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NANOBIO BIONANO

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NANOBIONANO NAB Vol. 1 | No. 1 | 2014

Potential Measurement of Biological Compound Concentration using Capacitive Sensor

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History

Received: May 12, 2014

Revised: June, 2014

Accepted: July, 2014

Available online: July, 2014

Keyword

Capacitive,

sensor,

solution,

biological compound,

enzyme

Monitoring of biological compounds related to health issue is an important activity. A device that is low in cost, simple and portable is an advantage for biological compound monitoring. In this work, a device was developed using a capacitance (C) concept with two plates system. Linear relationship between frequency (f) and sample concentration was obtained proving that the relationship between f and the dielectric constant (k) can be used to develop sensor for biological compounds. The behavior of sample reactions can be monitored in the presence of free enzymes in solution. By using capacitance as measurement concept linear relationship was obtained for the concentration of histamine from 100 until 300 ppm.

Measurement of biological compound is very important especially at the nanoscale level. There are many types of important biological compounds for food and health benefit. Conventional methods of analysis have certain disadvantages and even well-equipped, organized analytical laboratories do not provide analytical results in time especially in urgent situations. A need for direct measuring device that would make it possible to carry out nondestructive analysis with possibility of data transmission is greatly needed. Furthermore there are also increasing needs for new analytical tools that are sensitive, reliable and easy to handle. Despite the description of numerous systems in the literature, only a few techniques have met the basic requirements for practical utilization. Conventional procedures for the measurement of biological compound are usually based on chromatographic techniques, which require fluorescence derivatization and expensive instrumentation [1]. Enzyme-based amperometric biosensors using histamine oxidase [2,3], methylamine dehydrogenase [4,5,6], and quinohemoprotein amine dehydrogenase [7] have also been developed for the determination of histamine (toxin in fish). However, these methods have serious disadvantages in substrate specificity [4,5,6,7] and interference [2,3].

Comparing to other sensing principles, capacitive sensing has several advantages for sensor applications such as the simplicity of a capacitive sensing and its compatibility with the standard IC process technology that allows for a great amount of flexibility in design and fabrication. In addition, the sensing output is more repeatable and sensor performance is less likely to degrade over time. The capacitive sensing also has high sensitivity and it is more resistant to the change of operating temperature [8]. The basic structure of a capacitive sensor consists in an asset of plates of surface, A, separated by a distance, d. Capacitance value can be obtained at, $C=k(A/d)$, where, k, is dielectric constant of a medium between the plates. In order to achieve this goal a method for development of capacitive sensor was explored. The objective of this research is to study the concept of capacitance to the reaction of histamine in the presence of free enzyme (diamine oxidase) in solution.

Materials and Method

Histamine dihydrochloride, sodium hydroxide and sodium phosphate dibasic dehydrate were purchased from Sigma. Other chemicals were of analytical grade and used without further purification. All aqueous solutions were freshly prepared using deionized water. The activity of enzymes diamine oxidase and concentration of histamine were 0.16unit/ mg and 100-300 ppm respectively

The experimental set-up and an electrode with "a stable" multivibrator operation circuit (in C-R circuit) were constructed based on Wasoh et al. [9] and the operation circuit was modified from Wagiran et al. [9,10]. In this analysis, the system proposed by Boylestad and Nashelsky [11], was applied. The first step of this research was to examine a potential of capacitance (based on parallel plate system) to determine different histamine concentration (in this analysis, histamine was used as a sample of biological compound). No enzyme or membrane was used in this step. The distance between electrode plates (*d*) and the different in dielectric material (histamine sample in different concentrations) were tested. These two parameters were chosen because capacitance was depended on *d* and dielectric constant (*k*) by referring to equation $C=(kA)/d$ [11]. Computer with LabVIEW software was used in this experiment [9]. Another step was a test for histamine biosensor to detect the changes occurred in solution in the presence of free enzyme.

$$k = (1.44)(d) \,/\, f(A)\,(R_1+2R_2) \qquad \ldots (1)$$

This equation was derived from the equation given by Boylestad and Nashelsky [11]. In which, A= 15 mm x 5 mm= 75 mm^2, d= 2 mm, ε_o= 8.854 x 10^{-12} Fm^{-1}, R_1= R_2= 10 kΩ

$$k = 3.19 \times 10^9 / f \qquad \ldots (2)$$

Sensor response in *f* was changed into *k* value by using equation (2). Bar and line graph were plotted using Microsoft excel in order to see the relationship between *f* and *k* toward individual histamine solution.

Result and Discussion

In this experiment, the function of capacitor-resistor circuit was tested using different sample concentrations (in this case, histamine). This step was considered as a basic test before the circuit can be further used in the development of capacitive sensor for measurement of biological compound. The value of histamine concentration with good reading and in acceptable range was obtained with fixed capacitor (0.0022 μF) and resistor (10 kΩ). Value that is too high was avoided because it can give false output signal. Boylestad and Nashelsky [11] reported that the capacitor-resistor circuit gave a good performance in specific range of capacitance value.

Relation between Distance (*d*) and Frequency (*f*). In this section, capacitor with two parallel plates was used to observe the effect of '*d*' to the sensor response especially in a simple dielectric material. Frequency for different concentrations when *d*=1.0 and 0.5 mm is shown in Fig. 1 Two different

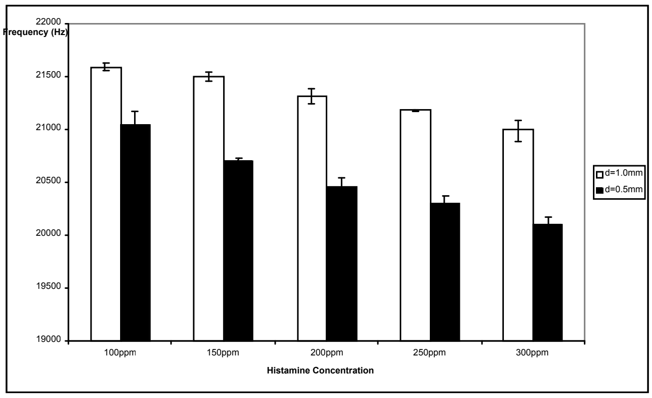

Figure 1: Frequency of histamine concentration when *d*=1.00.1cm and 0.50.1cm. Error bars represent standard deviation from the mean (n = 5).

values of distances were tested in order to test the ability of the sensor in providing different readings. In both cases a lower histamine concentration (100 ppm) yielded higher frequency value and a higher histamine concentration (300 ppm) yielded lower frequency value. Longer distance (1.0 mm) between capacitor plates gives higher frequency reading than shorter distance (0.5 mm), which meant that the frequency (*f*) was directly proportional to the distance (*d*). Based on the equation [$C=(kA)/d$] [11], *C* value is inversely proportional to *d* and based on the equation [$f(R_1+2R_2)=(1.44)/C$] [11], *f* is inversely proportional to *C*. Another formula can be derived by substituting the first *equation* into the second *equation* and resulted in *f* is directly proportional to *d*. The result obtained from the experiment is in agreement to the result formulated from the equation. Jia Ming [12] also reported capacitive sensor can show difference *f* values in difference sample concentrations.

Relation between (*k*) and Frequency (*f*). This test was carried out in histamine solution with different dilution as an indicator to dielectric material with different *k* values. The dielectric constant values were then calculated from *equation 1*. Result from the experiment (Fig.2) shows frequency (*f*) is inversely proportional to the concentration whereas dielectric constant (*k*) is directly proportional to the concentration. Regarding to the equation [$C=(kA)/d$] and [$f(R_1+2R_2)=(1.44)/C$], from Boylestad and Nashelsky [11], capacitance is directly proportional to dielectric constant ($C \alpha k$) and frequency is inversely proportional to capacitance ($f \alpha 1/C$) of nonconductive material. Based on these two equations it is suggested that frequency is inversely proportional to dielectric constant ($f \alpha 1/k$). This result is well in agreement with the theory explained in *equation 1* which was derived from equation given by Boylestad and Nashelsky [11]. Higher histamine concentration possessed weaker electric field strength, developed higher capacitance value and lower frequency reading. In reverse, lower histamine concentration possessed stronger electric field strength, developed lower capacitance value and higher frequency reading.

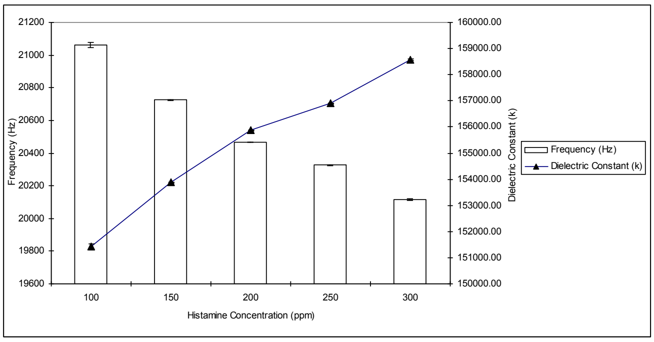

Figure 2: Relation of histamine solution to frequency (*f*) and dielectric constant (*k*). Error bars represent standard deviation from the mean (n = 5).

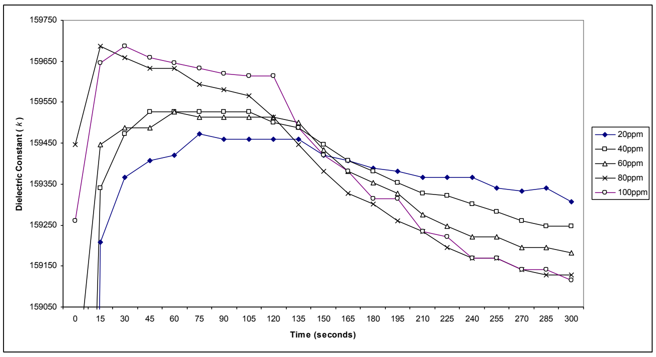

Figure 3: Dielectric constant (*k*) for histamine reaction with free enzyme.

A change in the capacitance is proportional to *k*, the dielectric constant of the material caused by having dielectric material between the plates [11]. This phenomenon occurred due to the molecules of histamine (in solution) is polarized by the electric field, producing concentrations of charge on its surfaces that create an electric field opposed (antiparallel) to that of the capacitor. Thus, a given amount of charge produces a weaker field between the plates than it would without the dielectric material, which reduces the electric potential. Considered in reverse, this argument means that, with a higher concentration of histamine, a given potential causes the capacitor to accumulate a larger charge and increase *k* value. The changes of bulk solution with respect to a fixed electrode established a changing capacitor value between the electrodes in aqueous solution and all relied on a measurement of changes in dielectric constant [11]. When dielectric material was placed in electric field, electrons in molecules were pulled toward positive plate and positively charged nuclei were pulled toward negative plate. The charges remained bound in their atoms or molecules, but the center of negative charge in a molecule was shifted with respect to the center of positive charge. The dielectric remained electrically neutral because the positive and negative charge remained bound, but this displacement produced a layer of negative charges on the surface of the dielectric closed to the positive plate and a layer of positive charges on the surface closed to the negative plate. These surface charges reduced the electric field strength within the dielectric material because they produced an electric field in the opposite direction from that capacitor plates.

Capacitance Behavior of Histamine Reaction in the Presence of Free Enzyme. Fig. 3 shows the changes of dielectric constant for histamine in the presence of free enzyme in solution along 180 seconds reaction time (300s-120s). This result was presented to show the behavior of sensor response to dielectric constant during enzymatic reaction of histamine at different concentration. Base on the graph, *t*=0s to 15s was a transition process for capacitance to become stable due to the change of dielectric material or change in *k*. Enzyme was added at *t*=120s and the response started to decrease indicating dielectric constant decreased during the reaction of histamine in the present of free enzyme in solution. After enzyme addition, a bigger change was obtained with high concentration of histamine (100 ppm) compare to low concentration of histamine (20 ppm) because high concentrations supplied more substrate for enzymatic reaction.

During the reaction many tiny bubbles were observed in the solution. In this case, perhaps the changes in capacitance reading were dominated by the production of hydrogen peroxide or oxygen. Oxygen was probably produced from oxidation of hydrogen peroxide which was produced from decarboxilation of histamine. Hydrogen peroxide was unstable and easily converted into oxygen.

This experiment showed that high histamine concentration produced big changes in dielectric constant whereas low histamine concentration produced small changes in dielectric constant. Base on equation *[C=(kA)/d]* [11], the dielectric constant *(k)* increases when capacitance (*C*) increases indicating enzymatic reaction under high histamine concentrations produced high *k* value and low histamine concentrations produced low *k* value. Perhaps, the enzyme may also be involved in dielectric changes due to its ability to dilute in reaction mixture or to produce link with other molecules because all of them were part of dielectric material and gave an effect to electric field strength. Yi et al. [13] reported that the dielectric properties of molecules depend on electron transfer, atomic bonds and the large-scale molecular structure. Weaker electric field strength may be generated in enzymatic reaction of high histamine concentrations compare to low histamine concentration. Silbey and Albert [14] reported weak electric field strength within the dielectric material can produce high changes in capacitance. The enzyme which was used for this particular experiment proved that the capacitance biosensor can be used to detect chemical changes in enzymatic reaction. This experiment also showed that the

the reaction of histamine could be monitored in the presence of free enzyme in solution. Therefore it may show good potential to detect the presence of histamine in the reaction system.

Conclusion

From the experiment, the capacitance in system was found to give linear relationship to the concentration of histamine (from 100 to 300 ppm). This experiment led to a conclusion that this type of sensor (using capacitive concept) can be applied to measure biological compound. In conclusion, this experiment shows the relationship between frequency, dielectric constant and histamine concentration can be manipulated in order to develop histamine biosensor in a further research. Therefore, the capacitance concept has a potential to be applied in measuring different biological compound concentrations.

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