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Speed Improvement of a Pathogenic Micro-Organism Population Detection with LAPS System by a Magnetic Bead Separation and a pH Detection.

H.S. Moon[1], S. K. Ryu[2] , D. Y. Yum [1] and H. C. Kim[2]
[1]Department of Biomedical engineering, Seoul National University, Seoul Korea
[2]InBioNET Corporation , Taejon, Korea

***Abstract*— In this paper, a magnetic bead based immobilization method and pH Detection method is applied to the LAPS (Light Addressable Potentiometric Sensor) system to detect a pathogenic micro-organism population. Magnetic beads are very small, superparamagnetic particles (0.8~5.0 μm in diameter) that are able to sustain a magnetic domain under excitation and do not exhibit residual magnetization when the external field is removed. By using magnetic beads as an immobilization method, other bulky and complex method can be alternated. To verify the method, an urease labeled anti-*salmonella typhimurium* antibody is used to detect a pathogenic micro-organism(*S. typhimurium*) population by a bias voltage maximum slope detection.**

Keywords— **LAPS, Magnetic Bead, Immobilization, separation, pathogenic micro-organism population detection**

I. Introduction

Recently biosensors based on the light-addressable potentiometric sensor (LAPS) [2], [3], [4] technique has become popular in various biological applications such as the detection and verification of *Salmonella typhimurium* [5] or Escherichia coli *O157:H7* [6], bacterial DNA [7], and biological warfare [8].

The LAPS is a kind of the ion-sensitive field effect transistor (ISFET), which has a electrolyte /insulator /semiconductor structure. By illuminating parts of the sensor surface with infrared light, a photocurrent flow of which the amplitude depends on the local surface potential or local pH value. In this way, the surface potential distribution can be obtained by scanning the light pointer across the surface of LAPS. LAPS has several advantages to name a few;. First, LAPS is easy to fabricate. LAPS chip is a simply silicon wafer without any complex structure, that is covered with an insulator at the front side and has an ohmic contact layer at the backside. Second, the concept of addressing the point of measurement with a light pointer, therefore no pre-patterning of sensor surface is required. Third, LAPS has a completely flat surface. This leads to an optimization of the microchamber design and of the hydraulics. It can be good advantage to applications with microfluidic system.

We have been developing a portable detection system based upon the LAPS for human pathogenic micro-organisms such as *salmonella typhimurium*. In this project, our major concern is to develop a system faster and easier than the conventional Enzyme-Linked Immunosorbent Assay (ELISA) system. In the previous attempt, the antibody was immobilized on biotin-coated nitrocellulose membrane, which is then brought in contact with sensor surface [5]. This approach requires a separate membrane kit and a plunger to press the membrane against the chip surface, which makes the total system bulky and inconvenient to use.

In this paper, we are investigating the feasibility of using magnetic beads coated with antibodies and enzymes required for detection of micro-organism and signal generation reaction, respectively. Recently micrometer-sized beads are widely used in various applications including filtration of cell, DNA and RNA [10] [11]. Since magnetic beads can be mixed with samples in liquid phase, there is no need for using a separate membrane kit and the resultant immuno-complex can be easily kept in contact with sensor surface by applying magnetic field from the beneath of the LAPS chip.

In order to confirm the feasibility of this idea, the in-house developed LAPS system was tested with different pH value solutions with and without magnetic bead attachment on the sensor surface. And **Urease labeled anti-*salmonella typhimurium* antibody is used to detect a pathogenic micro-organism (*S. typhimurium*) population by a bias voltage maximum slope detection.**

II. Experimental

1. Sensor fabrication

The Light-Addressable Potentiometric Sensor (LAPS) is a kind of Ion-sensitive FET. Basic setup and Structure of LAPS system is shown in Fig. 1. The LAPS ship used in this study is fabricated of a n-type semiconductor (ρ = 5~20 Ω· ㎝), with 1000Å Si3N4 layer and 300? SiO2 layer at the front side as a insulator, and AlO2 at the back side for ohmic contact. This chip was fabricated in Integrated Optics Sensor Lab at Kyung-pook University in Korea.

The LAPS sensor detects the surface potential at illuminated regions of the interface between the electrolyte and the Si3N4 insulator. When the sinusoidal modulated Infra-Red LED illuminates the back side of silicon structure, it produces an alternating photocurrent with a sigmoid-shape I-V curve changing according to the concentration of analyte (Fig. 2). The induced charges charge up the silicon-oxide-electrolyte capacitance and the pH can be computed from the resulting signal (sensitivity: 56mV/pH).

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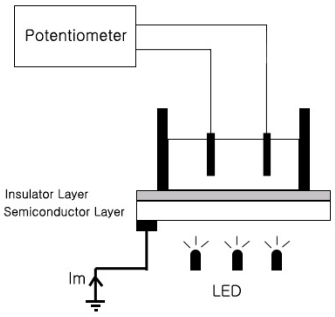

Fig 1 schematic diagram of LAPS system

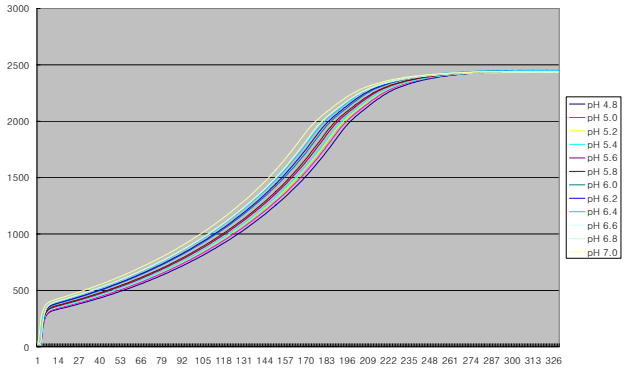

Fig 2 characteristic I-V Sigmoid curve of LAPS

2. Measurement System

To drive the LAPS System, A potentiostat was used to apply a bias voltage between the electrolyte and the LAPS chip, and to convert the photocurrent to a voltage with a factor of 10^6 V/A. the Bias Voltage Rage from -1 to +1V with 12bit. A detected photocurrent is amplified, high pass-filtered to remove an ambient noise, rectified and then acquired with 12bit AD converter. An acquired signal transferred to a PC by Serial communication. All function is controlled by Microcontroller (ATMEL AVR processor). A Data acquisition and processing PC software is made by LABVIEW™ (National instrument), Block Diagram is shown in Fig.3.

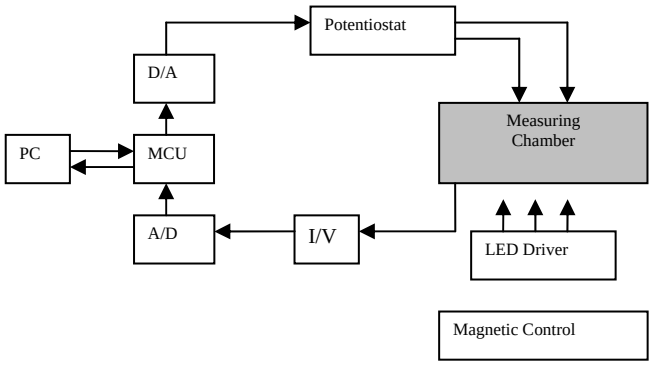

Fig 3 Block Diagram

3. Magnetic bead based bio separation

The Magnetic beads are very small, superparamagnetic particles (0.8~5.0 μm in diameter) that are able to sustain a magnetic domain under excitation. These Beads, which have high magnetic susceptibility, exhibits magnetic dipoles when placed in a magnetic field, but do not exhibit residual magnetization when the external field is removed. After the external magnetic field is removed, these beads are dispersed in the solution.

A simple concept of the magnetic bead based bio-separation and detection with the LAPS system is shown in a Fig. 4. Magnetic beads are introduced on the LAPS surface and immobilized by applying magnetic field. While holding the antibody-coated beads, antigen is injected into the electrolyte. Only target antigens are immobilized and other antigens are washed. For the alternation of other immobilization method such as argarose gel or membrane, LAPS can measure a pH with or without magnetic beads attached. Magnetic field is applied by a permanent magnet. Mechanically moving a magnet, magnetic field is controlled.

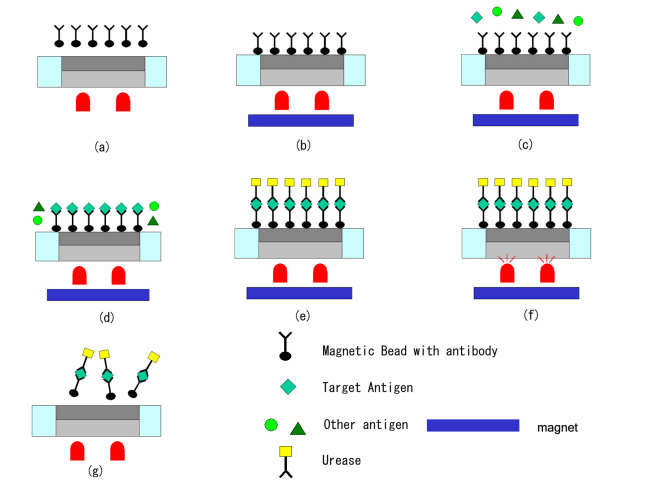

Fig 4. Magnetic bead based Separation

4. anti-*S. Thyphimurium* specific antibody with urease and magnetic bead

To immobilize the target pathogenic micro-organism on the surface of LAPS, Magnetic beads conjugated anti-*S. Thyphimurium* specific antibody (Fig. 6) is used with magnet (Fig 4(d)). And to detect the concentration of pathogenic micro-organism, an Urease labeled anti-*S. Thyphimurium* specific antibody (Fig 5) is injected to the immobilized pathogenic micro organism + magnetic bead complex (Fig 4 (e)). The Change in the redox potential is due to the production of NH3 by a Urease-Cell complex. This change is read by LAPS system as a pH Variation proportional to signal response (Fig 7).

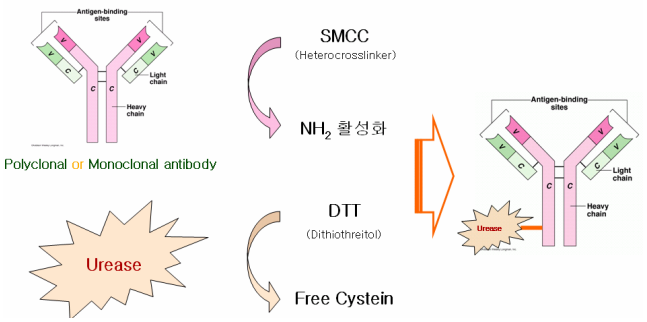

Fig 5 Urease labeled anti-S. typhimurium specific antibody

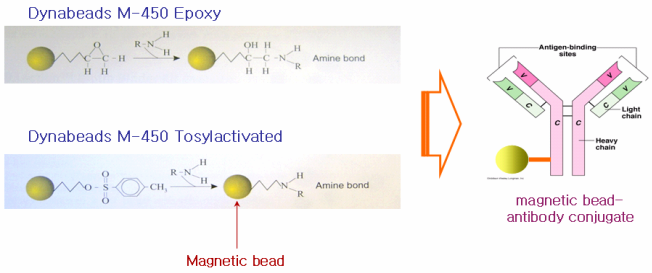

Fig 6 Magnetic beads conjugated anti-S. Typhimurium specific antibody

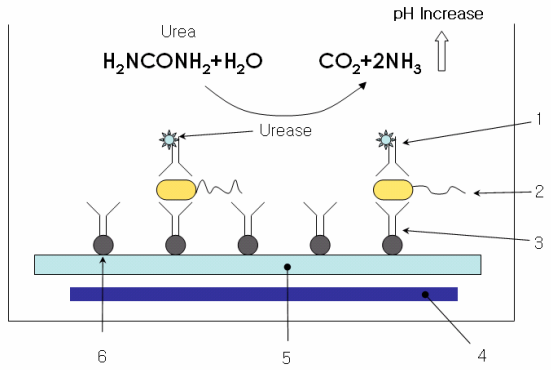

Fig 7 biological component of sensor system
(1). Urease conjugated anti-Salmonella antibody (2). *Salmonella thyphimurium* (3). Anti-Salmonella antibody conjugated magnetic bead (4). Magnet (5). Semiconductor layer (6). Magnetic bead

IV. Result and DISCUSSION

The concentration of bacterial cells in the samples was read by the LAPS system as a pH variation proportional to signal response. The presence of pathogenic cells induces a pH change proportional to the cell concentration (Fig 8). The system is very sensitive to the small change of cell concentration: 10^2 and 10^1 CFU (Colony forming Unit) can be detected over a control blank solution.

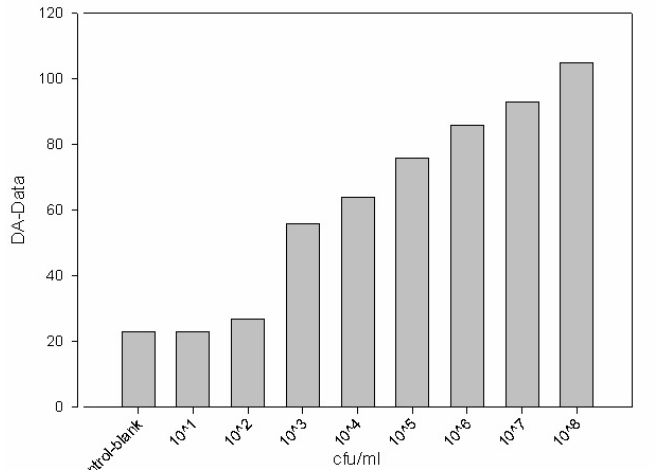

Fig 8 bias voltage variant vs. CFU/ml

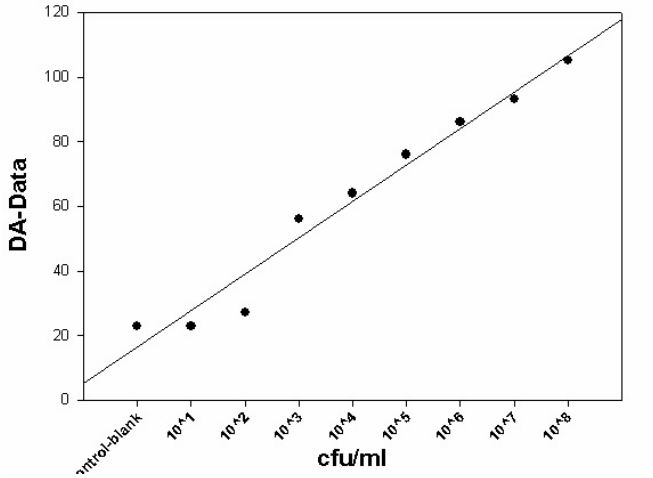

Fig 9 bias voltage variant vs. CFU/ml

V. CONCLUSION

In this paper, we demonstrate how an Magnetic bead based bio separation on the surface of the LAPS was able to specifically detect *Salmonella thyphimurium* By immunoassay. Because Magnetic bead is used for separation method, every process can be automated by fluidic system and electromagnet control. By changing the antibody-magnetic bead complex, we can have the feasibility of the target pathogenic micro-organism without changing a sensor component. By this advantage, we can improve the whole speed of detection process.

ACKNOWLEDGMENT

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