A label free colorimetric assay for detection of sulfite root based on $\text{Cu}^{2+}$

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Abstract. The reaction between sulfite and hydrogen peroxide causes the absorbance decrease, which provides the basis to determine the sulfite content that is sensitive to the absorbance change. $\text{Cu}^{2+}$ can catalyze the chemical reaction between hydrogen peroxide ($\text{H}_2\text{O}_2$) and the 2,2’-Azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) effectively, which will cause the change of absorbance. In this paper, we presented an experiment system to study the influence of pH value, $\text{H}_2\text{O}_2$ content, $\text{Cu}^{2+}$ content and reaction time on the reaction system. The comparison of experiment system results indicated that the optimal condition was pH value of 7.0, $\text{H}_2\text{O}_2$ of 500 μM, $\text{Cu}^{2+}$ of 250 μM and 5 minutes reaction time. Under the optimal conditions, the absorbance (Y) and $\text{SO}_3^{2-}$ content (C) have a linear relationship $Y = -861.35C + 0.8923$. The coefficient of determination ($R^2$) in the linear relationship is 0.9983, which indicates a strong correspondence between C and Y. Then, the established method was used to detect the sulfite in wine. The recovery rate was between 96.01% and 106.90%, which proves this method is efficient for sulfite determination.

1. Introduction

Sulfites have been widely used as a food additive in the food industry to maintain color, prevent bacteria and retain freshness[1-3]. Most of wines contain sulfites that are produced naturally during the fermentation process[4]. During the wine production, sulfites (or sulfur dioxide) are also added as preservative and inhibitor to arrest fermentation and prevent oxidation[5-6]. Although sulfites are safe for most people, they are a threat for 1% of the nonasthmatic population and 5% of asthmatics population who are sulfite sensitive[7-8]. Thus, it is necessary to detect the concentration of sulfites in the wine with a high precision and label them on the package prominently.

There are usually two methods for quantifying total sulfites in foods and beverages. One method is direct iodometry, which uses the color change to judge whether the chemical reaction is ended or not. Although the iodine direct titration method is relatively simple, there is a subjective error, and an iodine complex may be formed during the titration to cause interference. This method is easily interfered by compounds formed by iodine in the process of titration[9]. The accuracy of this method is questionable. Another method is European Union usually adopt more formal (AOAC recognized) Monier-Williams (OMW) based on optimization Detection method, which needs a long-time sample preparation and cannot be used in the rapid visual inspection on-site[10]. To address the challenge, great efforts have been made to detect the sulfite assays with better performance, such as Capillary electrophoresis[11], electrochemical analysis[12], fluorescence analysis[13-14], Chemiluminescence[15], colorimetry [16]. Although these methods have made some progresses in sulfite detection, they still have some...
shortcomings. For instance, Capillary electrophoresis with the wide, application range has a poor stability and is difficult to achieve good repeatability. The fluorescence analysis is fast methods, which requires large instruments and a large one-time investment. Although the electrochemical analysis method has high sensitivity and wide measurement range, it requires cumbersome modification and washing steps. It is precisely because of these shortcomings that these methods cannot be used for on-site inspection of sulfite, which cannot meet the needs of relevant law enforcement agencies for spot sampling. Therefore, it is of great practical significance to establish a research system that can be used for rapid visualization determination of sulfite on site.

Spectrophotometry has the advantages of being simple and fast and does not need large and expensive instruments. However, there are not many reports on the application of spectrophotometry to the detection of sulfite. Therefore, it is still a great challenge to build a determination of sulfite based on spectrophotometry. The chromogenic reaction of 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) and hydrogen peroxide (H$_2$O$_2$) is widely used in spectrophotometric studies. ABTS will be oxidized to green cation under the action of appropriate oxidants. Relevant studies have shown that ABTS can react quickly with hydroxyl radicals To form colored ABTS$^*$-[17-20]. Cu$^{2+}$ reacts with H$_2$O$_2$ to form hydroxyl radical, which can be used to catalyze the oxidation of ABTS[21-23].

In this study, we found that Cu$^{2+}$ has the ability to catalyze the oxidation of ABTS by H$_2$O$_2$ to generate colored ABTS$^{*+}$ and bring the change of absorbance. When SO$_3^{2-}$ are added to the system, the absorption of ABTS$^{*+}$ is reduced (Scheme 1). Because the H$_2$O$_2$ is consumed in the reaction between SO$_3^{2-}$ and H$_2$O$_2$, it reduces the absorbance of the system. Therefore, the new sensing method established based on the principle that sulfite consumes hydrogen peroxide and reduces the absorbance of reaction system can be applied in the detection of SO$_3^{2-}$. The proposed method does not need any chemical labeling, making it convenient, fast and low-cost.

Scheme 1. Schematic illustration of the colorimetric sensing system for SO$_3^{2-}$ detection.

2. Material and Methods

2.1. Reagent and chemicals
2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich Chemical Co. Copper chloride. H$_2$O$_2$ and Anhydrous sodium sulfite (Na$_2$SO$_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the other reagents in this work were of analytical grade. The ultraviolet-visible (UV-vis) absorption spectra were recorded using a Cary UV-1750 spectrophotometer.

2.2. Detection of SO$_3^{2-}$
The sample solution of 1500 μL mixture containing 250 μM Cu$^{2+}$, 10 mM tirs-HCl (pH 7.0), 500 μM H$_2$O$_2$ and various concentrations of SO$_3^{2-}$ was incubated for 5 min at room temperature. After that, ABTS
(2 mM) was added and after adding ABTS reaction for 5 min. Finally, the UV-vis spectra were recorded at room temperature 5 min later by using a UV-1750 UV-visible spectrophotometer (Shimadzu, Japan).

3. Results and discussion

3.1. Cu^{2+}-catalyzed H_{2}O_{2} + ABTS reaction

The catalytic ability of Cu^{2+} was first verified in the study (Figure 1a). In the absence of Cu^{2+}, there is no significant difference in terms of the absorption spectrum and color between ABTS solution (vial 1) and ABTS + H_{2}O_{2} mixture (vial 2). When Cu^{2+} was added into the ABTS solution (vial 3), absorption spectrum and color change were not obvious. However, great changes of absorption spectrum and color were observed after the addition of Cu^{2+} to ABTS + H_{2}O_{2} mixture. The color of the Cu^{2+} + ABTS + H_{2}O_{2} mixture becomes greener as the concentration of Cu^{2+} increases (from vial 4 to 6). These results indicate that Cu^{2+} can catalyze the oxidation of ABTS by H_{2}O_{2} to form colored ABTS^{+}, which increases the absorbance of the sensing system.

3.2. Cu^{2+}-catalyzed H_{2}O_{2} + ABTS reaction

When SO_{3}^{2-} is added to the ABTS + H_{2}O_{2} + Cu^{2+} system, SO_{3}^{2-} reacts with H_{2}O_{2} to generate hydroxyl radicals, which results in the H_{2}O_{2} consumption and corresponding reduction of ABTS^{+}. SO_{3}^{2-} reacts with H_{2}O_{2} to generate hydroxyl radicals, consuming H_{2}O_{2} and resulting in the reduction of. It can be inferred that SO_{3}^{2-} can reduce the absorbance of ABTS + H_{2}O_{2} + Cu^{2+} system. In order to explore whether the sensing system can be used for the detection of SO_{3}^{2-}, the feasibility of reducing ABTS + H_{2}O_{2} + Cu^{2+} reaction by SO_{3}^{2-} is investigated. As shown in Figure 1b, it can be known that when sulfite was added into the ABTS + H_{2}O_{2} + Cu^{2+} system, the absorbance of the system decreases at 415 nm and the color fades. Furthermore, with the increase of SO_{3}^{2-} concentration, the absorbance gradually decreases and the color of the sample solution becomes lighter (vial 2, 3, 4). However, in presence of SO_{3}^{2-}, absorption spectrum and color change of the ABTS + Cu^{2+} system were not obvious (vial 5). The results confirmed that the absorbance of the reaction system with SO_{3}^{2-} decreases because of the reaction of SO_{3}^{2-} + H_{2}O_{2} consumption. These results show that the principle designed in this research is feasible, and the sensor system can be used for the quantitative detection of SO_{3}^{2-}.

![Figure 1. UV-visible absorption spectrum of different sample solutions. (a) Inset: color change diagram of different sample solutions. 1: ABTS; 2: ABTS+H_{2}O_{2}; 3: 300 μM Cu^{2+}+ABTS; 4: 200 μM Cu^{2+} + ABTS+H_{2}O_{2}; 5: 300 μM Cu^{2+}+ABTS+H_{2}O_{2}; 6: 500 μM Cu^{2+}+ABTS+H_{2}O_{2}. (b) Inset: color change diagram of different sample solutions. 1: ABTS+H_{2}O_{2}+Cu^{2+}; 2: 60 μM SO_{3}^{2-} +ABTS+Cu^{2+}+H_{2}O_{2}; 3: 30 μM SO_{3}^{2-}+ABTS+Cu^{2+}+H_{2}O_{2}; 4: 600 μM SO_{3}^{2-}+ABTS+Cu^{2+}+H_{2}O_{2}; 5: 60 μM SO_{3}^{2-}+ABTS+Cu^{2+}. The concentrations of H_{2}O_{2}, ABTS and Cu^{2+} were 2 mM, 2 mM and 500 μM respectively.](image-url)
3.3. Optimization of experimental conditions

In order to improve the sensitivity of the ABTS+H$_2$O$_2$+Cu$^{2+}$ system for detecting SO$_3^{2-}$, many experimental conditions were investigated and optimized.

The pH value of the buffer solution has a certain influence on the absorbance of the ABTS+H$_2$O$_2$+Cu$^{2+}$ system. The A/A$_0$ under different pH were explored (where A is absorbance in the absence of SO$_3^{2-}$ and A$_0$ is absorbance in the presence of SO$_3^{2-}$). As shown in Figure 2a, the A/A$_0$ increases with the increase of pH, and then A/A$_0$ 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.

As the reactant, the concentration of H$_2$O$_2$ has a great influence on the absorbance of the reaction system. Therefore, the concentration of H$_2$O$_2$ is optimized in this experiment. From the Figure 2b, it can be seen that the absorbance of the system increases gradually when the final concentration of H$_2$O$_2$ reaches 500 μM. It can be seen that the absorbance of the system is gradually stable, so the optimal concentration of H$_2$O$_2$ is 500 μM.

Cu$^{2+}$ as catalyst has a certain influence on the colorimetric sensing system, so the Cu$^{2+}$ concentration was optimized in this experiment. It can be seen from Figure 2c that with the increase of Cu$^{2+}$ concentration, the absorbance also increases. When it increases to 250 μM, it can be seen that the absorbance gradually tends to be stable. Therefore, the optimal Cu$^{2+}$ concentration determined in this experiment is 250 μM, and this concentration will be used for subsequent research.

The reaction time of SO$_3^{2-}$ with H$_2$O$_2$ was also optimized. It can be seen from Figure 2d the absorbance of the system after the reaction time of SO$_3^{2-}$ and H$_2$O$_2$ exceeds 5 min. The change is not obvious, so the optimal reaction time of SO$_3^{2-}$ and H$_2$O$_2$ in this experiment is 5 min.

![Figure 2.](image)

Figure 2. (a) The absorbance of the systems in the absence or in the presence of SO$_3^{2-}$ 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 SO$_3^{2-}$ with H$_2$O$_2$ reaction time. The concentrations of ABTS and SO$_3^{2-}$ were 2 mM and 400 μM respectively.
3.4. Assay performance of the present method for SO$_3^{2-}$ detection

Under the optimal conditions, the absorption spectrum of SO$_3^{2-}$ with different concentrations was scanned at the wavelength of 405 ~ 480 nm, the color photos of sample solution and the drawing of standard curve were taken. It can be seen from Figure 3a that when the concentration of SO$_3^{2-}$ increases gradually, the absorbance value of its absorption spectrum decreases gradually at 415 nm. In Figure 4c we can see that when the concentration of SO$_3^{2-}$ was in the range of 20 μM ~ 1 mM, there is a good linear relationship with the absorbance. The linear equation is $Y = -861.35 C + 0.8923$ ($R^2=0.9983$) ($Y$ is the peak fluorescence intensity and $C$ is the SO$_3^{2-}$ concentration). With the increase of SO$_3^{2-}$ concentration, the color of the solution gradually changed from dark green to light. In addition, the relative standard deviations (RSDs) were 2.8%, 3.1% and 2.5% in three repetitive assays of 20 μM, 400 μM and 1 mM SO$_3^{2-}$, which shows that the method has good stability.

![Figure 3](image1.png)

**Figure 3.** (a) UV-visible absorption spectra in the presence of SO$_3^{2-}$ at different concentrations from 0 to 1 mM. (b) Color change in the presence of different concentrations of SO$_3^{2-}$. (c) SO$_3^{2-}$ 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, six different ions and SO$_3^{2-}$ were selected for comparison under the same reaction conditions, so as to verify the selectivity of ABTS+H$_2$O$_2$+Cu$^{2+}$ system to SO$_3^{2-}$. It can be seen from Figure 4 that when Na$^+$, Mn$^{2+}$, K$^+$, Cl$^-$, SO$_4^{2-}$, NO$_3^-$ with higher concentration than SO$_3^{2-}$ are used, it is found that these ions have little effect on the absorbance of the system, while the absorbance of the system decreases rapidly when SO$_3^{2-}$ with smaller concentration is added, which shows that the system is highly selective for SO$_3^{2-}$.

![Figure 4](image2.png)

**Figure 4.** Selectivity of this method. Concentrations of ABTS, Cu$^{2+}$, H$_2$O$_2$, and SO$_3^{2-}$ were 2 mM, 250 μM, 500 μM and 1 mM, respectively. Concentration of other metal ions were 2 mM. Error bars were estimated from three replicate measurements.
3.6. Determination of actual samples

In order to use the sensor system established in this experiment for the detection of actual samples, this experiment has carried out the analysis and determination of wine. In the actual sample wine, different concentrations of sulfite standard solution have been added as samples to be determined. The results show that the absorbance value of the sample decrease with the increases of sulfite concentration added in wine. In addition, the standard addition recovery experiment on wine samples was carried out, the standard addition recovery rate of sulfite in wine is between 96.01% and 106.90% (Table 1). The standard addition recovery experiment shows that the method established in this experiment has potential application for the detection of sulfite.

6.5. Positioning figures

Table 1 Experimental results of determination of sulfite in wine

| Sample | Added(μM) | Found(μM) | Recovery(%) | RSD (n=3, %) |
|--------|-----------|-----------|-------------|--------------|
| 1      | 0         | 0         | -           | -            |
| 2      | 100       | 106.90    | 106.90      | 4.9          |
| 3      | 300       | 294.99    | 98.33       | 2.6          |
| 4      | 500       | 480.05    | 96.01       | 3.4          |

4. Conclusion

Based on the principle that SO$_2^2$ can reduce the absorbance of Cu$^2^+$+ABTS+H$_2$O$_2$ reaction system, a simple and rapid spectrophotometric method was developed for the detection of sulfite and the sensitive analysis of sulfite was realized. This method has some obvious advantages. It does not need any chemical synthesis and enzyme participation. It has low cost and high stability. Because the whole detection process does not need to go through the complex steps such as washing, it is very convenient. In addition, the method has high selectivity. The sensor system established in this paper is applied to the detection of real sample wine. From the recovery of standard addition, it can be concluded that the method is feasible, and the result is reliable. Therefore, the sensor system established in this paper provides a new way for the detection of sulfite.

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