Study on the Effects of Electrode and Microchannel Sizes on the Performance of MBEB

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Abstract

Background/Objectives: The design and fabrication of Microfluidics-based electrochemical biosensor with high quality and the optimal effective surface area are of primary importance for more accurate result. The effects of the microelectrode size with variation in the microchannel size was investigated. **Methods/Statistical Analysis:** The effect of the microchannel size on the performance of the sensor was then investigated using cyclic voltammetry testing for different sizes of the fabricated channels (100, 400, 700 and 1000 μm) at electrode size of 100μm and 200μm. **Findings:** The electrokinetic properties of the microchannel were found to be affected by the size of both the microchannel and the electrode. The highest sensitivity of the sensor was reported at microchannel size of 700 and electrode size of 200μm. **Application/Improvements:** High accuracy and fast responding electrochemical biosensor are expected to be produced through the optimization of the microchannel size and the electrode surface area.

Keywords: Microfluidics, Electrochemical Biosensor, Microscope, Microchannel, Electrode.

1. Introduction

The microfluidics-based biosensor is a new field that integrates biosensor with the microfluidics systems. Specialist part of this area calls electrochemical biosensor base on microfluidics. There are several types of electrochemical biosensors as well several applications¹⁻³[Gopinath, 2016 #544]. The majority of its implementation is in the biomedical applications.

Several studies had covered the basic principles, fabrication materials, biological conditions and targets disease using this type of biosensors⁴⁻⁶. For example Heo and Crooks⁷, have presented in their paper a microfluidics-based biosensor for the detection of glucose and galactose. The hydrogels were placed in the middle of the channel after entrapping the enzymes. The hydrogels were reported to enhance the detection ability and to increase the sensitivity of the sensor. Further improvement was reported in the presence and absence of microchannel. The study concluded that the microchannel played as enhancer for the sensitivity which allows the detection of DNA up to 100 pM level.

Further research was conducted by⁶ when they studied the effects of on indirect electrochemical biosensor with catalytic enhancement using the simple glucose base biosensor. The findings of the experiment were reported to be positive where it was concluded that the flow rate is of high importance where it needs to be optimized in order to prevent the electrons washout.

The current innovation and developments of microfluidics-based biosensors are giving more attention to the electrodes materials. However, less attention was paid to the other parts such as the microchannel and electrode size. The investigations on the microchannel and electrode size of microfluidics-based biosensor impact on the performance weren't studied in details. This paper presents the effect of variation of the microfluidics-base electrochemical biosensor.

2. Materials and Methods

2.1 Materials and Preparation

In this study, raw materials were used to test the sensors. Potassium ferricyanide was purchased from

Sigma Aldrich. The redox electrolyte was prepared by dissolving 16.5mg of potassium ferricyanide and 2.53 mg of potassium nitrate in a 25 ml volumetric flask of water. The solution was prepared as a stock solution with the concentration of 10mM of potassium ferricyanides.

2.2 Electrochemical Calibration

Prior to cyclic Voltammetry measurements with a standard redox species, the potential of the on-chip Ag/AgCl reference electrode is calibrated. The potential difference was measured between the fabricated on-chip reference electrodes and a commercial Ag/AgCl reference electrode, which is immersed in a 3M NaCl and 1M KNO₃inner electrolyte.

2.3 Cyclic Voltammetry Experiments

Initially, measurements were taken for the sensor at which the channels were totally empty. The potentiostat was set up for the voltage range of -0.2 to +0.5V and the scan rate were then changed between 100, 200, 500 and 1000 mV/s accordingly. A respective amount of the potassium ferricyanide solution then was loaded into the microchannels using the syringe pump as supporting electrolyte. The channels were fully filled with electrolyte fluid and measurement was taken using the potentiostat using the same setting. Repetitive measurements were taken for the different size of the electrode and the size of the channels as described in Table 1. The results were recorded for each run and graphs are being generated using the potentiostat software.

Table 1.	Sets o	f Experii	ments	Set-up
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Electrode with size of	Electrode with size of	
100 μm	200 μm	
100µm microchannel	100µm microchannel	
400µm microchannel	300µm microchannel	
700µm microchannel	700µm microchannel	
1000µm microchannel	1000µm microchannel	

3. Results and Discussion

3.1Electrochemical Calibration Result

The reference electrode calibration is important to ensure that the result you obtained is valid. The reading of the potential differences between the commercial and the fabricated using 3 M NaCl solution was found to be zero. This result confirms that the fabricated reference electrode is the same type as the commercial reference electrode. A similar experiment was conducted using 1M KNO₃ for further confirmation. The reading of the electrode was fund to be equal to that obtained when using NaCl. This result shows more stable respond can be obtained using the fabricated reference electrode.

3.2 Cyclic Voltammetry Results

Designing the electrochemical biosensor base on microfluidics channels was illustrated to compare between the microchannels and the electrode size. During the measurement of the cyclic voltammetry, the channel size was observed to have an effect on the response of the sensor. As shown in figure 1 for the electrode size of 100 µm the response current peaks was eventually increasing then decrease. An optimum response was obtained at microchannel size of 400µm. This behaviour of the response can be as explained as a result of shifting the limitation from mass transport to electrochemical reaction limits caused by the uncompensated solution resistance. Another reason for this behaviour is that the charges at higher bulk volume are accumulated at the surface of the electrode, which results in creating a barrier between the analyte and the electrode surface



Figure 1. The cyclic voltammetry curves for 100, 400, 700 and 1000 μ m microchannel in 100 μ m electrode size at a scan rate of 1000 mV/s.

There was a significant increase in the current density (signal) with the increase in the microchannel size from 100 to 400 μ m. However, this improvement in the current signal was found to decreases with the increase in the microchannel size to 700 and 1000 μ m.

Similarly, the microchannel effects on the response were increased and then decreased when the electrode size was 200μ m. Figure 2 shows the variation in the

response current with the change in the microchannel size. At this point, the optimum response was then shifted at 700 μ m. This behaviour of decaying in the response at higher microchannel size can be as explained earlier in this paper, is due to the limitation changed from the mass transport limit to the electrochemical reaction limits.



Figure 2. The cyclic voltammetry curves for 100, 400, 700 and 1000 μ m microchannel in 200 μ m electrode size at a scan rate of 1000 mV/s.

Another reason for this behaviour is that the charges at higher volume bulk are accumulated at the surface of the electrode which results in creating a prior between the analyte and the electrode surface. This agreed with findings reported by¹⁰ when they studied the effects of microchannel size in the performance of microfluidicsbase electrochemical biosensor where they conclude that the microchannel size variation dramatically changes the response of the sensor. Nevertheless, the response at 200 µm electrode was observed to be higher than the obtained at 100 µm electrode. As illustrated in figure 1 and figure 2, the ability of the sensor shows improvement with increase in both microchannel and electrode size. This can be explained as result of increasing the number of electrode at the detection site which in order increase the current signal. However, it was notice that there was an optimum size of both microchannel and electrode where further increase has no impact on the signal. This due to the transition from mass transfer limitation to the reaction limitation which doesn't not affects by the geometry of the sensor.

With respect to the sensor quality in term of sensitivity and detection range, the sensitivity and detection range at different microchannel sizes for electrode sizes of $100\mu m$ and $200\mu m$ are shown in figure 3 and figure 4 respectively. The effects of microchannel size and electrode size on the sensitivity and limit of detection of the sensor had been quantitatively investigated. Sensitivity was calculated as the slope of calibration curve. Detection limit of sensors and biosensors could be calculated according to the Equation1.

LOD = Response of blank + 3(standard deviation) (1)

The result shows an increase in both the sensitivity and the detection range versus the microchannel size. However, these increases in the sensitivity and the detection range values then dropped at higher microchannel sizes. For an instant, at electrode size of 100µm, the highest sensitivity was reported at channel size of 400 µm with a value of 0.031208AmM⁻¹cm⁻². On the other hand, at the electrode size of 200 µm the maximum sensitivity value was obtained at microchannel size of 700 μ m with a value of 0.017948 AmM⁻¹cm⁻². The increase in the sensitivity with channel size and electrode size showed that the sensitivity of the sensor affected as well by the bulk volume. Moreover, the sensitivity is higher at all channel sizes when the electrode size is at 200 µm compare to the sensitivity values obtained at electrode size of 100 µm.



Figure 3. The Sensor Sensitivity at different electrode size.



Figure 4. The Sensor Detection Limit at different electrode size.

The values of the detection limits show how low the sensor can detect the target analyte. For most of the applications, the detection limits are reported as low as 1 mM. However, in our sensor, we reach to lower than 0.066mM which makes the sensor is more reliable for an accurate result.

The sensor detection limits were found to behave in similar minor as the current signal where the detection limits were reported to be the lowest at the channel size of 400 when the electrode size is 100 μ m. Nevertheless, the values of the detection limits were the lowest at channel size of 700 μ m.

Comparing the result obtained from our sensor to the existence commercialized glucose sensors; with optimum conditions the sensitivity we obtained better results. For an instant, the detection limits for the Abbott glucose sensor device brand was reported to be 1.11 mM whereas we obtained at 700 μ m microchannel size and 200 μ m electrodes size detection limits of 154.1 μ M. These findings show that a lower detection limits can be achieved by optimizing of the design conditions.

4. Conclusion

The study of the electrochemical properties of the electrochemical biosensor employed in microfluidic devices is therefore useful for the development of advanced biosensor systems for decentralized analyses. In this paper, the progressive development for the integration of microstructured electrodes in a planar microchannel fabrication technology was reported. The findings show a proportional relation between the channel size and the sensitivity of the sensor (signal). Nevertheless, this proportionality, fall back at microchannel size of 700 µm at electrode size of 100µm, and at microchannel size of 1000µm at electrode size of 200µm. This drawback in the signal is believed to be due to the access bulk volume which shifts the limitation from mass transport limitation to the electrochemical reaction limitation. Additionally, the accumulation of the charges at the surface of the electrode may also have created a boundary between the analyte and the target.

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6. References

- Cao J, Sun T, Grattan KTV. Gold nanorod-based localized surface plasmon resonance biosensors: A review. Sensors and Actuators B: Chemical. 2014; 195:332–51.
- 2. Gomez FA. Biological applications of microfluidics: John Wiley & Sons. 2008.
- Gopinath PG, Aruna Mastani S, Anitha VR. Design of Interface Circuit and Biosensor Fabrication using Inkjet Printer for the Detection of Glucose. Indian Journal of Science and Technology. 2016 Dec; 9(15):1–15.
- 4. Kim J, Junkin M, Kim D-H, Kwon S, Shin YS, Wong PK et al. Applications, techniques, and microfluidic interfacing for nanoscale biosensing. Microfluidics and Nanofluidics. 2009; 7(2):149–67.
- Zhang X, Jones P, Haswell SJ. Attachment and detachment of living cells on modified microchannel surfaces in a microfluidic-based lab-on-a-chip system. Chemical Engineering Journal. 2008; 135(1):S82–S88.
- Abad L, Javier del Campo F, Munoz FX, Fernandez LJ, Calavia D, Colom G et al. Design and fabrication of a COP based microfluidic chip: Chronoamperometric detection of Troponin T. Electrophoresis. 2012 Nov; 33(21):3187–94.
- Lamberti F, Luni C, Zambon A, Serra PA, Giomo M, Elvassore N. Flow biosensing and sampling in indirect electrochemical detection. Biomicrofluidics. 2012; 6(2):24114–1413.
- Heo J, Crooks RM. Microfluidic biosensor based on an array of hydrogel-entrapped enzymes. Analytical chemistry. 2005 Nov; 77(21):6843–51.
- Chen I-J, White IM. High-sensitivity electrochemical enzyme-linked assay on a microfluidic interdigitated microelectrode. Biosensors and Bioelectronics. 2011 Jul; 26(11):4375–81.
- 10. Hayder AA, Esmail AB. Investigating the Effects of Microchannel Size on the Performance of Microfluidics-Based Electrochemical Biosensor. Current Analytical Chemistry. 2016; 12:1.