Building a Prototype for a Magnetic Nanoparticle Bead Based Biosensing Device

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***Abstract*—In the modern day society with its modern way of life, there is a growing problem with regard to the health of the people. One of the major concerns right now is the increasing number of overweight and obese people in the world. A way to determine whether the person has this problem is by detecting Adiponectin. The aim of the work is to make use of magnetic beads for future detection of the adiponectin, which is the target analyte of the work. This work will discuss the theoretical background of the target system as well as results for experiments using the prototype of the system implemented using commercially available components. The work shows a working prototype for a biosensing device that will be developed in the microscale level in the future.**

***Index Terms*—Magnetic Beads, Point of Care Testing (POCT), Hall Sensor, Adiponectin, Enzyme-Linked Immunosorbent Assay (ELISA)**

I. Introduction

The need for Point of Care Testing (POCT) has been growing for the past couple of years. It is a tool that may be used in diagnostics which plays an important part in the modern day. They can be separated into four different categories which include clinical diagnostics, veterinary diagnostics, environmental monitoring and food testing. Clinical diagnostics does not only involve detection of disease but also in its prevention and health care

This work will address the growing problem of obesity in the world. In order to deal with obesity, we must understand the physiology of an obese person. Obesity is defined as a medical condition wherein there is an excessive accumulation of body fat and has already reached a point where the person's health problem has increased and the life expectancy has decreased [1]. Therefore we must understand what is body fat. Body fat or fat is referred to as the adipose tissue in histology. It is a loose connective tissue that is composed of adipocytes or fat cells. Its main role in our body is to store energy in the form of fat. It also provides the body with cushion and insulation. It is made of several types of hormones, of which, have different effects on the body. One of the hormones that have been studied and are found to be a promising therapeutic tool in treating obesity and other human diseases is the adiponectin. There are a hundred ways to detect adiponectin, which from here on will also be referred to as the target analyte, but the most commonly used way is the enzyme-link immunosorbent assay (ELISA) protocol [2].

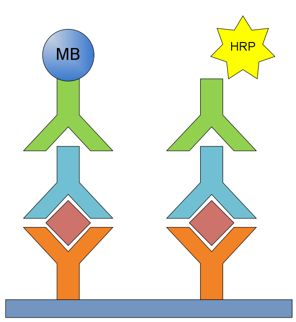

Figure 1. Capture of sample using sandwich ELISA. Illustration shows two ways of detection of sample presence; (left) magnetic bead, (right) HRP used in conventional ELISA

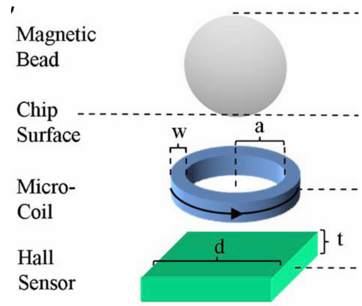

Figure 2. Magnetic Bead Sensing System [3]. The system make use of inductor as a source of polarization.

The work aims to develop in the future a portable POCT device that would utilize the characteristics of the magnetic beads to detect the concentration of the adiponectin protein hormone. Magnetic beads are ideal for such kind of bioassay application due to the following reasons: (a) cells exhibit few magnetic properties, (b) signals from magnetic beads are stable with time, (c) results from magnetic detection is independent of the color and clarity of the sample, and (d) magnetic labeling has functionalities that may be used to improve bioassay performance such as magnetic filtration and manipulation [3].

As a proof of concept for the microscale device, this work will present a working prototype developed using commercially available components. For the prototype, 2mm steel balls will be used in place of the magnetic beads that are superparamagnetic. Superparamagnetic behavior is an important property of magnetic beads used for detection since it prevents the beads from clustering at the absence of magnetization [4]. The components used to build the prototype are also discussed. There are several types of devices that can be used in the detection of magnetic field. Of which include

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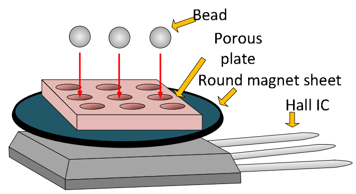

Figure 3 Circuit model of the sensing system. This includes steel balls, magnetic sheet and commercially available hall sensor IC.

superconducting quantum interference devices (SQUIDs), Giant Magnetoresistive (GMR) Sensors, spin valve sensor, Tunneling Magnetoresistive (TMR) sensors, and Anistropic Magnetoresistive (AMR) sensors. However, such devices are not ideal for dense arrays due to the presence of magnetic field caused by applied bias conditions [4]. For this work, a commercially available hall sensor, WSH 136, was used in the system and is characterized in the following article. The said device is ideal for such application since it is designed to track extremely small change in the magnetic flux density [5].

The work will give a theoretical background of the concepts of which the work is based on. Such discussion will include description of adiponectin, the ELISA protocol, the magnetic beads, and the sensor system. After which, a detailed discussion of the implementation of the circuit model will be given. This is then followed by the discussion of the experimental data and results，conclusion and future work.

II. Theoretical Background

A. Adiponectin

Adiponectin is a protein hormone that is secreted exclusively by the adipose tissue into the bloodstream. It is relatively abundant in plasma as compared other hormones. Its function includes modulation of a number of metabolic processes. These processes include glucose regulation and fatty acid catabolism. Study shows that the higher the level of adiponectin, the lower is the production of insulin. Our blood sugar is also better controlled. The increased number of adiponectin will reduce the risk of diabetes and heart disease. Furthermore, people with plenty of adiponectin will have a better controlled weight. Although the adiponectin is produced from body fat, it is found that the plasma level of adiponectin is inversely proportional to the body fat. Therefore, as adiponectin level is lowered, the weight of the person further increased. Some studies have shown that the adiponectin production is increased when a person does exercise.

B. Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) is a protocol dominantly used for medical diagnostics. The work will make use of its basic principle for the detection of the target analyte, which refers to the substance being to be analyzed by the procedure. The protocol will consist of three different antibodies. The first antibody will be the capture antibody that will be placed on the surface of the micro plate and is responsible for capturing the target analyte from the sample. The second antibody will attach itself to the target analyte captured. The third antibody is the detection antibody which will have horseradish peroxidase (HRP) attached to it and will bind to the second antibody. Figure 1 illustrates the architecture of the said antibodies and analyte. In general ELISA protocol, the concentration of the sample will be calculated based on the optical density measured from the result of the experiment. Optical density can be described as the amount of light absorbed by the target sample. HRP is used to increase the detectability of the target analyte. The number of detection antibody that will remain on the sample will be proportional to the concentration of the target analyte. The reaction between the HRP attached on the detection antibody and a substrate will change the opacity of the sample giving it a much denser color. The optical density of the solution is then measured and is presumed to be the concentration level of the target analyte in the sample. A low value of optical density will mean a higher concentration of the target analyte present in the sample. For this work, the ELISA protocol will slightly be altered. The detection antibody will be attached to magnetic beads instead of HRP. There will no longer be a need for a substrate to react with sample. The number of magnetic beads left in the sample will determine the level magnetic field detected by the sensing system. Figure 1 illustrates the difference between the typical ELISA protocol and the proposed structure with the magnetic beads. This process is often referred to as magnetic assays.

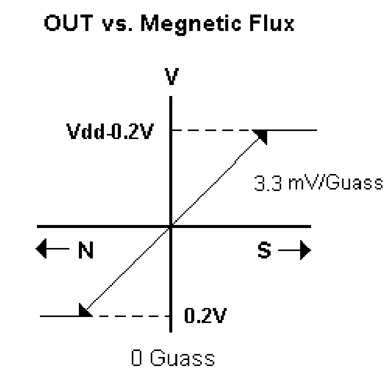

Figure 4. Characteristic curve of the WSH 136 commercial hall sensor IC. [5] The figure illustrates the output voltage of the component with regard to the sensed magnetic field

C. Magnetic Beads

Magnetic beads are made from $\gamma - Fe_2O_3$ or Fe_3O_4 nanometer crystals that are dispersed in a polymer matrix [4]. Their superparamagnetic behavior is an important property when used for biomedical detection. When the beads are coated with specific chemical substance they are able to bind to specific biological material. They can be used in POCT devices that use bioassays for detections and measurements. The super paramagnetic characteristics of these beads are ideal for such applications since they have little or no intrinsic magnetization. This means that there will be no clustering of magnetic beads at the absence of magnetic field.

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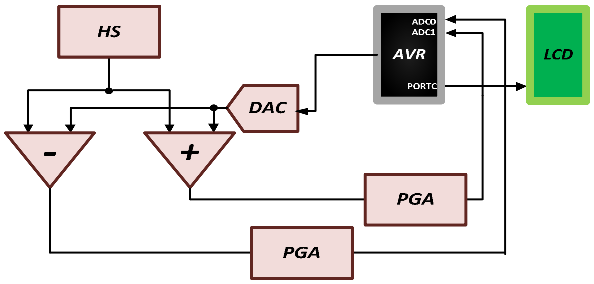

Figure 5. Illustration of the readout circuit diagram for the implementation of the large scale model. The system also includes a microcontroller that would process the data to human understandable number.

D. *Sensing System*

Figure 2 illustrates a sample magnetic beads sensing system [3]. The sensing system is composed of a hall sensor and a micro-coil. The system is implemented using the complementary metal oxide semiconductor (CMOS) technology. In order for the hall sensor to detect the magnetic beads, it needs to have a flux generator to induce magnetic field onto the super paramagnetic beads. A micro-coil is, therefore, placed on top of the hall sensor to serve such purpose. This, at the same time, will serve as a calibration mechanism for the system. Since there is a magnetic field source specific for the system, the environmental magnetic fields are negligible. The hall sensor would then be connected to a source that will provide an electrical signal running on a uniform direction. And another distinct electrical signal will be measured. An example of such electric signal is having a constant current fed through your hall sensor. And for the measured electrical signal; the voltage across the hall sensor shall be taken. At the presence of additional magnetic field which will come from the super paramagnetic beads, at constant supply current, the value of the measured voltage will change in proportion to the number of magnetic particles present in the system.

III. Circuit Model Implementation

A. *Sensing System*

For this work, the magnetic beads are replaced with steel ball with a 2mm diameter. The steel balls are ideal for this work

Table I. List of Components Used in Implementation of the Large Scale System

Component	Model
Shift Register	74LS164
Digital to Analog Converter (DAC)	DAC0808
Operational Amplifier (OPA)	LMC6484
Microcontroller	ATmega8535
Hall Sensor	WSH 136

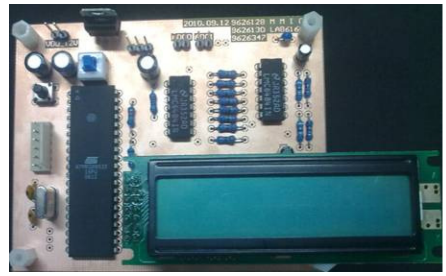

Figure 6. A picture of the whole large scale system implemented on a custom made PCB board.

since they are unpolarized and get magnetized when placed near a magnetic field which is similar to the characteristic of magnetic beads. The downside, though, is that they will not automatically demagnetize when taken away from a magnetic field. Therefore, this work also made use of a demagnetizer circuit to demagnetize the steel balls after each use. The system was made such that the system would be able to test up to a quantity of nine steel balls. A porous plate was made to hold the steel balls in place. In place of the micro-coil, a round magnetic sheet is placed for polarization of the steel balls. On the bottom is the commercially available hall sensor integrated circuit (IC). The hall sensor already have amplifier that would preprocess the reading from the hall sensor. The illustration of the described sensing system is shown in Figure 3.

B. *Characteristic of the Hall Sensor IC*

The work made use of a commercially available hall sensor IC made by Winson. The model used in this work is the WSH 136. Integrated into this component is a hall sensing element, linear amplifier, sensitivity controller, and emitter follower output stage [5]. The component operates in a voltage range of 3.0V to 12V. It has a sensitivity of 3.3mV/Gauss. Figure 4 illustrates the operating curve of the sensor IC at a given range of magnetic field.

C. *Circuit Implementation*

The circuit for readout circuit is illustrated in Figure 5. The system includes the following components: shift register, digital to analog converter (DAC), operation amplifier (OPA), microcontroller, and the hall sensor IC. The system consists of an adder and a subtractor. These components are used to compute the analog signal from the hall sensor. The system simultaneously adds and subtracts the output the DAC from hall sensor IC.

Figure 7. LCD display output of the whole sensor system.

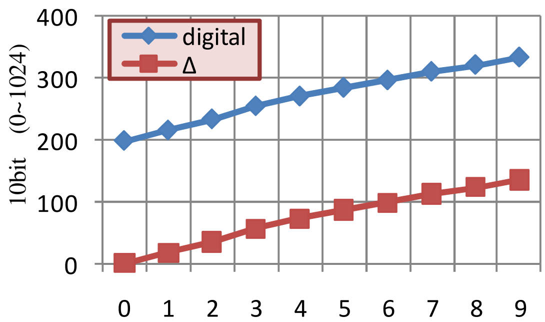

Figure 8. Digital output value for the system generated from 0 to 9 numbers of steel balls placed on the sensor system.

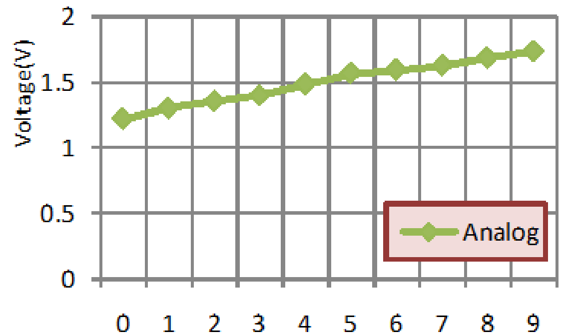

Figure 9. Analog output value for the system generated from 0 to 9 numbers of steel balls placed on the sensor system.

Their output is then fed into a programmable gain amplifier that is designed using OPAs. Since they are of the same potential and current we are able to derive equations (1) and (2). Although the adder and the subtractor work simultaneously, only one path will be used for the measurement of the result. This will be done by the microcontroller.

The system will perform an Auto Zero calibration or AZC which is performed when no steel ball is placed on the system. This is done to eliminate the magnetization coming from the surrounding. This will be done every time the device is turned on and is used to account for the offset given by the magnetic field from the surrounding. Then when the steel balls are placed, the computation of the magnetization measurement will start. The output from the adder and the subtractor will be fed into the microcontroller wherein the internal analog to digital converter (ADC) will be used to convert the analog signal to a digital value that could be processed by the microcontroller. The two values are then averaged and compared to the values from the adder and subtractor through the use of an XOR operation until the desired value is reached.

$$HS - DAC = V- \quad (1)$$

$$HS + DAC = V+ \quad (2)$$

The implemented system is illustrated in Figure 6. And the list of commercially available components used in the building of this system is listed in table 1.

D. Data Display

Figure 7 shows a picture of the LCD display for the reading of the system. Table 2 shows the description of each value in the LCD display. “AZC” stands for the Auto Zero Calibration which is the initial reading value of the system. The initial value is the reading when there is no steel ball placed on the system. The “initial reading” will account for the magnetic field surrounding the system. The magnetic field from the surrounding will be the offset value of the system. This value will change at different locations. Therefore, auto zero calibration will be time each time the device is used. This also means that the “initial value” will vary each time the device is used. The “corrected value” stands for the value of the reading when a certain number of steel balls are placed on the system. And “MAG” will stand for the magnitude of the readout. The “measured value” will have the same value as that of the corrected value. The “variation” will stand for the change in the output value between the first reading and the second reading.

IV. Data and Results

Figure 8 shows the digital output value of the system while Figure 9 shows the analog output value of the system. The digital output shows a linearity of 0.98. And the delta in the figure represents the difference between the initial value and each count of steel balls present in the sensor system ranging from 1 to 9. This result also shows a linearity of 0.98. The analog output of the system also shows a linearity of 0.98 same as that of the digital output. The output voltage range, as shown in Figure 8 is ranging from 1.217V to 1.728. This shows a system sensitivity of 56.77mV for each steel ball.

V. Conclusion and Future Work

A circuit model was implemented with components having the same function as that of their counterparts on the submicron technology scale to be implemented. The steel balls were used in place of the magnetic beads, the round magnetic sheet were used in place of the micro-coil and the hall sensor IC with internal preprocessing circuit was used in place of the CMOS based hall sensor. The results show that the quantity of steel balls present on the sensor will affect the output voltage of the system. The result also shows high linearity for the results. From this we can conclude that the system is able to give an accurate result for sensing the quantity of beads present on the system. For the future work, the system shall be implemented on a submicron CMOS technology for its intended application.

From the results shown in this work, the number of magnetic beads captured by the sensor using magnetic assay should

Table II. Description of the displayed values of the LCD display panel.

AZC	initial value	corrected value
MAG	measured values	variation

reflect the concentration of the target antigen. The sensor will use BioMEMS technology for implementation. This will provide the sensor to have greater binding capabilities to biological materials. And it will also provide a smaller distance between sensor and magnetic beads due to its post process etching capability, thereby increasing the sensitivity of the sensor. The post data processing of the said device would be done using an 8051 microcontroller. The data gathered from the device would be converted to a value that is understandable by the user. The signal quality of the system will be taken into consideration. Additional filters will be applied to the system as deemed needed to reduce noise levels during the development of the micro-scale system. The said device shall not be limited to the testing of a specific analyte. The device may be altered to the need of the user. This is simply done with the change antibodies used for the reactions.

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