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Study on the Reaction Constant and Concentration Detection Algorithm of Enzyme Injection Glucose Biosensor

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Because of its good stability, immobilized enzyme biosensor has become one of the main methods to prepare enzyme electrode, which is widely used in biosensors. The sensor cannot be used online due to the high temperature sterilization process. It cannot control the amount of glucose in real time, which affects the quality and yield of fermentation. For this reason, some scholars put forward the idea of using enzyme instead of immobilized enzyme membrane. The glucose biosensor of enzyme injection has reached the standard of practical application.
However, there is a slight difference in the detection time, stability and other performance indicators compared with the mature enzyme membrane sensor. In this paper, a new algorithm is designed to calculate the reaction constant and concentration of enzyme injection glucose biosensor based on the kinetic equation of enzyme reaction.
The accuracy of the algorithm is verified by experiments. The main content of this study is to detect the glucose solution of 1mg/ml and 2mg/ml by enzyme injection sensor. By recording the voltage versus time curve, the linear slope of the curve is obtained. The Michaelis constant K_m of the enzyme involved in the reaction was calculated to be 1.59. The change of voltage gradient of 3mg/ml glucose standard solution can be obtained. In addition, the algorithm is incorporated into the concentration equation. The concentration of the measured object is 3.04mg/ml. The accuracy of the algorithm is 98.67%.

1. Introduction

Enzyme electrode has been widely used in biosensors because of its high stability. Enzyme protein is immobilized on the electrode surface by immobilized enzyme technology, which realizes the preparation of enzyme electrode (Jafari, et al., 2016). Although this method improves the stability of the enzyme, it will weaken the catalytic activity of the enzyme (Chen, et al., 2015; Zaki et al., 2017; Andrade et al., 2017; Masutti et al., 2016; Pinotti et al., 2017).
Michaelis constant K_m is an important kinetic constant in the Michaelis Menten equation. Michaelis constant K_m is an important kinetic constant in the Michaelis Menten equation. In addition, due to the inactivation of enzyme by high temperature sterilization, the immobilized enzyme biosensor cannot realize the on-line detection of glucose in fermentation process. In addition to the immobilized enzyme biosensor, Kriz.D proposed a biosensor for the identification of a substance to be injected into the reaction pool for the patent application in 1995. The sensor injected the enzyme into a reaction tank in a liquid form and reacted with the liquid to be tested.
However, it is easy to be affected by environmental factors due to the instability of the enzyme structure, such as temperature, pH value (Ertek, et al., 2016). Its expression in the dynamic equation is the change of Michaelis constant Due to the difference of the Michaelis constant of immobilized enzyme and liquid enzyme, special consideration should be given to the design of enzyme injection biosensor.
In the immobilized enzyme biosensor, the slope of the initial linear phase of the response voltage curve of the sensor is proportional to the concentration of the reactant (Rahaman, et al., 2016). But the idea is not entirely correct in enzyme-injected biosensors. In view of this, this study designed a detection algorithm based on the kinetics of enzyme injection biosensor and verified the algorithm.

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2. Materials and methods

2.1 Experimental materials and equipment

In the experiment, SBA-40E biosensor was selected as a mature sensor, which was compared with enzyme injection glucose biosensor (Lee, et al., 2016). Glucose standard solution was used to calibrate the biosensor. The phosphate buffer was prepared by adding distilled water to a special buffer of the SBA series analyzer, and the buffer was used to provide a reaction environment suitable for the oxidase activity. Glucose oxidase solution was prepared by adding G7141 glucose oxidase (312U/mg, American Sigma Company) to phosphate buffer. Enzyme injection glucose biosensor made by laboratory.

2.2 Research methodology

In order to improve the detection performance of glucose biosensor of enzyme injection and improve the detection performance, this paper analyzes the original detection algorithm based on the mechanism of enzymatic reaction. On this basis, a new detection algorithm is designed, and the accuracy of the algorithm is verified by experiments.

(1) Detection algorithm analysis

The glucose concentration was measured by hydrogen peroxide electrode in enzyme injection glucose biosensor. In the process of reaction, hydrogen peroxide has two processes of generation and consumption, which can be expressed by Eq (1) and (2). The two processes are carried out simultaneously.

$$\frac{dP}{dt} = \frac{k_2[S_0][E_0]}{[S_0](k_{-1} + k_2)/k_1} \tag{1}$$

$$\frac{d[P]}{dt} = k[P] \tag{2}$$

As shown in the Eq (1) and (2), *dp/dt* represents the formation rate of reaction product. [*P*] is product concentration; [S_0] is initial substrate concentration; [E_0] initial enzyme concentration; K_1 indicates the reaction rate constant of the reaction between enzyme and substrate; K_2 indicates that the reaction rate constants of complexes are decomposed into enzymes and substrates; K_{-1} represents the reaction rate constants of the complex decomposition enzymes and products; *K* represents the generation rate of resultant. When the two processes are the same, the concentration of hydrogen peroxide does not change, which reflects in the current as the current stability. The glucose concentration can be solved by combining Eq (1) and (2).

This algorithm can be used to calculate the concentration of the analyte in the laboratory, but it also has some disadvantages. Firstly, the glucose concentration can be determined by the glucose standard solution, which can be used to determine the background current (Lin, et al., 2016). However, in the actual measurement, the liquid to be tested is the liquid mixture removed from the fermentation tank. The pH value of the mixed solution will be adjusted according to the growth trend of bacteria. The mixture is filled with an ionizable substance that changes the background current. At this time, the steady state current value is no longer the change value based on the original background current, while it is the total current value after adding the additional current value produced by the electrolysis of the electrolyte in the fermentation liquid. The background current of the sensor can be measured by calibration. However, due to the difficulty of measuring the current produced by the electrolysis of the substance in the fermentation liquid, the calculation of the concentration of a given point cannot get accurate results (Ramanathan, et al., 2016). Secondly, due to the absence of catalase in the sensor, the generated hydrogen peroxide is naturally decomposed around the platinum electrode, resulting in slow growth rate of hydrogen peroxide decomposition. Therefore, the stability time of hydrogen peroxide concentration becomes longer. Therefore, the algorithm can increase the detection time and reduce the detection speed of the sensor. After analysis, there are many unreasonable points in the original detection algorithm. This algorithm will weaken the detection performance of the sensor (Li, et al., 2016).

(2) Detection algorithm design

Because the hydrogen peroxide concentration is very low at the beginning of the reaction, the reaction is an enzymatic reaction, that is, the generation of hydrogen peroxide, which can be expressed by Eq (1). At the same time, with the increase of time, the function of the index part weakened gradually, and the hydrogen peroxide concentration in the enzymatic reaction can be approximately linear with the time. In this study, the relationship between the concentration of glucose and the concentration of glucose was found by measuring the slope *K* of the linear region, which can be expressed by the Eq (3):

$$k = \frac{k_2[S_0][E_0]}{[S_0](k_{-1} + k_2)/k_1} \tag{3}$$

After simplifying the Eq (3), we can obtain the Eq A= $K_2[E_0]$, and the Michaelis constant is $K_m=(K_{-1}+K_2)/K_1$. Then the Eq (4) can be obtained:

$$k = \frac{A[S_0]}{[S_0] + K_m} \tag{4}$$

In the Eq (4), the slope K represents the generation rate of the product, and A is the former factor, which is also known as the frequency factor.

Since the concentration of hydrogen peroxide in the reaction tank is directly proportional to the response voltage, the formation rate of the reactant can also be expressed by the rate of change of the response voltage. Therefore, the Eq (4) can be used to describe the change rate of the voltage in the sensor. According to this principle, a new concentration detection algorithm is designed. The new detection algorithm needs to measure the slope of the linear region of the curve and the Michaelis constant of the detection environment. Therefore, it is necessary to determine the parameters in the algorithm by means of experiments and calibration. Firstly, the linear phase of the response voltage curve is determined by experiment. Secondly, the linear slope of the response voltage is determined by measuring the two known concentrations of glucose solution. The current detection environment of the Michaelis constant value can be calculated according to the Eq (5) and Eq (6):

$$k_1/k_2 = \frac{\frac{A[S_1]}{[S_1] + K_m}}{\frac{A[S_2]}{[S_2] + K_m}} = \frac{[S_1][S_2] + [S_1]K_m}{[S_1][S_2] + [S_2]K_m} \tag{5}$$

The Michaelis Menten constant in real time can help the model to be closer to the real liquid enzyme reaction process, thus increasing the detection accuracy and stability of the sensor. As shown in Eq (6), after determining the Michaelis constant, the Michaelis constant is incorporated into the model to detect the unknown concentration solution.

$$[S_{unknow}] = \frac{[S_{know}]K_m K_{unknow}/K_{know}}{[S_{know}] + K_m - [S_{know}]K_{unknow}/K_{know}} \tag{6}$$

In the Eq, [$S_{unknown}$] indicates the concentration of glucose solution to be detected, and it is an unknown quantity. [S_{know}] indicates the concentration of glucose solution. $K_{unknown}$ represents the linear partial slope of the unknown concentration glucose solution detected by sensor. K_{know} represents a linear partial slope of a known concentration of glucose solution detected by sensor.

Because the linear part only exists in the early stage of the reaction, the algorithm can rapidly get the liquid concentration. In addition, the detection time relatively has been greatly improved.

(3) Accuracy verification of detection algorithm

In order to test the accuracy of the detection algorithm, the related experiments are designed to verify the algorithm. However, it is necessary to verify the linear variation of the voltage versus time in order to verify the accuracy of the algorithm. The algorithm needs to calculate the glucose concentration by the slope of the linear change of the voltage with time, so the selection of the linear phase is directly related to the accuracy of the slope. If the selected linear phase is too small, the resulting slope may be affected by interference due to the lack of full utilization of the linear part of the data. If the linear range is too large to exceed the linear range of the voltage, the slope cannot represent the linear change of the response voltage. There is a big error in the calculated concentration. Therefore, it is necessary to determine the range of the linear change of the curve by experiment.

The glucose solution of 0.5mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml, 2.5mg/ml, 3mg/ml was detected by enzyme injection glucose biosensor, and the curve of voltage with time was obtained. The data points in different time ranges are selected and the data points are fitted by the straight line. The linearity of the curve within the range was determined by fitting the correlation coefficient R2 of the straight line and the data points. Then the Michaelis constant K_m of the enzyme involved in the reaction was calculated. Finally, the voltage change curve and the linear partial slope are obtained by measuring the glucose solution of another known concentration. The concentration equation is established and the concentration of the measured object is calculated.

3. Results

6 glucose solutions with different concentrations were detected by enzyme injection glucose biosensor. The change curve of the voltage with time in the reaction of the known concentration glucose solution is recorded and the slope of the linear part of the curve is obtained. The experimental results are shown in Figure 1, and the fitting results of the current voltage curves are shown in Table 1.

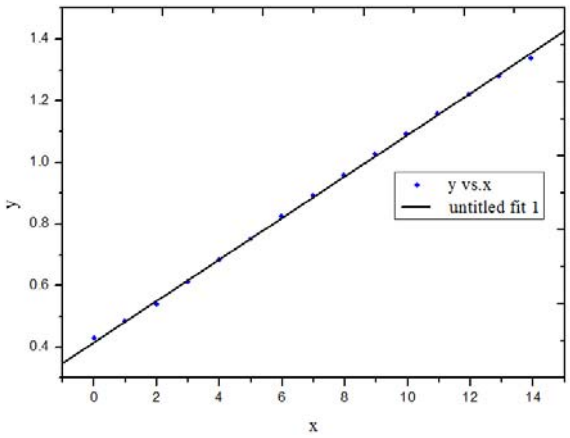
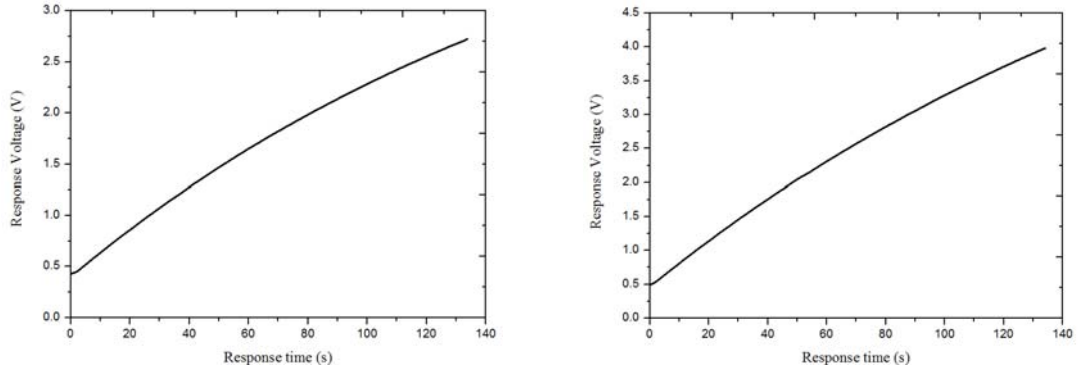


Figure 1: Fitting results of voltage curve linear part

Table 1: Curve fitting linear degree

Glucose solution concentration (mg/ml)	Range of curve liner slope(s)					
	0-5	0-10	0-15	0-20	0-25	0-30
0.5	0.9971	0.9988	0.9991	0.9982	0.998	0.9976
1	0.9933	0.9987	0.9991	0.9986	0.9974	0.996
1.5	0.9899	0.9979	0.9992	0.9987	0.9978	0.9966
2	0.9901	0.998	0.9993	0.9992	0.9982	0.9974
2.5	0.9858	0.9929	0.9993	0.9987	0.9971	0.9964
3	0.9937	0.9982	0.9993	0.9989	0.9989	0.9988

It can be seen from Table 1 that the response voltage curve has the highest linear degree when the curve is selected from 0 to 15 seconds. It is assumed that the voltage is proportional to time in this period.
Therefore, the slope of the curve between 0 and 15 seconds is chosen as the slope of the linear phase curve in the initial stage of the algorithm.
The Michaelis constant of the enzyme involved in the reaction should be detected before the concentration detection. The slope can be obtained by measuring the concentration of glucose solution, and the Michaelis constant K_m can be obtained by the Eq (5). The linear region of the reaction was detected by enzyme injection glucose biosensor, and the concentration of the analyte was deduced by Eq (6). The glucose standard solution with 1mg/ml and 2mg/ml concentration was detected, and the change of the voltage in the sensor with time was recorded. The slope of the linear change of voltage is taken as the K in Eq (5). The Michaelis Menten constant of glucose oxidase in the reaction environment can be obtained by comparing the slope Eq of different glucose concentrations. The glucose concentration of the glucose solution was measured with enzyme injection glucose biosensor, and the linear part was calculated by the linear part. The current concentration of the glucose solution can be calculated by converting the detected slope value of the known concentration solution and the concentration slope value into the Eq (6). Finally, the accuracy of the method is verified by comparing with the actual concentration. The glucose standard solution of 1mg/ml and 2mg/ml was detected by enzyme injection glucose biosensor, and the change curve of response voltage with time was analyzed. The slope of the linear part of the slope can be expressed by the enzymatic reaction kinetics equation (4). The Michaelis constant K_m can be determined by comparing the slope. The voltage and time data of the sensor are inputted into the MATLAB, and the curve of the voltage change with time is drawn by the drawing instruction. Figure 2 (a) is the 1mg/ml glucose standard solution when the voltage changes with time curve. The linear partial slope at Initial reaction is K_{1mg}/ml =0.02952.



(a) Voltage curve of 1mg/ml glucose solution *(b) Voltage curve of 2mg/ml glucose solution*

Figure 2: Voltage curve of 1mg/ml glucose solution and 2mg/ml glucose solution

Figure 2 (b) is the change curve of voltage with time changes in the decomposition period of 2mg/ml glucose standard solution. The linear partial slope at Initial reaction is K_{2mg}/ml ==0.03438. The two slopes were added to the Eq (5), and the Michaelis Menten constant of glucose oxidase is K_m=1.59.
Enzyme injection glucose biosensor was used to detect the voltage change curve of 3mg/ml glucose standard solution, and the linear partial slope of response curve can be obtained. At the same time, the slope and the slope of the 1mg/ml solution were added into the Eq (6), and the concentration of glucose solution was calculated by the Eq (6). The accuracy of the method is verified by comparing it with known concentrations. Figure 3 shows the voltage versus time curve in the decomposition of 3mg/ml glucose solution, and the slope K_{3mg}/ml = can be obtained by selecting its linear part.

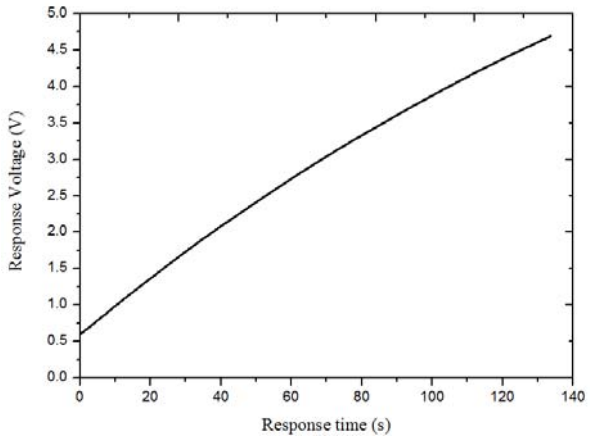


Figure 3: Voltage curve of 3mg/ml glucose solution

The Eq K_{3mg}/ml=0.05022 can be obtained by the tested data of sensor. K_{1mg}/ml=0.02952, K_{3mg}/ml=0.05022, [S_1]=1 mg/ml and K_m=1.59 were brought into Eq (6), the measured concentration [S_2]= 3.04mg/ml can be obtained. The concentration of glucose solution was 3mg/ml. The error rate was 1.33%, and the accuracy was up to 98.67%. In this paper, the detection algorithm is used to calculate the concentration of the analyte with a high accuracy.

4. Conclusions

In order to improve the detection efficiency of glucose biosensor of enzyme injection, the parameters should be applied to the standard. In this paper, a new method is designed to detect the concentration of enzyme biosensor based on the mechanism model of enzyme injection glucose biosensor. The accuracy of the method is verified by experiments. The voltage versus time curve was recorded in the experiment when the concentration of glucose solution had a reaction. The linear partial slope of the curve is obtained. Secondly, the Michaelis Menten constant of the oxidase was calculated by the established kinetic equation, which was K_m=1.59. Finally, the voltage change curve and the linear partial slope are obtained by measuring the glucose

solution of another known concentration. The algorithm has been incorporated into the concentration equation, and the concentration of the measured object is 3.04mg/ml. The experimental results show that the accuracy of this algorithm is 98.67%.

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