Piezoresistive Microcantilever Biosensor Potentiometric Signal Transduction For Human Stress Measurement.

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Abstract: - This paper deals with the development of Piezoresistive Microcantilever biosensor and the signal transduction to detect human stress by using salivary alpha amylase activity. A Piezoresistive Microcantilever biosensor can be used to detect saliva-amylase activity by deflecting upon interaction with a specific receptor. By measuring the amount of bending the microcantilever beam experiences in response to interactions with the molecules, and the amount of analyte in the solution can be quantified. When the Microcantilever beam deflects it caused the stress change within the microcantilever beam and applied strain to the piezoresistor material thereby causing the resistance change which can be measured with the Wheatstone Bridge circuit. The Piezoresistive Microcantilever sensor integrated with transducer components converts the biochemical signal into measurable signal when it react with salivary amylase enzyme. The enzyme concentration signal is converted to a voltage signal by the transducer. The device was designed specifically that it enables the small resistivity change due to the enzymatic reaction to be measured.

Key-Words: - Biosensor, Piezoresistive, Microcantilever, Signal Transduction, Resistance change, Saliva, Alpha Amylase

1 Introduction

Stress is the emotional and physical strain caused by response to pressure from the outside world. Stress comes in many forms and affects people of all ages and races. The degree of stress in our lives is highly dependent upon external factors which include the physical environment (job, relationship, home, challenges, difficulties and expectations confronted on a daily basis) and internal factors, which determine the body's ability to respond to and deal with the external stress-inducing factors such as nutritional status, overall health, fitness levels, emotional well-being and the amount of sleep and rest. When a person is stress, it indicates the overall cardiovascular function such as heart rate. The physiological state of stress may also be due to different sources such as physical condition, mental stress, low level or resources (example a burnout condition), or even emotional arousal[1].

Conventionally, there are two methods in determining stress, heart beat and blood pressure. Heart monitoring is the most common way of monitoring physiological stress. The wide use of heart period is related to the availability of electrocardiograph (ECG) acquisition device for noninvasive monitoring. Another object of invention is by providing a personal monitor and process for work and heat stress which has a heart beat sensor producing output electrical signals indicating a user's heart beats and the personal monitor[2] or computer system[3] stores heart beat information corresponding to the heart beat signals produced over a predetermined time interval and is connected to receive the heart beat information[4-6].

Though measurement based on cardiovascular activity is common this method required bulky equipment and consume much time. As alternative, the enzymatic activity could be used. Several researches revealed that psychological stress could produce physiological effects that are similar to

those produced by physical challenges in a variety of physiological systems[7].

Two primary systems involved with the stress response are hypothalamus-pituitary-adrenocortical axis (HPA) and sympatho-adrenomedullary (SAM) system. The activation of the HPA axis involves corticotrophin-releasing hormone and the secretion of glucocorticoid cortisol[8] into circulation. Meanwhile, the faster acting component which involves activation of the SAM axis and releases catecholamines such as norepinephrine. The HPA axis and sympathetic nervous system activity can be measured non-invasively in saliva. Human saliva is secreted from the organ called the salivary gland in the mouth and the activity of the salivary gland is dominated by the sympathetic nervous system.

Currently, technical advances in the assessment of saliva as biomarkers enabled researchers to study stress response in the society. The ability to measure biological variables noninvasively in saliva has created many opportunities for behavioral and social scientist to test biosocial models of individual differences and intra-individual change in mood, cognition, social behavior and psychopathology[9].

Salivary α-amylase is an enzyme which is contrast to the majority of salivary biomarkers employed on a regular basis in biobehavioral research (e.g. cortisol, testosterone)[9]. α-amylase is produced by the salivary gland in the oral mucosa and it is relatively high concentrations under normal condition[9]. α-amylase is also a digestive enzyme and its secretion is innervated by the sympathetic nervous system where changes can be expected in its activity that is related to level of stress. The measurement of salivary α-amylase activity is a useful tool for evaluating the sympathetic nervous system with the aim of developing a simple quantitative measurement technique to monitor human stress.

In 1996 Chatterton et. al reported a significant correlation between salivary α-amylase to the sympathetic nervous system (SNS) component of the stress response. During physiology stress such as aerobic exercise the α-amylase increases 3-fold and both NE and epinephrine (EP) increased 5-fold over control levels but during written examination, α-amylase and NE concentrations increases but not EP[10].

The determination of alpha-amylase had plays an important role for diagnosing other disease[3, 5, 7, 10, 11] depending on the type of complications and measurements. With the flow-injection-type enzyme-based biosensor, the collected saliva sample were measured by using enzymatic method reagent (ESPA AMY-FS, Nippo Co., Japan) such as G4-β-CNP as the reagent substrate of α-amylase and a flow –injection-type device where the detecting current was measured by changing the different flow rate and concentration of maltopentaose produced by glucose and maltose coexist in saliva.

The great development of biosensors for numerous diagnosis of infectious diseases, detection of oxidizing of free radicals in saliva[1], glucose determination[2-5] and also stress measurements[6] has lead to the technological advancement of microsensors for biological sensing.

Biosensors can be coupled to physiochemical transducers that convert this recognition into a detectable output signal. Typically biosensors are comprised of three components: the detector, the transducer and the output system which involves amplification and display the output in an appropriate format.

Microtechnology has been identified as the most promising technologies of this century because of their potential for making affordable enhanced-functionality high-performance microscale systems and devices. A batch-fabricated integrated microsystem, Microelectromechanical System (MEMS) integrates motion and motionless microdevices (actuators, sensors, transducers, etc), microscale driving/sensing circuitry, controlling/processing integrated circuits (ICs) and energy sources. Microsystems can be examined utilizing the biomimetics paradigm examining microbiosystems which are vital part of all living biosystems, exist in nature in enormous variety. Through biosystems, analogy and biomimicking, a variety of electromechanical systems such as microtransducers, actuators, sensors and other have been designed, made and implemented for physical, chemical and biological sensing[12, 13]. These sensors have several advantages over the conventional analytical techniques in terms of high sensitivity, low cost, simple procedure, low analyte requirement (in μl), non-hazardous procedures and quick response. With the ability of high throughput analysis of analytes and ultra sensitive detection, this technology holds tremendous promise for the next generation of miniaturized and highly sensitive sensors.

The miniaturization of sensors recently involves the fabrication of a micro-device suitable for continuous sampling and analysis such as glucose, tuberculosis[14],etc. Their advantage is in term of their small size and integration into a variety of devices. Microcantilevers for example have been proven to be an outstanding platform for chemical and biological sensors, where the modified microcantilevers can recognize target molecules

through specified biological binding which results in deflection of the cantilever.A microcantilever biosensor is a device that can act as a physical, chemical or biological sensor by detecting changes in microcantilever bending or vibrational frequency. Microcantilevers are simple mechanical devices. They are tiny plates or leaf springs, typically 0.2-1μm thick, 20-100μm wide, and 100-500um long, which are connected on one end to an appropriate support for convenient handling.

2 Problem Formulation

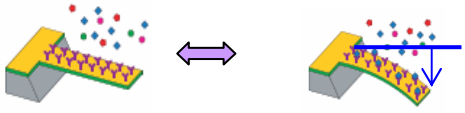
Recently, researchers have shown a keen interest in the application of biosensors because they are easy-to-use, cheap and highly sensitive methods for recognition of biomolecules. Biosensor uses biological materials in collaboration with appropriate instrumentation to determine the substrate in the samples.

Biosensing applications demand fast, easy-to-use, cheap, and highly sensitive methods for the recognization of biomolecules. A high degree of parallelization is also desirable because of the demands made by the pharmaceutical industry for high-throughput screening.

Micro-Electro-Mechanical Systems (MEMS) has proven itself to be of vital importance in the design of micro-devices that can act as sensors as well as actuators. An increasing number of reports confirm the potential of Microcantilever (MC) sensors for environmental such as gas detection, mass effect and gas sensitivity[15] and biomedical application[16-18]. Diverse applications of microcantilever in the field of biosensor have been explored by many researchers, such as monitoring of blood glucose [19], hybridization of DNA [20], and detection of salivary amylase enzyme [21, 22]. The use of piezoresistive microcantilever sensor in the measurement of human stress based on salivary amylase enzyme is new [21, 22].

The sensitivity of a microcantilever biosensor depends on its ability to convert biochemical interaction into micromechanical motion of the microcantilever. The deflections of the microcantilever biosensor are usually of the order of few tens to few hundreds of a nanometer. Such extremely low deflection requires an advanced instrument for accurately measuring the deflections.

As a consequence, most of the applications of microcantilever biosensors are done in laboratories equipped with sophisticated deflection detection and readout techniques. This paper proposes and analyses a self-sensing Piezoresistive Microcantilever for electrical measurement of microcantilever deflection. Microscale cantilever beams can be used to detect biomolecules by deflecting upon interaction with a specific biomolecule as in Fig. 1[23-27].



Immobilisation of bioreceptor produces Biochemical Event

Conversion of Biochemical Event into Deflection of micromachined cantilever beam

Figure 1 Microcantilever beam response

By measuring the amount of bending each microcantilever beam experiences in response to interactions with the molecules, the amount of analyte in the solution can be quantified.

3 Methodology

A. Circuit Simulation Design

The software design and simulation was performed using the OrCAD Capture CIS 15.7. Its offers a comprehensive solution for entering, modifying, and verifying complex system designs quickly and cost effectively. PSpice is a derivative of the original Spice program which supports integrated circuit design and general circuit design. The simulation study begins with theoretical analysis of all the components in the design.

The component values of choice must be realistic to realize the design on the circuit board. A new project is started by clicking on *Create a new project* function on the taskbar. The new project must be created in ORCAD by using analogue or mixed component mode. It is necessary to populate the library list with component libraries. The simulation profile must be set up by using the P-Spice simulation profile to run or simulate the circuit design. Simulation can be run in bias point, DC sweep, AC sweep or time domain mode.

B. Hardware Construction

The hardware construction was initiated using resistor decades, OP-27 operational amplifiers, resistor, capacitor and a voltage source. The Op-27

operational amplifier was used in this design for its low-noise with high precision performance. The DC power supply voltage for OP-27 at V+ and V- is +12V and -12V respectively. The capacitors C1, C2 and resistors R9, R10 as in Figure 3 are used to keep the circuit stable, especially in cases where the same DC power supplies are used for several stages. The output measurement was taken using digital multimeter. In order to obtain the precise value of the resistor decade equivalent to the fix resistor value, digital meter is used.

The construction of the hardware circuit implemented by testing the input and output of all stages on the breadboard. A lot of modification and troubleshooting done to achieve the desired result as in software design.

C. Piezoresistive Microcantilever Deflection Detection

The microcantilever beam deflection is revealed and associated with the beam interaction with an external (physical, mechanical, chemical, biologica, etc) stimulus (Figure 1). The microcantilever beam deflection is related to asymmetric, mechanical stress generated in the microcantilever.The functionalization of a bioreceptors onto the microcantilever surface where the most common forms of bioreceptors used in biosensing are based on proteins, antibody/antigen or nucleic acid interactions. When the analyte molecules are adsorbed onto the finctionalized cantilever surface, surface stresses generated bend the cantilever[23]. The cantilever deflection depends on the molecular species and its concentration therefore by measuring the cantilever deflection the concentration of the attaching species can be determined

Piezoresistive Microcantilever deflection method involves the embedding of a piezoresistive material such as doped polysilicon at the top surface of the microcantilever to record the stress change [8]. When the microcantilever beam deflects a stress change occurs within the beam that will apply strain to the piezoresistor. Thereby causing a change in resistance that can be measured by electronic instruments. The resistance of the piezoresistive material changes when strain is applied to it. The relative change in resistance as function of applied strain can be defined as

$$\frac{\Delta R}{R} = K\delta \qquad (1.1)$$

Where K is a Gauge Factor which is an important material parameter, δ is the strain in the material and R is the piezoresistor resistance.

D. Thin film Piezoresistive Microcantilever Fabrication

The fabrication of the piezoresistive microcantilever was performed at MIMOS semiconductor (MySEM). It uses the surface micromachining method since the structures involved several layers and so that the microcantilever beam can be "released" to allow it to move vertically. Besides being the common surface micromachining structural material, Polysilicon material could also be deposited with well-controlled and repeatable film stress level. The fabrication is based on four basic microfabrication techniques: deposition, patterning, doping and etching as illustrated in Figure 2

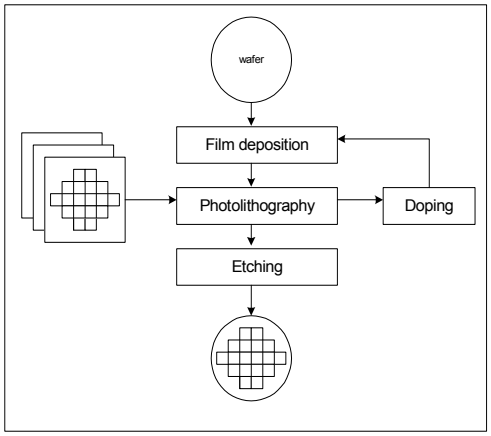


Figure 2 Flow diagrams of basic microfabrication techniques

There are two important processes in surface micromachining which is the deposition of low stress thin films that can be used as microcantilever beam and the sacrificial layer which allows the microcantilever beam to be released and therefore allowing the beam deflection. There are two different thickness of microcantilever beam fabricated that is 0.5μm and 1μm. The sacrificial layer which is made from BoronPhosphosilicateGlass (BPSG) also has two different thicknesses which is 0.9μm and 1.8μm. Several different thicknesses of microcantilever beam and sacrificial layer was designed and fabricated to investigate the optimum design suitable for the salivary amylase detection. Figure 3, shows a cross-sectional view of the piezoresistive microcantilever structure.

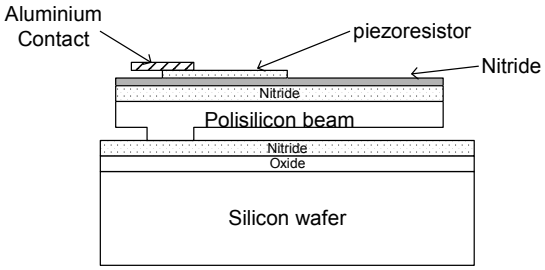


Figure3 Piezoresistive Microcantilever cross-sectional view

The fabrication process started from patterning a 0.9µm –thick photoresist of BoronPhosphosilicate Glass (BPSG) sacrificial layer on a silicon substrate by standard photolithography. The microcantilever beam is then formed by depositing a polysilicon layer of 5000A (0.5µm) thickness using Low Pressure Chemical Vapor Deposition (LPCVD). Next, a 500nm-thick Silicon Nitride (SiN) layer is deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) which will act as an insulator. Another polysilicon layer is then deposited with a dimension of 195µm x 75 µm u-shape resistor pattern and blanket implanted to achieve a resistor value of 1.2kΩ. Then the electrode pad was patterned and deposited with Aluminium and finally the cantilever beam is released by wet etching. The cross section SEM image of the designed piezoresistive microcantilever is as shown in Figure 4.

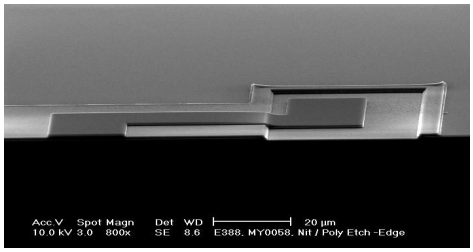


Figure 4 FESEM of microcantilever sensor cross section

E. Electrical Measurement using Wheatstone Bridge Circuit design

This project consists of three major parts, which are theoretical calculation, software design and hardware construction. The project was designed based on theoretical calculation. It is then divided into two parts that is construction of the circuit and software development. The software design and simulation was performed using the OrCAD Capture CIS 15.7. The detection method of the Piezoresistive Microcantilever sensor is a potentiometric transduction circuit as shown in Figure 5. It is made of two stages, the sensor and transduction stage, which is made up of a Wheatstone bridge and a differential op-amp, where variation in the resistance caused by the piezoresistive microcantilever deflection can be measured based on differential in voltage.

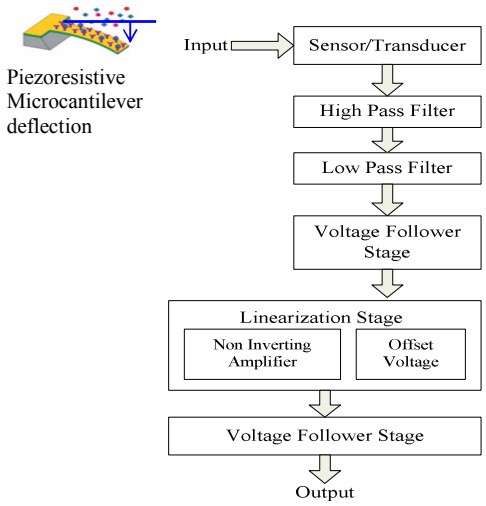


Figure 5: Potentiometric Circuit Block Diagram

The Piezoresistive (PZR) Microcantilever sensor developed embeds a layer of piezoresistive poly-silicon material near the top surface of the microcantilever to record the stress change occurred on the surface. When the analyte molecules are adsorbed onto the functionalized cantilever surface, the deflection of microcantilever undergoes a stress change that applies strain to the piezoresistor, thereby causing a change in resistance, R3, an arm of the Wheatstone bridge circuit, the sensor stage, as shown in Figure 6.

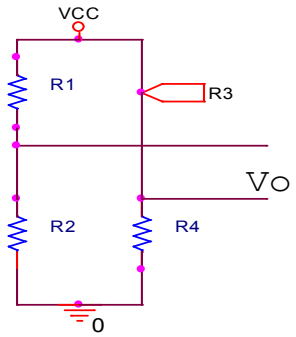


Figure 6. Wheatstone Bridge Circuit used for the PZR Microcantilever deflection detection.

For a piezoresistor embedded on to the surface of the microcantilever has a length of l µm, with cross-section area of Aµm^2 and a resistivity of ρ Ωµm, the

resistance is given by

$$R = \frac{\rho l}{A} \qquad \Omega \quad (1.2)$$

When the piezoresistor material is stressed mechanically by a load W newtons, a stress, σ occurs where

$$\sigma = \frac{W}{A} \quad (1.3)$$

By using a Taylor's series expansion method on resistance R, the resistance changes can be determined by:

$$\Delta R = \left(-\frac{L}{A^2}\right)\Delta A + \left(\frac{L}{A}\right)\Delta\rho + \left(\frac{\rho}{A}\right)\Delta L \quad \Omega \ (1.4)$$

Then, to obtain the fractional change in R, divide eqn. 1.4 with eqn. 1.2 and we will get

$$\frac{\Delta R}{R} = -\frac{\Delta A}{A} + \frac{\Delta\rho}{\rho} + \frac{\Delta L}{L} \ (1.5)$$

Figure 7 shows the integration of a Wheatstone bridge circuit with a differential op-amp to complete the transduction of enzyme concentration in response to change in human stress. A differential amplifier can be considered as two amplifiers with separate inputs, but a common output terminal, which delivers sum of two amplifier output voltages. Both amplifiers have the same voltage gain, but one has an inverting input, V_{in}-, while the other has a non-inverting input, V_{in}+. A differential amplifier is used to measure biomedical signals where it's applied between the inverting and non-inverting input of the amplifier. The signal therefore amplified by the differential gain of the amplifier. Figure 6 shows the sensor integration consist of Wheatstone bridge and different op-amp circuit.

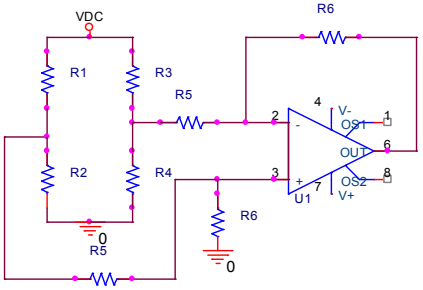


Figure 7: Sensor Integration Circuit

If the following resistor ratios equal, R6/R5 = R6/R5, the output voltage is:

$$\Delta V = V_o\left\{\frac{R_2}{R_1 + R_2} - \frac{R_4}{R_3 + R_4}\right\} \qquad (1.6)$$

Where $R_3 = R + \Delta R$

Next, the filtering stage will accommodate the stress signal in a range of 0.15-0.4Hz using the first order high pass filter and low pass filter after the first stage of amplification. A filter is a circuit that passes certain frequency and attenuates or reject all other frequencies. Its constructed using combination of resistors and capacitor.

The high pass filter is the inverse of the of low pass filter, so one can reasonably expect its frequency response characteristic to mirror that of the low pass filter. Its formed from a first order low pass type by interchanging component R_i and C_i as in Figure 8 below. The gain, A of the filter can be expresses as follows:

$$A = 1 + \frac{Rf}{Ri} \qquad (1.7)$$

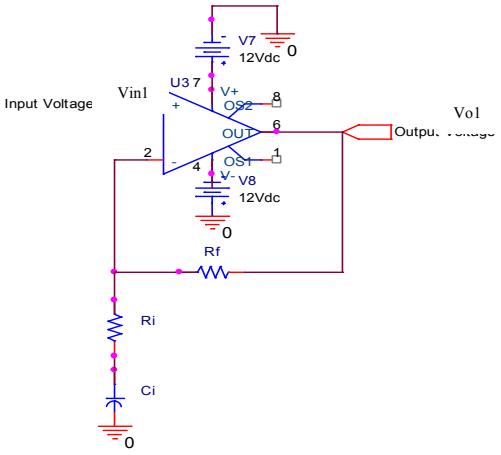


Figure 8:schematic for first order high pass filter

The circuit design will accommodate the stress signal range in frequency in a range of 0.15Hz. The cutoff frequency f_L, which is 0.15 Hz, is the frequency at which the magnitude of the gain is 0.707 times its pass band value. It can be obtained using the equation as in:

$$f_L = \frac{1}{2\Pi R_i C_i} \qquad (1.8)$$

The low pass filter is used to eliminate frequency higher than the stress signal which is 0.4Hz, and to eliminate the change of noise interruption or data

error. Low pass filter pass only the frequencies below the designed frequency, blocking all other frequency. Figure 6 illustrate the structure of low pass filter schematic circuit. The frequency at which the gain starts to decrease is controlled by C_3 and R_2. This type of circuit is usually configured as an inverting amplifier and is refered to as an active low pass filter. The circuit will pass low frequency signals on to its output gain, Av, but will attenuate signal of higher frequencies. The output gain for this circuit was calculated as:

$$Av = -\frac{R_2}{R_1} \qquad (1.9)$$

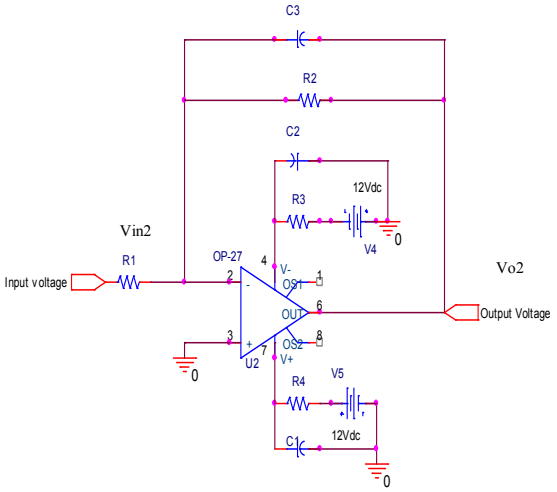


Figure 9: Schematic for first order low pass filter

The cut off frequency defines the end of the passband and its normally specified at the point where the response drops -3dB (70.7%) from the passband response. Cut off frequency is calculated by the familiar formula:

$$f = \frac{1}{2\Pi R_2 C_3} \qquad (1.10)$$

The linearization stage design involved the modification of a basic circuit so that the output was approximately linear functions of its input, in order to facilitate analysis of the system. For interface to an analog-to-digital converter, this needs to be linearizing between 0 to 5V. As a result, it is easiest to develop an equation for the output in terms of output. From this circuit can be envisioned.

$$Vout = mVin + Vo \quad (1.11)$$

The schematic circuit for linearization stage was developed as in Figure 10. It consists of two operational amplifier, (OP1 and OP2) and resistor network. OP1 is connected in non-inverting stage, while the OP2 is the simple differential amplifier construction. Note that a portion of Vo4 is feedback via R6 to the negative input of an op-amp. For the offset voltage, a variable resistor (R11) has been used, so both loading of the divider by the op-amp circuit and variation of the supply from exactly 5V can be compensated for by adjusting until the bias exactly 2.603V.

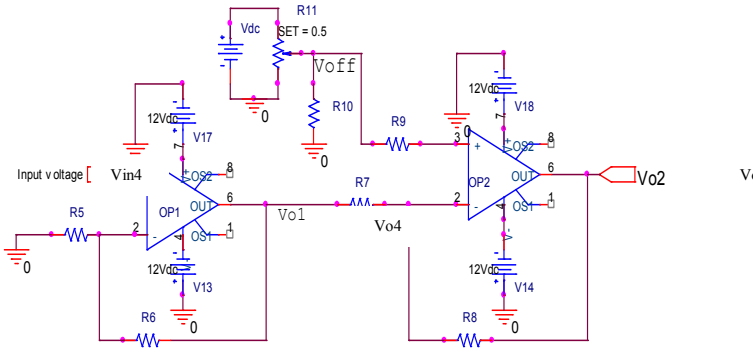


Figure 10: Schematic for Linearization Stage

4 Results

From testing with the actual Piezoresistive Microcantilever sensor, it is found to have a resistance value of 5.767 kilo ohms. Table 1 shows the voltage output from the bridge at and slightly off the null bridge conditions. It can be confirmed that the null bridge condition is obtained when R2 equals 6.245 kilo ohms for actual sensor testing.

Table 4.1 Nulling of Wheatstone Bridge Circuit

(R3 kΩ) PZR	(R2 kΩ) Rpot	Vout(mV) (theoretical Calculation)	Vout (mV) (experimental)
5.767	6.000	-49.40	-45.102
5.767	6.100	-28.88	-24.37
5.767	6.200	-8.500	-8.04
5.767	6.210	-6.500	-6.143
5.767	6.220	-4.400	-4.247
5.767	6.230	-2.400	-2.176
5.767	6.240	-0.400	-0.519
5.767	6.241	-0.180	-0.955
5.767	6.242	0.020	-0.481
5.767	6.243	0.220	-0.374
5.767	6.244	0.420	-0.059
5.767	6.245	0.620	0.414
5.767	6.246	0.820	0.616
5.767	6.247	1.021	0.883

5.767	6.248	1.221	1.241
5.767	6.249	1.420	1.481
5.767	6.250	1.600	1.623
5.767	6.260	3.600	3.723
5.767	6.270	5.600	5.432
5.767	6.280	7.600	7.250
5.767	6.290	9.600	9.021
5.767	6.300	11.50	10.768

With reference to experimental outcome on the deflection of Piezoresistive Microcantilever range, a range of 6.245 to 6.25 kilo ohms is chosen as variable resistance range. The output from the differential amplifier ranges from 0.616 millivolts to 1.623 millivolts on actual experiment. A discrepancy within 13.16% (Table 4.2) on the average is detected, which could be attributed to tolerances of electronic components and wiring.

Table 4.2: Integration of Sensor and Transduction Stage

(R3 kΩ) PZR	**(R2 kΩ) Rpot**	**Vo1 mV (Theoretical)**	**Vo1 mV (Experimental)**	**% Discrepancy**
5.767	6.246	0.820	0.616	24.88
5.767	6.247	1.021	0.883	13.52
5.767	6.248	1.221	1.241	-1.64
5.767	6.249	1.420	1.481	-4.30
5.767	6.250	1.600	1.623	-1.44

Figure 11 depicts the outcome from a comparative study between theoretical and experimental results with the integration of sensor and transduction stage. It can be observed that the voltage output from the differential amplifier is linearly related to the resistor, R2, the variable resistor.

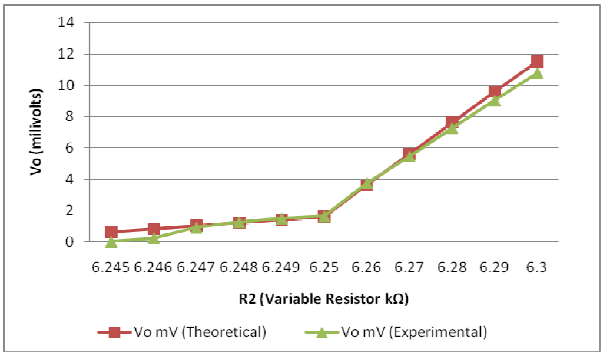


Figure 11 Comparative study between Theoretical, Simulation and Experimantal results on output voltage of Integration of Sensor and Transduction Stage

5 Conclusion

The sensing principle is based on immobilization of the bioreceptor to produce a biochemical reaction. The novel Piezoresistive Microcantilever sensor integrated with a transduction circuit converts this biochemical event into a measurable electrical signal. The Piezoresistive Microcantilever biosensor can be used to detect the small biological signal in response to the proposed biosensor system. The deflection of the Microcantilever beam caused a resistance change within the beam and therefore generated signal which is converted to voltage by the Wheatstone Bridge circuit. The transduction circuit designed enables the small change in resistivity due to the enzymatic reaction to be detected. The circuit has been designed and tested through theoretical, simulation and experimental studies.

By investigating the integration of the Piezoresistive Microcantilever sensor with the developed transducer, the result shows that the percentages different between the software simulation and the hardware developed transducer was very low and insignificant to each other. Thus, it is proven with theoretical result. The software simulation and hardware implementation have been successfully completed; this finding is useful for the future enhancement of the bioamplifier design.

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