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An AlGaAs/GaAs Photo-Transistor-Based Fluorescence Detection System for Human Serum Albumin

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This study reports a fluorescence detection system using bio-sensors, combining AlGaAs/GaAs photo-transistors with a biomarking technique for sensing human serum albumin (HSA). Following excitation, the fluorescence of biomarkers was used as the signal source and an AlGaAs/GaAs heterojunction photo-transistor with micro-flow channels and built-in amplification was used as the sensing device. The entire chip comprised a total of 808 connected photo-transistors and a total sensing area of 18.18 mm^2. Linear results were observed in the sensing range of 0.01 to 0.07 mg/mL with the sensing current of 1.75 to 2.62 μA, which covers the range from normal to slight proteinuria required for early warning applications. In addition, the HSA bio-sensor can be reused promptly after flushing the device with pure water.
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In clinical medicine, the medical condition of each organ in the human body can be determined by detecting changes in biomolecules such as glucose and proteins in the blood and urine. For example, in the clinical detection of nephropathy, glomeruli function can be determined by quantifying urinary protein.

The use of indicator papers is a traditional method with which to qualitatively analyze urinary proteins; however, this method can produce false positives or false negatives, resulting in faulty identification. Turbidimetric immunoassay, high-pressure liquid chromatography, and the detection of fluorescence are other methods that can be used for quantitative analysis. Turbidimetric immunoassay and high-pressure liquid chromatography precisely measure the quantity of proteins; however, they require complex operations and expensive equipment and reagents. Likewise, the detection of fluorescence requires complex optical instruments and the use of specific software for the analysis of optical signals. These three methods require an excess amount of time and money and fail to provide the convenience necessary for a practical detection system.

It would be highly advantageous to develop a compact low-cost sensor with high sensitivity and accuracy that is capable of detecting specific biomolecules and providing immediate results. Patients would not have to spend excessive time undergoing examinations and could even perform preliminarily exams on themselves.

Recently, the field of bio-sensors has drawn a considerable amount of interest because of the requirements of medical diagnostics. Optical bio-sensors are capable of detecting and identifying chemical or biological species. They offer a number of advantages, such as the ability to perform remote sensing with a high degree of selectivity and specificity and the ability to use unique bio-recognition schemes. In 1962, Clark and Lyon reported the fabrication of the first bio-sensor.[1] They constructed an "enzyme electrode," comprising immobilized enzymes on an electrochemical detector, with a substrate capable of responding to the concentration of enzymes. In the 1970s, Yellow Springs Instrument Company (YSI) commercialized a Clark invention, the enzyme membrane, resulting in the first practical use of a bio-sensor for the rapid, accurate measurement of blood sugar. Over the years, these advances have been extended to applications in bio-technology, health care, and sports medicine.

Tremendous progress in material sciences has enabled the development of low-cost bio-sensors of high sensitivity for the detection of human serum albumin (HSA).[2–6] In this study, an AlGaAs/GaAs-based heterojunction bipolar transistor (HBT) was used to fabricate a heterojunction photo-transistor (HPT) array as a sensing device, in which the fluorescence of the biomarker was used as a signal source. This study demonstrates a fluorescence detection system for detecting HSA in which the HPT of the sensing device is combined with a biomarking technique and micro-flow channels. Measurement results indicate that the AlGaAs/GaAs photo-transistor-based fluorescence detection system is capable of measuring the concentration of HSA in the range of 0.01 mg/mL to 0.07 mg/mL, thereby covering the range from normal to slight proteinuria required for early warning applications.

Design and Fabrication

Based on fluorescence, a biological signal was developed in which HSA is immobilized using an infrared dye ($C_{38}H_{46}ClN_2NaO_6S_2$) and infrared light emitting diodes (IR LEDs) are used as a light source for excitation.[7] The immobilized compound emits infrared light while returning to the ground state, and the emitted light is received by the sensing device to measure the concentration of HSA.

The buffer solution was prepared using Na_2HPO_4 dissolved in ultra-high-purity water at 10 mM and was adjusted to a pH of 7.4 by using H_3PO_4. The HSA powder was dissolved in a buffer solution to obtain the different concentrations of the HSA solutions. In addition, the dye solution was $C_{38}H_{46}ClN_2NaO_6S_2$ dissolved in CH_3OH, which was diluted in the buffer solution at 0.2 mg/mL. The HSA and dye solutions were prepared in terms of volume ratios (1:1) for use as the test solutions. The excitation spectrum of the dye solution was between 750 nm and 850 nm.[7]

In the sensing device, an AlGaAs/GaAs photo-transistor array was fabricated for use as the bio-sensor. The bio-sensor used in this study was based on the structure of a conventional AlGaAs/GaAs *npn* HBT without a base metal contact. The original contact area of the base was enlarged and adopted for use as the light-receiving (sensing) area. The energy bandgap of GaAs material in the base and collector structures enabled converting infrared light into an electrical current, and the photo-generated current was amplified by a built-in *npn* AlGaAs/GaAs HBT. The regular gain of the transistor current was approximately 50. The main structural layer of the AlGaAs/GaAs HBT is shown in Table I, where the base and collector layers form the main absorption regions. The sensitive wavelength of the photo-transistors, which depended on the GaAs material, was between 600 nm and 870 nm.[8] Both the cap and sub-collector layers were designed for Ohmic contact and the only metal contacts were on the emitter and collector layers. The size of the individual photo-transistor was 230 $\times$ 230 μm^2 including the surrounded collector metal. A pair of photo-transistors with sensing area of 150 $\times$ 150 μm^2 in the base area is shown in Fig. 1a. Fig. 1b shows a schematic cross-section of the device, in which most of the infrared light is absorbed in the base and collector layers and the generated carriers are amplified and collected using the surrounding collector electrode. The size of the entire chip

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Table I. Structure of the epitaxial layer of the AlGaAs/GaAs-based photo-transistor.

Layer	Material	Thickness (nm)	Doping (cm^{-3})
Cap	$In_{0.6}Ga_{0.4}As$	50	1×10^{19}
	$In_yGa_{1-y}As$ ($y = 0 \sim 0.6$)	500	1×10^{19}
	GaAs	150	5×10^{18}
Emitter	$Al_{0.3}Ga_{0.7}As$	45	4×10^{17}
Base	GaAs	160	4×10^{19}
Collector	GaAs	500	2×10^{16}
Sub-Collector	GaAs	500	5×10^{18}

was 15 × 15 mm^2 including bonding pads, and the layout is shown in Fig. 2. The entire chip comprised a total of 808 connected photo-transistors and a total sensing area of 18.18 mm^2. The layout of the chip was also designed to be integrated with micro-flow channels to provide a steady flow of tested solution onto the surface of the chip.

Fig. 3 shows the micro-flow channel system, in which the chip is bonded to a printed circuit board (PCB) and sealed within a micro-chamber with electrical connections beneath. Two wires were used to attach the PCB to the sensing device. In and out tubing was designed with a diameter of 2 mm, embedding it at a 45-degree angle with a space of 8 mm between input and output ports on the surface of the chip.

Figs. 4a–4b show the complete fluorescence detection system with a tubing pump and measurement equipment. The HSA and dye solutions were prepared in terms of volume ratios (1:1) for use as the HSA test solutions. Prior to the test, 5 mL of various concentrations of the HSA test solution were prepared and excited using the illumination of infrared LEDs for 5 min. After illumination and excitation, the test solutions were pumped through opaque tubing into the micro-chamber and onto the sensors. The corresponding electrical signals were measured using the photo-transistor sensor through optical-electrical conversion. The photo-generated current of the AlGaAs/GaAs photo-transistor array was measured at a forward bias of 1 V by using a parameter analyzer (Agilent B1500).

Results and Discussion

Before using a fluorescence detection system to detect HSA, titration experiments were performed. The HSA test solutions after illumination and excitation were titrated onto the surface of the chip (sensing device). The measurements that involved titration were repeated 7 times using various concentrations of HSA solution from 0.001 to 0.10 mg/mL. Fig. 5 shows one of the experiments in which the photo-generated current changed slightly after seven repeated titrations when the concentration of HSA was 0.03 mg/mL. The average measured current was 8.65×10^{-8} A, and the root-mean-square deviation was 3.39×10^{-9} A. The titration experiments involved measuring the HSA test solution in concentrations of 0.001 to 0.10 mg/mL, the results of which are shown in Fig. 6. The insert of Fig. 6 shows that a linear relationship was observed between the photo-generated

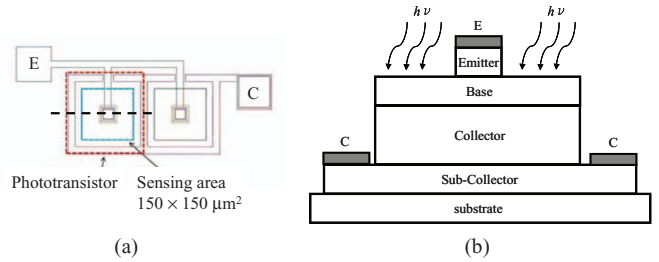

Figure 1. (a) A pair of photo-transistors showing the sensing area and emitter/collector contacts; (b) A schematic cross-section of the device with emitter/collector contacts and absorption layers in the base and collector.

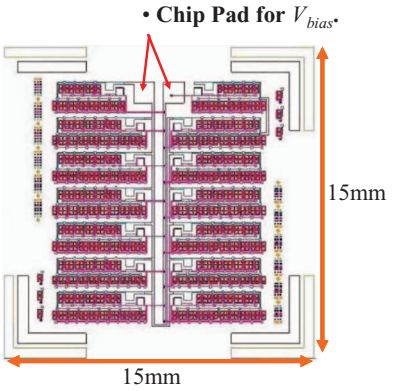

Figure 2. Layout of the entire chip including 808 photo-transistors covering an area of 15 × 15 mm^2 and bonding pads.

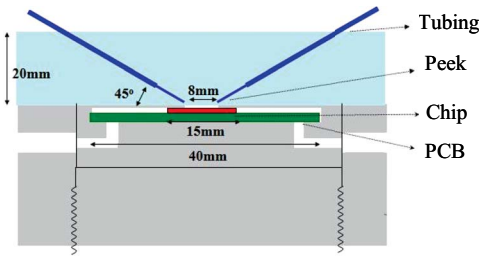

Figure 3. System of micro-flow channels with chips bonded on a PCB and sealed within a micro-chamber.

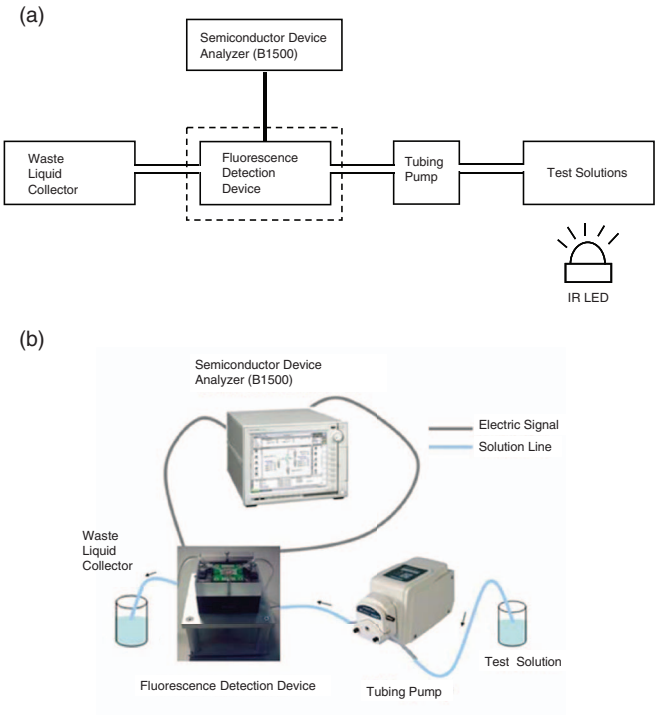

Figure 4. (a) Configuration of the fluorescence detection system in this study. (b) Complete fluorescence detection system with tubing, pump, and measurement equipment.

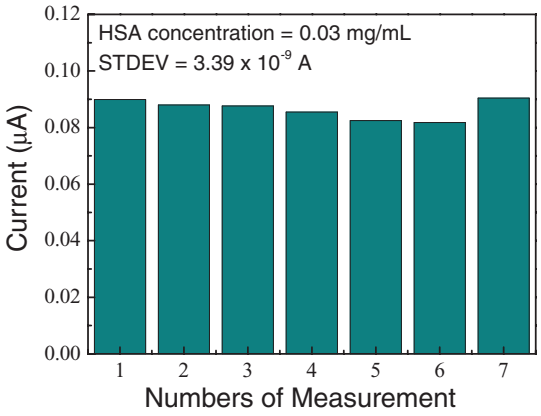

Figure 5. Photo-generated current for the HSA concentration of 0.03 mg/mL when using titration.

current and the concentration of HSA in the range of 0.01 mg/mL to 0.07 mg/mL ($Y = 5.72 \times 10^{-10} X + 7.13 \times 10^{-8}$). Based on the results of the titration experiments, the fluorescence detection system was used for the different concentrations of HSA test solution from 0.01 to 0.07 mg/mL by using a tubing pump.

The HSA test solution was prepared in concentrations of 0.01, 0.03, 0.05, and 0.07 mg/mL. The volume of each solution was 5 mL. After illumination and excitation, the HSA test solutions were pumped through opaque tubing into the micro-chamber and onto the sensors. When the tested solution flowed through the micro-flow channels on the surface of the sensor, the sensors received the emitted light. The generated photo-current varied according to the concentration of the HSA test solutions. The measurement of photo-current versus time for HSA test solutions of different concentrations is shown in Fig. 7. The measured photo-current from the bio-sensors for HSA test solutions of 0.01, 0.03, 0.05, and 0.07 mg/mL were approximately 1.75, 2.05, 2.35, and 2.62 μA, respectively. Between each measurement, pure water was circulated through the device to clean the micro-flow channels and surface of the sensor. The background current of the bio-sensor was approximately 0.61 μA. The values of photo-generated current versus time show that this HSA bio-sensor can be reused for the detection of HSA within a considerably short period of time.

Measurements were repeated 14 times, and the corresponding measurement data for the four different concentrations is shown in Fig. 8. A linear relationship was observed between the photo-generated current and concentration of HSA in the range of 0.01 mg/mL to 0.07 mg/mL ($Y = 1.38 \times 10^{-8} X + 1.60 \times 10^{-6}$). These results show that the photo-generated current increased by

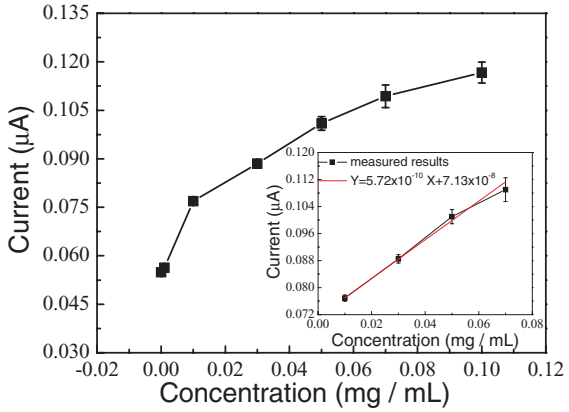

Figure 6. Photo-generated current versus concentration of HSA test solutions when using titration.

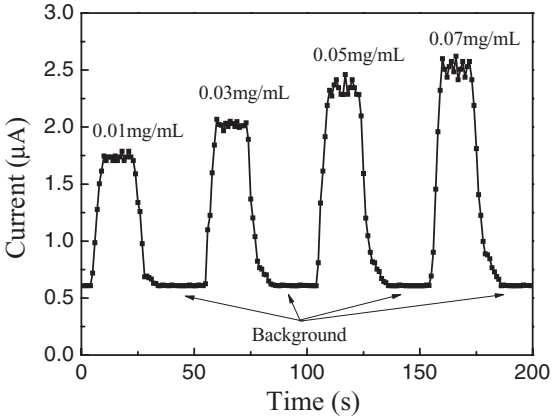

Figure 7. Photo-generated current versus time for the different concentrations of HSA test solution from 0.01 to 0.07 mg/mL when using a tubing pump.

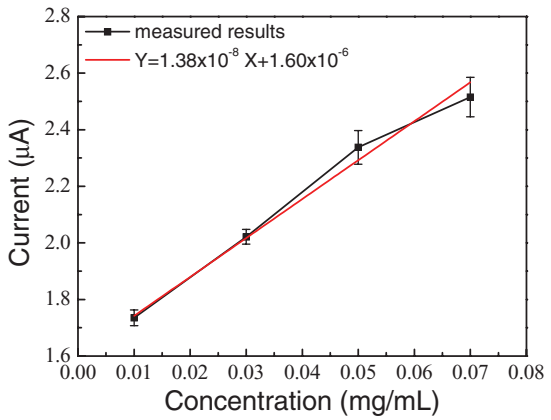

Figure 8. Photo-generated current versus concentration of HSA test solutions from 0.01 to 0.07 mg/mL when using a tubing pump.

13.8 nA when the concentration of HSA was increased by a microgram per microliter within the range of 0.01 mg/mL to 0.07 mg/mL. This is a clear indication that the fabricated sensor is capable of detecting the concentration of HSA in the range of 0.01 mg/mL (normal) to 0.07 mg/mL (slight proteinuria) required for early warning applications.

Conclusion

This study demonstrated an AlGaAs/GaAs photo-transistor-based fluorescence detection system for human serum albumin in which an AlGaAs/GaAs photo-transistor array was fabricated as a bio-sensor. According to the magnitude of the photo-generated current induced by emitted light, differentiating the concentration of HSA in the range from 0.01 mg/mL to 0.07 mg/mL is possible. In addition, the values of photo-generated current versus measurement time show that this HSA bio-sensor can be reused promptly after cleaning the surface of the sensor. This fluorescence detection system provides a feasible system for the early detection of HSA in urine for the diagnosis of kidney disease.

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