

Development of an Enzymeless Homocysteine Biomedical Sensor for the Precise Determination of Homocysteine

Congo Tak-Shing Ching,
Chia-Ming Liu and Wei-Hao Liu
Graduate Institute of Biomedicine and Biomedical Technology
National Chi Nan University
Nantou, Taiwan, ROC
tsching@ncnu.edu.tw

Tai-Ping Sun and Hsiu-Li Shieh
Department of Electrical Engineering
National Chi Nan University
Nantou, Taiwan, ROC
tps@ncnu.edu.tw

Abstract—Coronary heart disease (CHD) is responsible for 50% of all mortality in developed countries. And, homocysteine is a risk factor for CHD. Therefore, the aim of this study is to develop an enzymeless, simple, low-cost and reliable homocysteine biomedical sensor (HBS) for the precise determination of homocysteine. The HBS is a bowl cell consisted of 4 silver electrodes. Its working principle is based on the impedance measurement of homocysteine solution. The HBS on the detection of homocysteine at a specific frequency ranges (29.65–212.25 Hz) shows an excellent linear ($r^2 \geq 0.95$) response range (0–100 μ M), which covers the normal physiological and pathological ranges of plasma homocysteine levels. Intraclass correlation coefficient showed that all measurements had excellent reliability and validity (ICC=1.00). The HBS also showed excellent long-term stability (99% of its initial value of current response after 6 months of storage). In conclusion, an enzymeless, simple, low-cost and reliable HBS was developed. It is able to precisely determine homocysteine concentrations in both the normal physiological and pathological ranges.

Keywords- Homocysteine; Biomedical Sensor; Impedance

I. INTRODUCTION

Coronary heart disease (CHD) is responsible for 50% of all mortality in developed countries [1]. On the other hand, many retrospective case-control studies support that homocysteine is a risk factor for CHD [2] which has been confirmed by meta-analyses [3-5]. More importantly, studies have demonstrated that total plasma homocysteine levels correlate better than cholesterol levels with an increased risk of arteriosclerosis [6]. Therefore, it would be great if a reliable, quick and inexpensive screening method and a point-of-care testing are available for patients with cardiovascular diseases and etc.

Many methods are available for homocysteine determination. For instant, high performance liquid chromatography [7-10], gas chromatography-mass spectrometry [11-13], enzymatic [14-17], immunoassay [18-19], molecular imprinting [20], capillary electrophoresis [21-22] and electrochemical [23]. However, tedious analytical procedures and expensive reagents and instrumentation are

necessary for such determination methods. Therefore, enzymeless homocysteine biomedical sensor (HBS) for homocysteine determination is reported in this study. Several studies of homocysteine biosensors have been reported on in the literature [23-27]. However, the current study is the first to report an enzymeless approach for homocysteine determination and this is our aim and novelty.

The working principle of the enzymeless HBS is based on the impedance measurement of homocysteine solution. Impedance measurement is obtained by injecting low-level sinusoidal current in the homocysteine solution and measuring the voltage drop generated by the impedance of the homocysteine solution.

The aim of this study is therefore to develop an enzymeless, simple, low-cost and reliable HBS for the precise determination of homocysteine.

II. MATERIALS AND METHODS

A. Chemicals and reagents

All reagents used in this study, were commercially available and used without further purification: homocysteine and 10X phosphate buffered saline were purchased from Sigma Chemical Co. (St. Louis, MO). De-ionized water purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to dilute the 10X phosphate buffered saline in order to obtain a 0.01M phosphate buffered saline (pH 7.4), with 0.154M sodium chloride. The diluted phosphate buffered saline was then used to prepare homocysteine solutions with various concentrations.

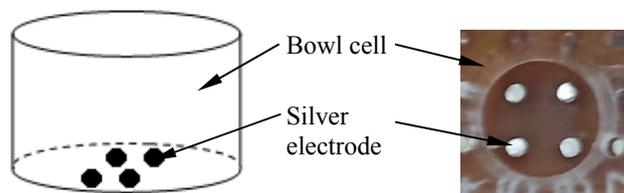


Figure 1. The homocysteine biomedical sensor.

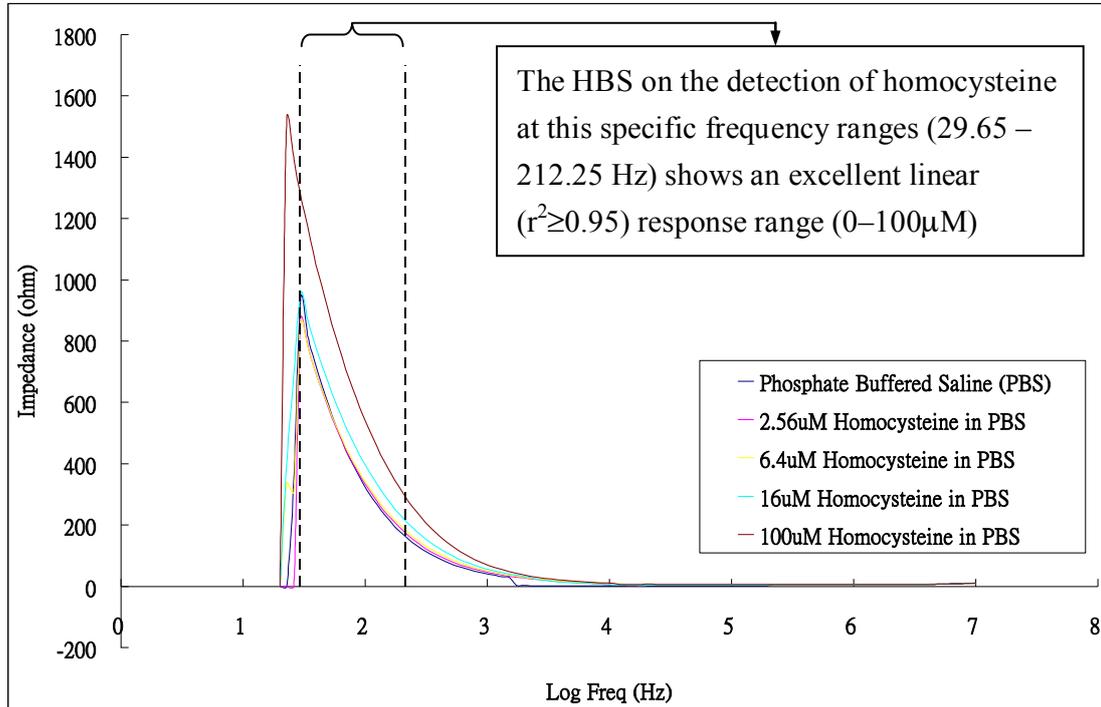


Figure 2. The impedance spectrum of homocysteine solution at various concentrations within the frequency range of $20\text{--}10 \times 10^6 \text{ Hz}$. Peak impedance was observed at the frequency of 29.65 Hz for all homocysteine solution at various concentrations (0–100 μM)

B. Instrumentation

An impedance analyzer (Precision Impedance Analyzer WK6420C, Wayne Kerr Electronics Ltd, United Kingdom) was used for all impedance measurements of homocysteine solutions with various concentrations.

C. The enzymeless HBS and its working principle

The HBS is a bowl cell incorporated with four 1-mm-diameter silver electrodes (2.5 mm between electrode centers) mounted in square configuration (Figure 1). Its working principle is based on the impedance measurement of homocysteine solution.

D. Measurements of the HBS impedance spectrum to homocysteine

Measurements were carried out at 25°C. The HBS was connected to the impedance analyzer. One hundred microliter of homocysteine solution (0, 2.56, 6.4, 16 and 100 μM) was added onto the HBS. Impedance spectrum of homocysteine solutions was recorded over the frequency range $20\text{--}10 \times 10^6 \text{ Hz}$ with 17 frequency points per logarithmic decade. The amplitude of the perturbing wave was limited to 200 mV. Three separate sets of measurement of a homocysteine solution were made in succession in order to check reliability of the measurements.

III. RESULTS AND DISCUSSION

The HBS was consisted of four-electrode, rather than two-electrode, configuration because this could lead to the measured impedance essentially independent of the contact

impedance between electrode and the testing homocysteine solution.

Figure 2 showed the impedance spectrum of homocysteine solution at various concentrations within the frequency range of $20\text{--}10 \times 10^6 \text{ Hz}$. It was found that peak impedance was observed at the frequency of 29.65 Hz for all homocysteine solution at various concentrations (0–100 μM). Therefore, it was speculated that the impedance of homocysteine is specific to this frequency. Because of this speculation, the impedance at the frequency of 29.65 Hz for all homocysteine solution at various concentrations was then used to plot a calibration curve as shown in Figure 3. It was found that there was an excellent linear relationship ($r^2=0.95$) between the concentration of homocysteine solutions and their corresponding impedance measured at the frequency of 29.65 Hz. This excellent linear response range (0–100 μM) covers the normal physiological and pathological ranges of plasma homocysteine levels.

On the other hand, other than the specific optimum frequency (i.e. 29.65 Hz.), a specific frequency range (29.65–212.25 Hz) was found, in which an excellent linear ($r^2 \geq 0.95$) response range (0–100 μM) could be obtained (Figure 3).

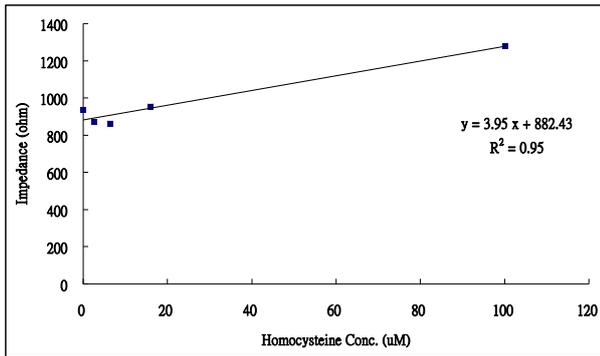


Figure 3. Calibration curve of the impedance measured by the HBS at the frequency of 29.65Hz to the homocysteine concentration (0–100µM). The line is that of best fit found by linear regression ($r^2=0.95$). The sensitivity of the HBS (sensitivity = 3.95Ω/µM) is the slope of the linear regression line.

In order to evaluate the ability of the HBS on the determination of the concentrations of homocysteine solutions at the frequency of 29.65Hz, evaluation study had been conducted (Table 1). It was found that the HBS was able to precisely determine the concentrations of homocysteine solutions (0-100µM) with the maximum percentage error of 2.48%.

The ICC is a measure used to quantify the reproducibility of a variable and together a measure of the homogeneity within groups of replicate measurements relative to the total variation between groups. It has been suggested that ICC values above 0.75 are indicative of good reliability and those below 0.75 should be considered as poor to moderate [28]. Moreover, Portney and Watkins [29] state; "For many clinical measurements reliability should exceed 0.90 to ensure reasonable validity". The ICC(3,1) measurement in this study was 1.00 which suggests they have excellent reliability and validity (Table I).

The long-term stability of the HBS was tested over a 6-month period at room temperature in the condition of fully nitrogen gas environment. The response of the HBS to 100µM homocysteine was found to be about 99% ($n=10$) of its initial value. The life-span of our HBS is excellent as it does not employ enzyme in its working principle. So, there is no enzyme deterioration problem. A possible explanation for the 99% long-term stability, rather than 100%, is that the four silver electrodes of the HBS might slightly get oxidation.

Screen printing technique is very common and therefore, the cost and the size could be further reduced.

Research is ongoing to study the impedance spectrum of homocysteine in blood. This result will be reported in due course.

IV. CONCLUSION

The first enzymeless HBS was successfully designed and developed. The HBS operated at the frequency of 29.65Hz is highly-sensitive, reliable, simple and low-cost. Other than 29.65Hz, a specific frequency range (29.65–212.25Hz) was

TABLE I. EVALUATION OF THE HBS ON THE DETERMINATION OF THE CONCENTRATIONS OF HOMOCYSTEINE SOLUTIONS AT THE FREQUENCY OF 29.65HZ.

Standard Homocysteine Solutions (µM)	Measured Homocysteine Concentration (µM)			Mean (µM)	SD (µM)	% Error	Intrarater Reliability (ICC 3,1)
	Trial 1	Trial 2	Trial 3				
10	10.20	10.20	10.30	10.23	0.06	2.33	1.00
20	19.70	19.70	19.80	19.73	0.06	1.33	
30	30.30	30.30	30.30	30.30	0.00	1.00	
40	39.80	39.90	39.90	39.87	0.06	0.33	
50	50.10	50.20	50.10	50.13	0.06	0.27	
60	61.30	61.50	61.30	61.37	0.12	2.28	
70	71.00	71.30	71.30	71.20	0.17	1.71	
80	81.50	81.40	81.40	81.43	0.06	1.79	
90	92.10	92.30	92.30	92.23	0.12	2.48	
100	98.80	98.80	98.70	98.77	0.06	1.23	

also found, in which an excellent linear response range could be obtained. It has excellent long-term stability with the life-span of no less than 6 months. The linear working range (0–100µM) of the HBS is capable of determining the normal physiological and pathological homocysteine plasma levels and this allows a quick and inexpensive screening method and a point-of-care testing for patients with coronary heart disease.

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