2 \qquad (1)$$

where i_D is the current flowing through CMOS FET from source to drain, kn' a constant, W the width of the channel of CMOS FET, L the length of the channel of CMOS FET, $V_{GS} = V_G - V_S$, and V_t the threshold voltage of the CMOS FET.
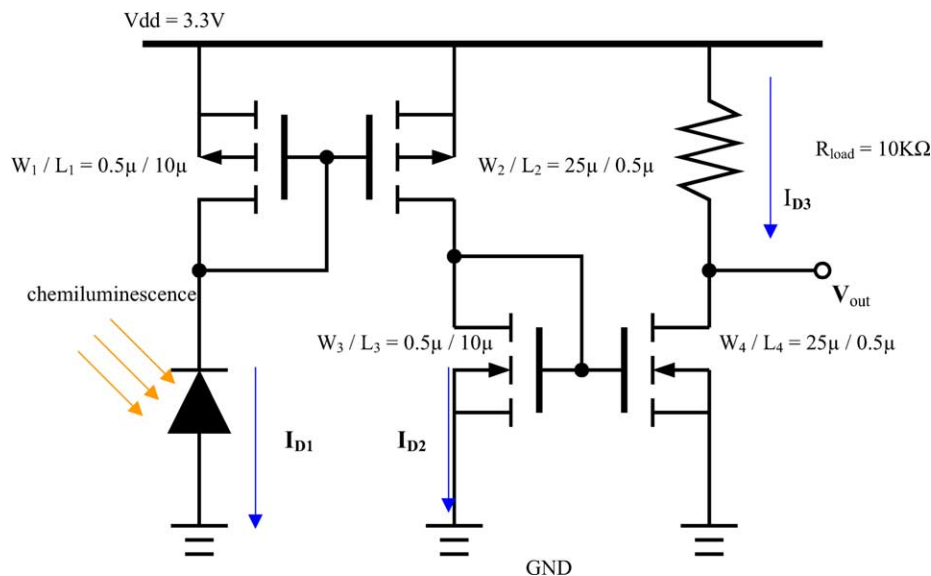

Fig. 2. Current amplifier design diagram and parameters of CMOS chemiluminescence sensor chip. The chip was manufactured by a 0.25 μm CMOS standard process and worked under constant voltage of 3.3 V. The current amplifier was designed with two stages of current mirror, each of them have the factor of 10^3 for amplifying the current. After two-stage amplification, i_{D_3/i_D1} would be 10^6. R_{load} (10 kΩ) was an external circuit resistant, which converted the i_{D3} to voltage signal.

One current mirror was composed of two FETs. In our study, kn', V_{GS}, and V_t were all under the same condition, therefore, the factor of amplification only depends on the ratio of W/L. After considering the range of original induced signal current i_{D1} (pico-ampere) and the range of resistor $(k\Omega)$, we designed the parameter of each FET as:

$$\frac{i_{D2}}{i_{D1}} = \frac{W_2/L_2}{W_1/L_1} = \frac{i_{D3}}{i_{D2}} = \frac{W_3/L_3}{W_2/L_2} = \frac{25\,\mu m/0.5\,\mu m}{0.5\,\mu m/10\,\mu m} = 10^3 \tag{2}$$

After two stages of amplification, $i_{D3}/i_{D1} = 10^6$, where i_{D1} is the current signal caused by chemiluminescence and i_{D3} the output current signal.

2.3. Chemicals and biochemicals

D-(+)-Glucose, HRP, luminol and bis–tris-propane were purchased from Sigma (St. Louis, MO, US). H_2O_2 (30% (w/w)) and H_2KPO_4 were purchased from Riedel-deHaën (Buchs, Switzerland). Glucose oxidase (from *Aspergillus niger*) was purchased from Fluka (Seelze, Germany). Tris–HCl buffer was purchased from Amersham Biosciences (Buckinghamshire, UK). K_2HPO_4 was obtained from J.T. Baker (Phillipsburg, NJ, US).

2.4. Enzyme preparation

HRP powder (1 mg or 80 U) was dissolved in 1 ml Tris–HCl buffer (0.1 M at pH 8.6). The powder of GOD (20 mg or 3000 U) was dissolved in 1 ml phosphate buffer (0.2 M at pH 7.0). The enzyme solution was stored at $-80\,°C$ before use. The HRP and GOD solutions were melted in ice bath just before use and were diluted with the specified buffer. One unit of HRP is defined as 1.0 mg of purpurogallin formed from pyrogallol in 20 s at pH 6.0 and $20\,°C$ by Sigma. One unit of GOD, defined by Fluka, will oxidize 1 μmol glucose/min at pH 7.0 and $25\,°C$.

2.5. Enzyme assay

The optimal pH value and temperature for the HRP–luminol–H_2O_2 system have been reported before (Thorpe and Kricka, 1986). In our experiment, the reaction mixture included luminol (1 mM), H_2O_2 (1 mM), Tris–HCl (100 mM at pH 8.6) and HRP (0.1–2 U) at $25\,°C$. An aliquot amount of HRP was first added into the cuvette, and followed by the injection of all other necessary reagents and samples. These processes were to make sure that the whole compounds were well mixed in the cuvette in a short time without extra shaking. The data was collected within 1 s after the final injection. All enzyme assay data were the average of three measurements.

3. Results

3.1. The design and construction of CMOS base light detecting instrument

Figs. 1–3 depicted the design of the CMOS system. The components of the system were diagramed in Fig. 1. Fig. 2
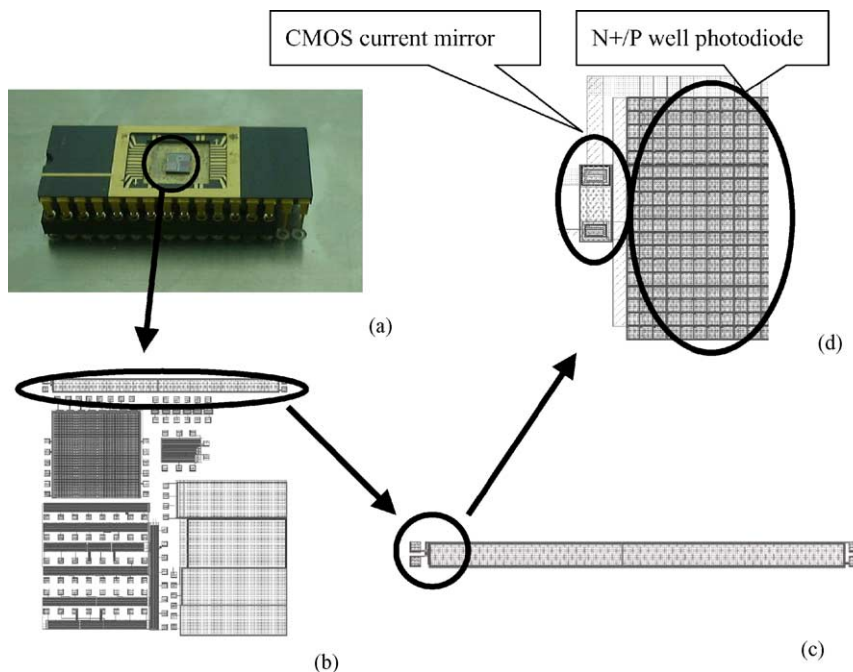

Fig. 3. Layout diagrams of CMOS chemiluminescence sensor chip. (a) CMOS chemiluminescence sensor chip. (b) The layout of the whole die. (c) The layout of photodiodes: the left side array (18×150) contains two sets of the current mirror. The array on right side (18×173) was a reference array without current mirror. (d) The magnified diagram of current mirror and photodiodes array: each pixel of photodiode was in the size of $10\,\mu m \times 10\,\mu m$, and two stages of current mirror amplify the current 10^6 times.

was the diagram of current mirrors included in this CMOS sensor chip. The CMOS chip shown in Fig. 3(a) was manufactured with a 0.25 μm CMOS standard process fabricated by Taiwan Semiconductor Manufacturing Company (TSMC), Hsinchu, Taiwan. The layout below the photodiode array in Fig. 3(b) was not used in this report. The chemiluminescence sensor chip was an 18×150 photodiode array with pixel size 10 μm $\times$ 10 μm for each photodiode. The current amplifiers were shown on the left of Fig. 3(c). The photodiode array on the right side was the reference array that did not contain the current mirror. This reference array was used to make sure that the current amplifiers make sense.

In Fig. 2, resistor $R_{load} = 10\,k\Omega$ was an external circuit resistant. R_{load} converts the current signal to a voltage signal. In addition, R_{load} was manually changeable. This allowed us to tune the output range of the voltage signal. As shown in Fig. 2, the voltage signal was:

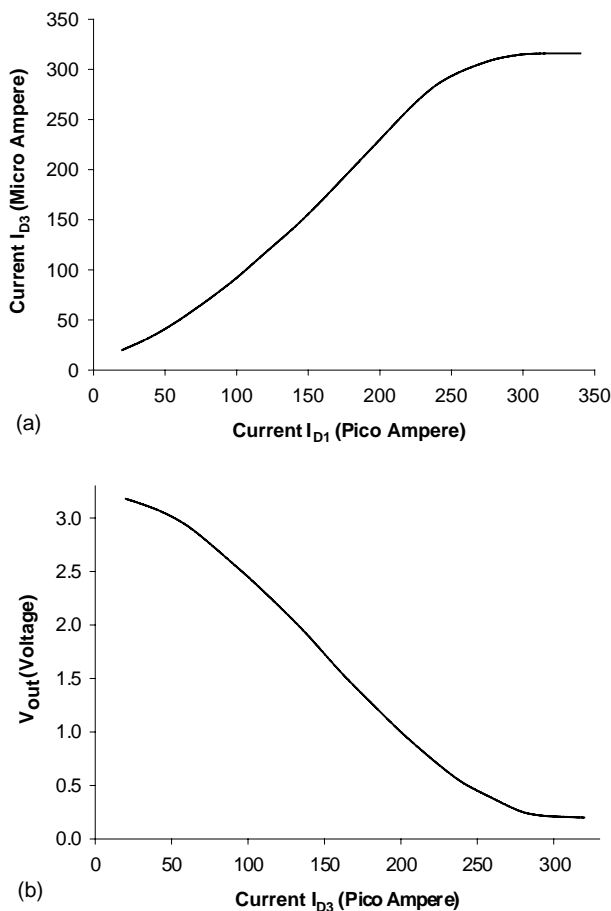$$V_{load} = V_{dd} - V_{out} \quad (3)$$

(a)

(b)

Fig. 4. Simulation data of the CMOS chemiluminescence sensor chip. The electric circuit simulation tool, Simulation Program with Integrated Circuit Emphasis (SPICE), kindly offered by department of Electronics Engineering, National Chiao Tung University, Hsinchu, Taiwan, was used to simulate the circuit of a chemiluminescence sensor chip diagramed in Fig. 2. (a) I_{D1} vs. I_{D3} simulation data. (b) I_{D1} vs. V_{out} simulation data.

The E3646A power supply supported a constant voltage $V_{dd} = 3.3\,V$ to the CMOS chemiluminescence sensor chip. The 34401A multimeter collects the V_{load} data and transferred it to a personal computer (PC) through GPIB connection as shown in Fig. 1 for the signal flow chart.

3.2. Circuit simulation

The simulation results of the current mirrors' (Fig. 2) amplification effects were shown in Fig. 4(a) and (b). The simulated relation between I_{D1} versus I_{D3} was shown in Fig. 4(a), and that of I_{D1} versus V_{out} was shown in Fig. 4(b). Chemiluminescence generated current I_{D1} was quite linear in the range of 30–240 pA. In Fig. 4(b), the I_{D1}–V_{out} curve shows that the V_{out} stayed at 0.2 V instead of linearly decreasing when I_{D1} was larger than 240 pA. While $V_{out} < 0.2$ V, V_{DS} of the FET in the second stage current mirror was too small to maintain the amplification properties.

3.3. Data processing of enzymatic reactions

The original data of $V_{netload}$ versus time was shown in Fig. 5. $V_{netload}$ was defined below as:

$$V_{net\,load} = V_{load} - V_{blank} \quad (4)$$

where V_{load} was defined in Eq. (3). V_{blank} was the voltage load caused by the dark current, and it was a constant 0.32 V.

The integrated $V_{netload}$ data that was processed manually with Excel (Microsoft) were shown in Fig. 6 as typical progress curves for enzymatic reaction. The tangent slopes at the beginning ($t = 0$) of the curves imply the initial rate of enzymatic reactions. According to the tangent slopes at the beginning ($t = 0$) of the curves, the enzyme kinetics analysis could be figured out, and the relation between HRP units and initial reaction rate was shown in Fig. 7. To obtain the tangent slope at $t = 0$, a function $y(t)$ was used to
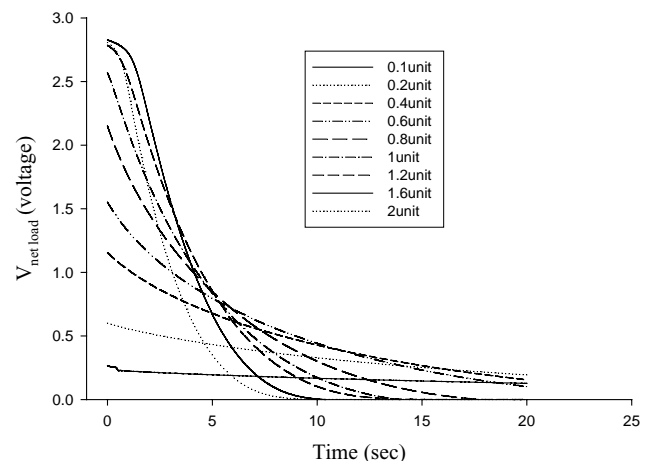

Fig. 5. Change of voltage induced by the HRP–luminol–H$_2$O$_2$ reaction system. The HRP–luminol–H$_2$O$_2$ reaction condition was described in Section 2.5. The $V_{netload}$ data was collected continuously with the CMOS base luminescence detecting system.
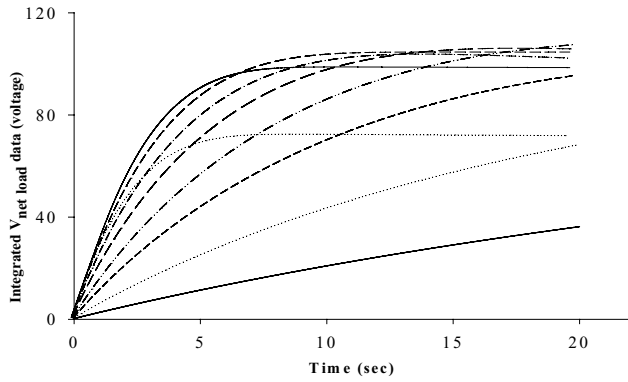
Fig. 6. Integration of $V_{netload}$ data generated by HRP–luminol–H_2O_2 reaction. The integrated $V_{netload}$ data obtained from Fig. 5 was processed manually with Excel (Microsoft).

describe each curve shown in Fig. 6, and calculated the $y'(0)$ for each curve which can be differentiated at $y(t)$, $t = 0$.

The Regression Wizard of software Sigma Plot 2001 was used to fit the curve shown in Fig. 6. The function:

$$y(t) = \frac{1 + at}{b + ct} \tag{5}$$

was selected to fit the first 5 s of the curves shown in Fig. 6(b), where a, b, and c are constant coefficients. Then, from Eq. (5), this equation was derived:

$$y'(t = 0) = \frac{ab - c}{b^2} \tag{6}$$

After the fitting, Sigma Plot 2001 returned the value of a, b, and c. The value of $y'(0)$ was calculated with the returned coefficient. The data in Figs. 7–9 were applied with the same process. The $R2$ values for the fitting in this report were all higher than 0.998.

3.4. Kinetic data obtained by CMOS detection system

The H_2O_2 standard curve obtained by the CMOS chemiluminescence sensor chip was shown in Fig. 8. A typical
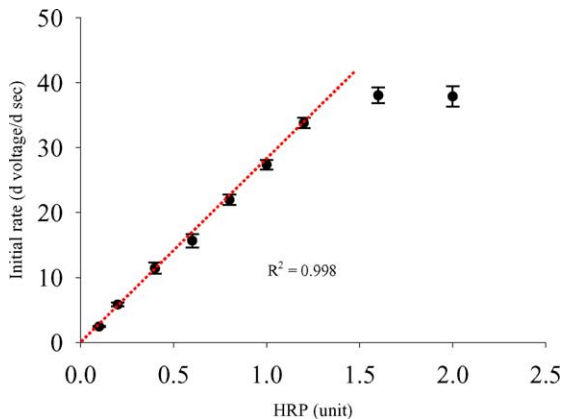

Fig. 7. HRP enzyme activities profile obtained by CMOS base luminescence detecting system. The enzymatic activities were the initial slopes of the reaction curves in Fig. 6.
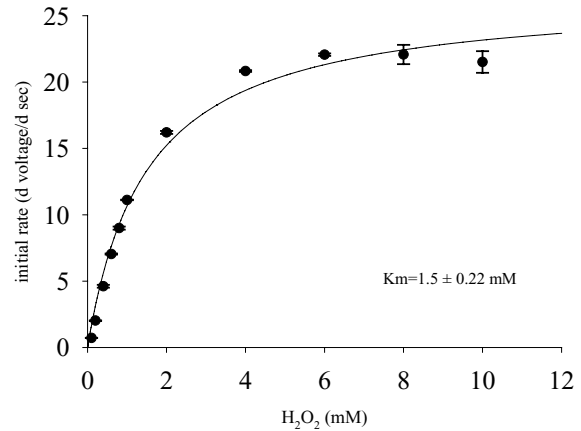

Fig. 8. Michaelis–Menten plot of H_2O_2 with the data obtained by CMOS base luminescence detecting system. The reaction mixture included luminol (1 mM), Tris–HCl (100 mM at pH 8.6), HRP (0.32 U), and H_2O_2 (0.1 mM to 15 mM) at room temperature. The experimental procedures were described in Section 2.5. The data process was described in Figs. 5–7 and in the text. The curve fitting and K_m value were obtained with the Michaelis–Menten equation using Sigma Plot 2001.
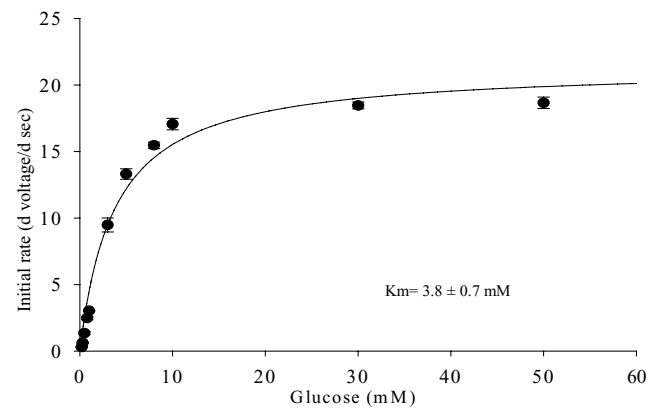

Fig. 9. Michaelis–Menten plot of glucose with the data obtained by CMOS base luminescence detecting system. The reaction mixture for this coupled enzymes system includes 150 U GOD, 0.32 U HRP, luminol (1 mM), and Tris–HCl buffer (100 mM, pH 8.6) in final volume of 1 ml at room temperature. The mixture of luminol, Tris–HCl buffer, glucose and GOD had been incubated for 10 min at room temperature prior to the addition of HRP to start the reaction. Each data point was the average of three measurements and was processed as described in Fig. 8.

Michaelis–Menten progress curve was obtained and the K_m of H_2O_2 was 1.5 mM as determined by a non-linear regression program. The couple enzyme system using glucose as the variant substrate was shown in Fig. 9. A typical Michaelis–Menten progress curve was also obtained and the K_m of glucose was 3.8 mM.

4. Discussion

The current mirrors played a key role in the CMOS chemiluminescence sensor chip. Because of the noise produced in regular electronic devices, it would be impossible for us to

detect and quantify the current signal at pico-ampere level unless the signal can be amplified without noise interference. The pico-ampere level electric signal can only be detected with some extremely sensitive instruments (e.g. HP 4145/4156 Semiconductor Parameter Analyzer). In this report, the current mirrors have been shown to faithfully amplify the signal obtained by the CMOS sensor, and allowed us to collect the data with a common commercial multimeter.

4.1. Analysis of chemiluminescence produced from enzymatic reactions

In typical enzyme assays using spectrophotometer, the signals observed by UV-Vis absorption or fluorescence were accumulated. However, the luminescence cannot be accumulated in the chemiluminescence assays. The HRP–luminol–H_2O_2 reaction was a flash type chemiluminescence reaction; the brightest emission happened at the beginning of the reaction as shown in Fig. 5. Thus, progress curve similar to a typical enzyme assay was reconstructed (Fig. 6). The progress curves shown in Fig. 6 precisely matched the expected result for typical enzyme assays. The integrated voltage became lower when the excess enzyme was used (1.6 and 2.0 U HRP). This result was also expected and defined the useful range for the enzyme assays in this report.

4.2. Comparison of the simulated and experimental data

As shown in Fig. 4(a) and (b), the responding curves were quite linear while chemiluminescence generated I_{D1} was located in the range of 30–240 pA. In Fig. 4(b), the I_{D1}–V_{out} curve shows that the V_{out} stayed at 0.2 V instead of linearly decreasing when I_{D1} was larger than 240 pA. While $V_{out} < 0.2$ V, V_{DS} of the FET in the second stage current mirror was too small to maintain the amplification properties.

The experimental data agreed with the result of the simulation. In Fig. 5, the initial $V_{netload}$ of the excess amount of HRP (1.2–2 U) reactions were almost the same (about 2.8 V). According to Eq. (4), we add the constant voltage load 0.32 V caused by the dark current to $V_{netload}$,

$$V_{load} = V_{net\,load} + V_{blank} = 2.8 + 0.32 \fallingdotseq 3.1 \text{ V} \qquad (7)$$

According to Eqs. (3) and (7), the V_{out} of the initial rate of HRP (1.2–2 U) is,

$$V_{out} = V_{dd} - V_{load} = 3.3 - 3.1 = 0.2 \text{ V} \qquad (8)$$

The result matched the phenomena in Fig. 4(b) that V_{out} stayed at 0.2 V, while I_{D1} was larger than 240 pA.

4.3. Quantification of HRP, H_2O_2, and glucose with a CMOS base chemiluminescence detection system

As shown in Fig. 7, the linear relation between HRP (unit) and initial rate $y'(0)$ stayed from 0.1 to 1.2 HRP units. Two

possible reasons may limit the linearly range in Fig. 7. One was that the MOS FET working characters, as described in the previous section. Biochemical design may be the other reason that gave the linear range limitation. Under high HRP units, the initial rate of HRP–luminol–H_2O_2 system would be too fast for us to collect. At this point, the linear range of the enzyme profile was good enough for our current research. The results defined the effective assay range of this CMOS system, and helped us to set the HRP conditions in our following experiments.

The HRP–luminol system could couple with many other enzyme reactions that produce H_2O_2 (Kricka et al., 2000; Yang et al., 2002). Thus, our ability to determine H_2O_2 indicates that many other enzymes and biochemicals which are important for clinical diagnosis and other bio-related research can be determined. The relation between luminescence intensity and H_2O_2 concentration is shown in Fig. 8. A typical curve that fits the Mechalis–Menten equation was obtained with our novel CMOS base instrument. The K_m value was 1.5 mM, which was in the same range with the K_m (1.1 mM) obtained from a standard PMT instrument, Hitachi F4500 (Yang et al., 2002; Lu et al., 2003).

Glucose plays an important role in metabolism and is an important target for biochemical diagnosis. To determine the glucose concentration, the coupled enzyme assays, GOD and HRP, were also performed with the CMOS base instrument. The enzyme kinetic analysis of a glucose standard curve observed with the CMOS base instrument is shown in Fig. 9. The K_m value of glucose was 3.8 mM by this method, which was in the same range with the K_m (3.4 mM) obtained from the standard PMT instrument (data not shown).

5. Conclusion

In this study, a CMOS based chemiluminescence biosensor for glucose and H_2O_2 have been reported. It established a foundation, in equipments as well as in methods, to determine various chemiluminescent assays on chip. Following the pace, not only chemiluminescence but also fluorescence and UV-Vis absorption CMOS based biosensors for various important assays could also be developed.

We have demonstrated that the combination of CMOS photodiode, IC design technique and commercially available electronic devices are useful to quantify biological enzyme assays. CMOS process, which is widely applied to digital and analog IC is very common and standard in the semiconductor industry. This means that CMOS based instruments have the advantages to be relatively low cost equipments for healthcare industry with mass production. In addition, CMOS showed great capability of logic processing and, in the future, allow the sensor chip to perform the data processing by itself. With some portable power supply, a new perspective to personal health care industry is expected. However, the insufficient sensitivity of CMOS photodiodes does handicap some applications. This may be

improved following the development of novel semiconductor manufacture processes. Overall, the characteristics of CMOS chips make them very attractive to be developed as personalized clinical diagnostic instruments.

Acknowledgements

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