Determination of Cysteine by Discoloration Spectrophotometry using Copper(Ⅱ)-Bis-Cyclohexanone Oxalydihydrazone

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Abstract. In the alkaline medium of pH＝9.18, Cu^{2+} can be reduced to Cu^{+} by the sulfhydryl (-SH) of cysteine, and it result in the decrease the amount of Cu^{2+} in the system. The decrement of Cu^{2+} is directly proportional to the addition of cysteine, then using bis-cyclohexanone oxalyldihydrazone (BCO) as chromogenic reagent for Cu^{2+} to determinate the content of cysteine indirectly by discoloration spectrophotometry. A new method for the determination of cysteine by discoloration spectrophotometry using Copper(Ⅱ)-BCO has been established. The influencing factors of the determination of cysteine is investigated. The results show that the maximum absorption wavelength of chromogenic system was 602 nm, in the range of 0.008000～0.06800 mg/mL, the linear relationship between the decrease of absorbance and the mass concentration of methimazol is A=0.2162+2.4824C (mg/mL), and the linear correlation coefficient is r=0.9959. The method has been applied to the determination of cysteine in food, and the results are basically consistent with those determined by pharmacopoeial method.

1 Introduction

Cysteine(the molecular structure is shown in Figure 1) is an indispensable amino acid containing sulfhydryl in human body. It plays important roles in protein synthesis, detoxification metabolism and so on. Cysteine deficiency can causes many symptoms, such as children grow slowly, liver damage sleepiness and so on. However, at higher than normal levels, it turns to cause the cardiovascular diseases, osteoporosis, etc. Therefore, it is important to establish a simple method for the determination of cysteine.

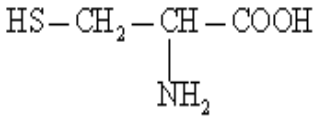

Fig.1. The molecular structure of cysteine

So far, Various different methods have been applied for the determination of cysteine, such as colorimetric determination[1], diffuse reflectance spectroscopy[2], electrochemiluminescence[3], fluorescence detection[4-5], HPLC[6], etc.

In the alkaline medium of pH＝9.18, Cu^{2+} can be reduced to Cu^{+} by the sulfhydryl (-SH) of cysteine, and it result in the decrease the amount of Cu^{2+} in the system. The decrement of Cu^{2+} is directly proportional to the addition of cysteine, then using bis-cyclohexanone oxalyldihydrazone (BCO) as chromogenic reagent for Cu^{2+} to determinate the content of cysteine indirectly by discoloration spectrophotometry. A novel method for the determination of cysteine by discoloration spectrophotometry using Copper(Ⅱ)-BCO is established. The maximum absorption wavelength of chromogenic system is 602 nm, in the range of 0.008000～0.06800 mg/mL, the linear relationship between the decrease of absorbance and the mass concentration of methimazol is A=0.2162+2.4824C (mg/mL), and the linear correlation coefficient is r=0.9959. The method has been applied to the determination of cysteine in food, and the results are basically consistent with those determined by pharmacopoeial method.

2 Experimental

2.1 Equipment and reagents

UV-2401 UV-visible spectrophotometer (The Shimadzu Corporation,Japan); 722S spectrophotometer (Shanghai Precision & Scientific Instrument Co,.Ltd).

Cu^{2+} solution: 1.000 g/L, prepared by $CuSO_4 \cdot 5H_2O$; Cysteine standard solution: 1.000 g/L； BOC solution: 1.000 g/L; pH=9.18 buffer solution: prepared by solium borate.

All reagents are of analytical reagent grade, and bidistilled water are used.

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2.2 Method

1.000 g/L cysteine standard solution(1.00 mL) or cysteine sample solution(appropriate amount), 1.000 g/L Cu^{2+} solution(0.80 mL), 1.000 g/L BOC solution(4.00 mL) and pH=9.18 buffer solution(3.20 mL) are transferred into a comparison tube(25 mL). The mixed solution is diluted to 25 mL with distilled water, mixed well. In another comparison tube(25 mL), the blank solution(Cu^{2+} solution+BOC solution+pH=9.18 buffer solution) is prepared in the same way. Then the two solutions are reacted for 30 min at 85℃in water both and cooled back to room temperature. After placing 20 min, the absorbance(A_1) of the blank solution(Cu^{2+} solution+BOC solution+pH=9.18 buffer solution) and the absorbance(A_2) of the determination solution(cysteine solution+Cu^{2+} solution+BOC solution+pH=9.18 buffer solution) are measured at 602 nm using water as reference. The $\triangle A$(A_1-A_2) is calculated.

3 Results and discussion

3.1 Absorption spectrum

On the basis experimental method, the absorption spectrums of the blank solution(Cu^{2+} solution+BOC solution+pH=9.18 buffer solution) and the determination solution(cysteine solution+Cu^{2+} solution+BOC solution +pH=9.18 buffer solution) in the range of 520～680 nm are shown in Figure 2. It can be seen that the maximum absorption wavelength of the blank solution and the determination solution are at 602 nm. So, 602 nm is selected for determination wavelength.

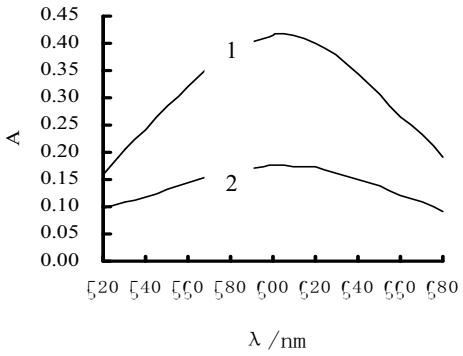

Fig. 2. Absorption spectrum
1-the blank solution (Cu^{2+} solution+BOC solution+ pH=9.18 buffer solution);
2-the determination solution (cysteine solution+Cu^{2+} solution+BOC solution+pH=9.18 buffer solution);
Cysteine solution:1.00 mL; Cu^{2+} solution:0.80 mL; BOC solution:4.00 mL; pH=9.18 buffer solution:3.20 mL; reaction temperature:85℃; reaction time:30 min; placing time:20 min.

3.2 Reaction temperature, reaction time and placing time

The effect of the reaction temperature(70～100℃) on the absorbance($\triangle A$) is seen in Figure 3. Figure 3 show that the $\triangle A$ of solution reaches maximum value and maintain basically stability when the temperature is 80～90℃. So, 85℃ is chosen.

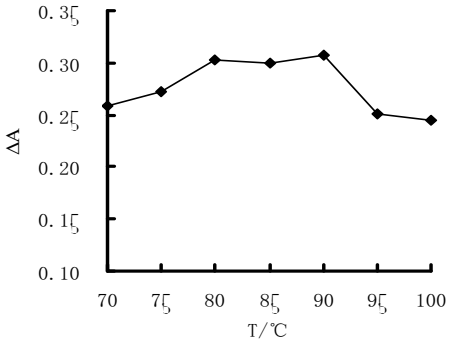

Fig. 3. Effect of the reaction temperature
Cysteine solution:1.00mL; Cu^{2+} solution:0.80mL; BOC solution:4.00mL; pH=9.18 buffer solution:3.20mL; reaction time:30min; placing time:20min.

Keep other parameters constant, when the reaction temperature is 85℃, the effect of the reaction time(10～60 min) on the absorbance($\triangle A$) is studied. The results show that the $\triangle A$ of solution reach maximum value and are basically identical when the reaction time is 20～30 min. hence, 25 min is selected.

Keep other parameters constant, When the reaction temperature is 35℃ and the reaction time is 25 min, the effect of the placing time (10～180 min) is researched. The experiments show that the absorbance($\triangle A$) of solution reach maximum value and remain constant when the placing time is 30～60 min. Therefore, 35 min is used.

3.3 pH=9.18 buffer solution amount

The effect of the pH=9.18 buffer solution amount on absorbance($\triangle A$) can be seen in Figure 4. The results show that the △A reaches its larger value and when the pH=9.18 buffer solution amount is up to 2.60 mL. Furthermore, the △A do not change with a further increase in the dosage of pH=9.18 buffer solution. Hence, 2.80 mL of the pH=9.18 buffer solution amount is chosen.

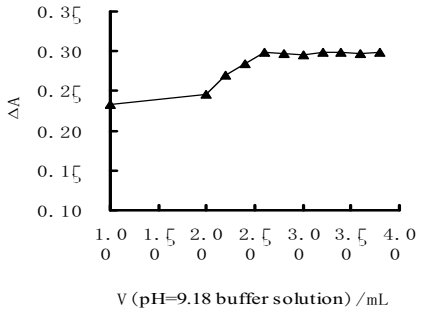

Fig. 4. Effect of pH=9.18 buffer solution amount
Cysteine solution:1.00mL; Cu^{2+} solution:0.80mL; BOC solution:4.00mL; reaction temperature:85℃; reaction time: 25min; placing time:35min.

3.4 Cu^{2+} solution amount

The effect of Cu^{2+} solution amount on absorbance(△A) is discussed (Figure 5). We can seen from Figure 5 that the △A attain maximum value and maintain basic stability when the Cu^{2+} solution amount is 0.50 mL～0.80 mL. So, 0.60 mL Cu^{2+} solution is applied.

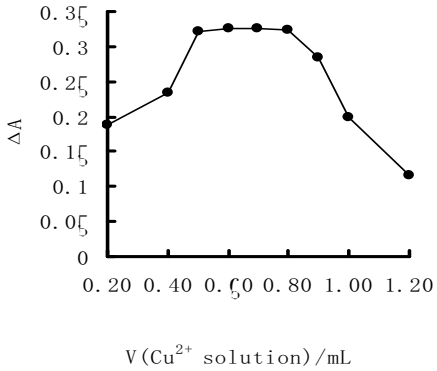

Fig. 5. Effect of Cu^{2+} solution amount
Cysteine solution:1.00 mL; BOC solution:4.00 mL; pH=9.18 buffer solution:2.80 mL; reaction temperature:85℃; reaction time:25 min; placing time:35 min.

3.5 BOC solution amount

The effect of BOC solution amount on absorbance(△A) can be seen Figure 6. Figure 6 show that the △A run up to maximum value and keep basically unchanged when the BOC solution amount is 3.40 mL～4.40 mL. So, 3.60 mL BOC solution is employed in the subsequent studies.

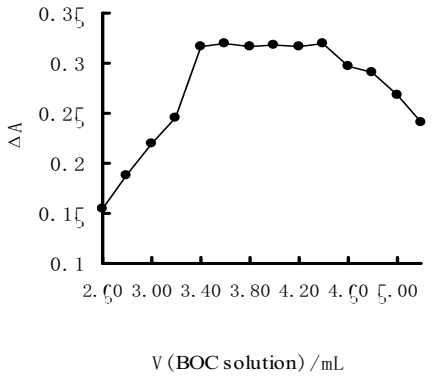

Fig. 6. Effect of BOC solution amount
Cysteine solution:1.00 mL; Cu^{2+} solution:0.60 mL; pH=9.18 buffer solution:2.80 mL; reaction temperature:85℃; reaction time:25 min; placing time:35 min.

3.6 Calibration curve

Under the best selected conditions, a series of standard solutions of cysteine standard solution are prepared. The absorbance($\triangle A$) is measured at 602 nm according to the experimental method, then the calibration curve is drawed(Figure 7, concentration of cysteine standard solution at horizontal coordinates, $\triangle A$ at vertical coordinates). In the range of 0.008000～0.06800 mg/mL, the linear regression equation is ΔA=0.2162 +2.4842C (mg/mL), and the correlation coefficient is 0.9992.

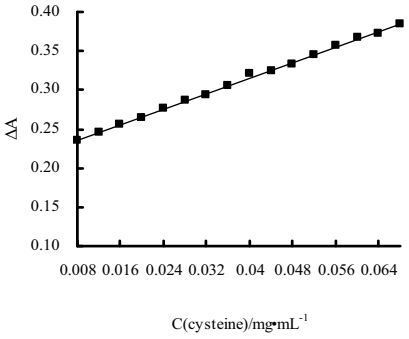

Fig. 7. Calibration curve
Cysteine solution:1.00 mL; Cu^{2+} solution:0.60 mL; BOC solution:3.60 mL; pH=9.18 buffer solution:2.80 mL; reaction temperature:85℃; reaction time:25 min; placing time:35 min.

3.7 Determination of food grade cysteine

0.0500 g power of food grade cysteine is weighed, then the power is dissolved in bidistilled water and is transferred into a 100 mL volumetric flask, the solution is diluted to the 100.0 mL and mixed well. This is the food grade cysteine sample solution.

Under the optimal conditions(Cu^{2+} solution:0.60 mL; BOC solution:3.60 mL; pH=9.18 buffer solution:2.80 mL; reaction temperature:85℃; reaction time:25 min; placing time:35 min), 1.60 mL food grade cysteine

sample solution are added. The content of the food grade cysteine is determined by proposed method, and content of the food grade cysteine is determined by standard method also. Meanwhile, the recovery tests of standard addition are performed. The results as show in Table 1.

Table 1. The determination results of food grade cysteine n=5

Sample	Food grade cysteine
Proposed method($mg \cdot g^{-1}$)	971.1
RSD（%）	1.8
Standard method ($mg \cdot g^{-1}$)	973.5
Added ($\mu g \cdot mL^{-1}$)	8.000 16.00
Recovered($\mu g \cdot mL^{-1}$)	7.568 15.22
Recovery(%)	94.6 95.1

4 Conclusion

A new method for the determination of cysteine by discoloration spectrophotometry using Copper(Ⅱ)-bis-cyclohexanone oxalyldihydrazone (BCO) has been established. The determination result(content of food grade cysteine) by this proposed method consistent with the result by standard method. Obviously, this method has certain practical significance.

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