Detection of Glucose by Copper Ion Catalytic ABTS-H$_2$O$_2$ Spectrophotometry

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Abstract. Cu$^{2+}$ can catalyze the chemical reaction between hydrogen peroxide (H$_2$O$_2$) and the 2,2’-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) effectively and bring the change of absorbance. Glucose oxidase (GOD) can catalyze glucose oxidation to produce H$_2$O$_2$. The content of glucose can be determined indirectly by measuring the content of H$_2$O$_2$. The pH value, H$_2$O$_2$ concentration, Cu$^{2+}$ concentration, reaction temperature and reaction time were investigated and optimized in the experiment. Under the optimal conditions, a linear relationship between the concentration of the glucose root and absorbance could be obtained over the range of 100 μM ~ 10 mM. The linear relationship is $A = 79.803B + 0.1224$ ($R^2 = 0.9958$) ($A$ represents the absorbance, $B$ represents the concentration of glucose).

1. Introduction

In recent years, due to the improvement of living standards, changes in diet structure and unhealthy lifestyle, the incidence of diabetes has been rising all over the world. Diabetes has threatened human health [1]. In view of this, early diagnosis and early treatment of diabetes are very important. Real-time and rapid monitoring of glucose levels in the blood is important for the control of this disease. At present, researchers have developed many methods to detect glucose, such as fluorescence [2], chromatography [3-5], electrochemical [6,7] and so on. Although the sensitivity of fluorescence analysis is high, this method requires the use of large instruments and a large one-time investment. High performance liquid chromatography (HPLC) has many advantages, such as high separation efficiency, good selectivity, high detection sensitivity, automatic operation and wide application range, but it has high equipment configuration and daily maintenance costs, complicated pretreatment process and long detection time. Although electrochemical analysis has high sensitivity and wide measurement range, its selectivity is poor, the electrode life is limited, and it is easily affected by external conditions such as temperature. Even though each of these methods has its merits, their popularization and application are limited by the high cost of analysis or the complexity of the analysis process, which makes the application inconvenient or wastes time. Consequently, it is of great practical significance to establish a simple and rapid glucose detection analysis method.

Among various analytical methods, spectrophotometry is favored for its advantages of simplicity, rapidity, no need of expensive instruments, and visual inspection. Nonetheless, most of them rely on nanoparticles, such as gold nanoparticles [8,9] and cerium oxide nanoparticles [10]. Moreover, nanoparticles need to be synthesized and generally need to be treated with water solubility, which is a
complicated process. Therefore, a simple and rapid spectrophotometric detection of glucose remains a major challenge. The chromogenic reactions of 2, 2-azidebis (3-ethylbenzothiazoline) -6-sulfonic acid (ABTS) and hydrogen peroxide (H$_2$O$_2$) have been widely used in spectrophotometric studies. With appropriate oxidant action, ABTS will be oxidized into green cations. Relevant studies have shown that ABTS can react quickly with hydroxyl radicals to form colored ABTS$^{•+}$ [11-14]. Cu$^{2+}$ reacts with H$_2$O$_2$ to form hydroxyl radicals, which can be used to catalyze the oxidation of ABTS$^{[15,17]}$. Compared with other nanoparticles, the Cu$^{2+}$ has the advantages of low cost and excellent stability. Glucose oxidase (GOD) can catalyze glucose oxidation to produce H$_2$O$_2$. Furthermore, Cu$^{2+}$ can catalyze the ABTS-H$_2$O$_2$ system to increase the absorbance. The content of glucose can be determined indirectly by measuring the content of H$_2$O$_2$. Accordingly, this study established a spectrophotometric method using Cu$^{2+}$ catalyzed ABTS-H$_2$O$_2$ system and applied this method to the detection of glucose.

2. Material and Methods

2.1. Reagent and chemicals
2,2-azidebis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) was purchased from Sigma-Aldrich Chemical Co. Copper chloride. H$_2$O$_2$ was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glucose oxidase (GOD) was purchased from Sinopharm Chemical Reagent Co., Ltd. All the other reagents in this work were of analytical grade. The absorbance is measured by using a UV-752 UV-visible spectrophotometer (Shanghai Metash Instrument co. Ltd)

2.2. Detection of glucose
The sample solution containing 20 mM phosphoric acid buffer (pH 7.0), 1 mg/mL GOD and various concentrations of glucose was incubated at 37°C for 30 min. Then 250 mM Cu$^{2+}$, 10 mM tirs-HCl (pH 7.0) and 2 mM ABTS were added to the solution, with a final volume of 1.5 mL. After the reaction for 5 min, the glucose content in the system was determined. The absorbance of the sample solution was measured at 415 nm.

3. Results and discussion

3.1. Method design and experimental principle
Cu$^{2+}$ has the ability to catalyze the oxidation of ABTS by H$_2$O$_2$ to produce colored ABTS$^{•+}$, which increases the absorbance of the sensing system [15]. GOD can catalyze glucose oxidation to produce H$_2$O$_2$. Under the action of catalyst Cu$^{2+}$, H$_2$O$_2$ generated hydroxyl radical, which rapidly oxidized ABTS to ABTS$^{•+}$ and caused the change of absorbance. The experimental principle is shown in Scheme 1. The color changes and the absorbance at 415 nm were proportional to the concentration of glucose within a certain range. Therefore, glucose can be detected by changes in absorbance.

3.2. Cu$^{2+}$-catalyzed H$_2$O$_2$ + ABTS reaction
Relevant studies have shown that ABTS can react quickly with hydroxyl radicals to produce colored ABTS$^{•+}$, while Cu$^{2+}$ reacts with H$_2$O$_2$ to generate hydroxyl radicals. Therefore, Cu$^{2+}$ has the ability to catalyze the reaction between ABTS and H$_2$O$_2$, so this experiment explored the catalytic effect of Cu$^{2+}$. As shown in fig. 1, the solutions in no. 1-3 centrifuge tube are respectively ABTS, ABTS + H$_2$O$_2$, and glucose oxidase (GOD) reaction solution.
ABTS + Cu²⁺, and all of them are nearly colorless. When different concentrations of Cu²⁺ were added to tubes 2 showed significant green after the same reaction time, and the color of the solution became greener as the concentration of Cu²⁺ increased (4-6 centrifuge tube). It is concluded that Cu²⁺ can catalyze the oxidation of ABTS by H₂O₂ to produce colored ABTS•⁺, which increases the absorbance of the sensing system. The comparison of no. 3 and no. 5 showed that the solution had no color when H₂O₂ was absent. GOD can catalyze glucose oxidation to produce H₂O₂, so this method can determine the content of glucose.

Fig. 1 Color change diagram of different sample solutions. 1: ABTS; 2: ABTS+H₂O₂; 3: 300 μM Cu²⁺+ABTS; 4: 20 μM Cu²⁺+ABTS+H₂O₂; 5: 300 μM Cu²⁺+ABTS+H₂O₂; 6: 500 μM Cu²⁺+ABTS+H₂O₂.

3.3. Optimization of experimental conditions

To detect glucose, the sensitivity of ABTS+H₂O₂+Cu²⁺ system needs to be improved, so we need to optimize the factors that affect the absorbance value. Therefore, this experiment optimized the pH, the concentration of Cu²⁺, the reaction time and the reaction temperature of GOD were investigated and optimized in the experiment. In the following experiments, H₂O₂ was directly used to replace H₂O₂ produced by glucose decomposition.

The pH value of the buffer solution has a certain influence on the absorbance of the system. The A/A₀ under different pH were explored (where A is absorbance in the presence of H₂O₂ and A₀ is absorbance in the absence of H₂O₂). As shown in Fig. 2A, the A/A₀ increases with the increase of pH, and then A/A₀ reached the maximum at pH 7.0. Thus, the optimal pH value is determined to be 7.0, which will be used in subsequent research.

Here, the concentration of H₂O₂ is optimized to provide a reference for the H₂O₂ generated by the decomposition of glucose, as well as a reference for the subsequent addition of glucose. Fig. 2B illustrates the absorbance increase H₂O₂ concentration system also gradually increases, when the concentration of H₂O₂ reached 500 mM, and then continue to increase the concentration of H₂O₂, it can be seen that the system of the absorbancy gradually stable, so we chose the optimal concentrations of H₂O₂ as 500 mM.

Cu²⁺ as catalyst has a certain influence on the colorimetric sensing system, so the Cu²⁺ concentration was optimized in this experiment. It can be seen from fig. 2C that as the concentration of Cu²⁺ increases, the absorbance also increases. When the concentration increases to 250 μM, it can be seen that the absorbance gradually tends to be stable. Therefore, the optimal concentration of Cu²⁺ in this experiment is determined to be 250 μM, which is used for subsequent studies.

The temperature and time of GOD reaction had an important influence on the results of the experiment. Therefore, this experiment explores time and temperature. As can be seen from fig. 2D, the reaction rate at 25°C is lower than that at 37°C. At 25°C, with the increase of decomposition time, the peak of absorption occurred at 415 nm kept increasing, and the equilibrium was not reached, indicating that the reaction was not complete. At 37°C, with the increase of decomposition time, the absorption peak at 415 nm gradually increased, and when the decomposition time reached 30 min, the absorbance basically stopped changing and became stable. Therefore, the optimal decomposition time was selected as 30 min, and the optimal reaction temperature was 37°C, and the temperature and time were used for subsequent experiments.
Fig. 2 (A) The absorbance of the systems in the absence or in the presence of H$_2$O$_2$ under different pH. (B) The effect of the amount of H$_2$O$_2$ on the assay performance. (C) The effect of the amount of Cu$^{2+}$ on the assay performance. (D) The optimization of the of reaction time of GOD.

3.4. Assay performance of the present method for glucose detection

Under the optimal experimental conditions, the standard glucose curve was drawn according to the absorbance of glucose at different concentrations. As shown in fig. 3, the absorbance value at 415 nm has a good linear relationship with the concentration of glucose in the range of 100 μM ~ 10 mM. The linear equation is, $A = 79.803 \times B + 0.1224$ ($R^2= 0.9958$) (where A is the absorbance and B is the concentration of glucose), and the correlation coefficient is $R^2= 0.9958$.

Fig. 3 Glucose standard curve. The error bars represent the standard deviation of the three measurements.

3.5. Selectivity of the present method

In order to further verify the feasibility of this sensing system, three glucose analogues fructose, lactose,
maltose and glucose were selected for comparison under the same reaction conditions, so as to verify the selectivity of ABTS+H2O2+Cu2+ system to glucose. As shown in fig. 4, it can be observed that glucose has a significant signal response, with a relatively large absorbance and a blue color, while other sugars basically have no signal and a small absorbance value, with a colorless color. Therefore, it indicates that other sugars have no effect on the selective determination of glucose, and this method can be used for the analysis and detection of glucose, which has certain practical significance.

Fig. 4 Selectivity of this method. Concentrations of ABTS, Cu2+, and glucose were 2 mM, 250 μM, and 10 mM, respectively. Concentration of fructose, lactose and maltose were all 10 mM. Error bars were estimated from three replicate measurements.

4.Conclusion
In this study, a simple and rapid spectrophotometric method for glucose detection was established by using Cu2+ catalyzed ABTS-H2O2 system. This method has several advantages: first, it does not require any chemical modification and synthesis. Second, it also does not require any enzyme to participate. Thirdly, the cost is very low. Further, detection in homogeneous solution is very convenient without a series of complicated operations such as separation and washing. In addition, this method has high selectivity and specificity. Therefore, the spectrophotometry established in this paper provides a new way to detect glucose content.

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