Determination the content of cysteine in food by potassium dichromate spectrophotometry

Xinrong Wen* and Changqing Tu

School of Chemistry and Environment, Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, Jiaying University, Meizhou, Guangdong, China

Abstract. In this paper, a new method for the determination of cysteine by potassium dichromate spectrophotometry is established. In the H_2SO_4 medium, potassium dichromate has strong oxidation. Potassium dichromate can react with the hydrosulfuryl(-SH) in cysteine, which makes potassium dichromate fade, and the fading degree of potassium dichromate is proportional to the amount of cysteine added. Therefore, the content of cysteine can be quantitatively determined by determinating the decrease value of absorbance. The various influencing factors for the determination the content of cysteine by potassium bichromate spectrophotometry are investigated. Under the optimal conditions, the content of cysteine in food is determined, and the results are satisfactory.

1. Introduction

Cysteine(Cys, the structure is shown in Figure 1) is the only reducing amino acid with sulfhydryl in the amino acid. It is one of the essential and important amino acid for the human body, and it is widely used in the fields of food, cosmetics and medicine. In biological metabolism, cysteine is involved in the reduction process of cells and has the role of regulating and

protecting the liver, the imbalance of cysteine metabolism can lead to a series of functional diseases of the immune system. Therefore, the studies on the determination method of cysteine is of great significance in life science.

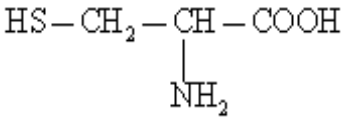

Fig. 1 The structure of cysteine

According to the literature, the determination methods of cysteine mainly include spectrophotometry[1-2], colorimetric analysis[3-4], electrochemical analysis[5-6], electrochemiluminescence analysis[6], fluorescence analysis[8-9], flow-injection analysis[10-11], etc.

In this paper, a new method for the determination the content of cysteine by potassium dichromate spectrophotometry is established. In the presence of H_2SO_4, potassium bichromate can oxidize cysteine, which makes the absorbance of potassium dichromate decreases. The decrease value of absorbance is proportional to the amount of cysteine added. The relationship between the decrease value of absorbance ΔA and the cysteine concentration is $\triangle A = 0.0161+2.2861C$(mg/mL) in the range of 0.002400 ~ 0.1680 mg/mL. Therefore, the content of cysteine can be quantitatively determined by determinating the decrease value of absorbance. Under the optimal conditions, this method is used to determine the content of cysteine in food, and the results are satisfactory.

2. Experimental

2.1 Equipment and reagents

UV-2401 UV-visible spectrophotometer and 722S spectrophotometer are used.

$K_2Cr_2O_7$ standard solution: 1.200 mg/mL; Cysteine standard solution: 0.6000 mg/mL, 0.3000 mg/mL; H_2SO_4 solution: 3.0 mol/L.

Analytic reagent and bidistilled water are used.

2.2 Methods

In a 25mL comparison tube, 7.00 mL $K_2Cr_2O_7$ solution, 4.00 mL H_2SO_4 solution and 5.00 mL cysteine standard solution or appropriate amount of the cysteine sample solution are added. Then the solution is diluted to the mark with distilled water and mixed well. This is the determination solution. In another 25mL comparison tube, the blank solution(7.00 mL $K_2Cr_2O_7$ solution + 4.00 mL H_2SO_4 solution) is prepared in the same way. The two

* Corresponding Author: wxrong5093@sohu.com

solutions are reacted for 25 min at 85℃ in water both and cool down using the running water for 5 minutes. After placing 5 min, the absorbance(A_1) of the blank solution and the absorbance(A_2) of the determination solution are measured at 435 nm against water, the decrease value of absorbance $\triangle A$(A_1-A_2) is calculated.

3. Results and discussion

3.1 Absorption spectrum

According to experimental method, the absorption spectrum in the range of 400 ~ 500 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 435 nm. So, 435 nm is used for the whole experiment.

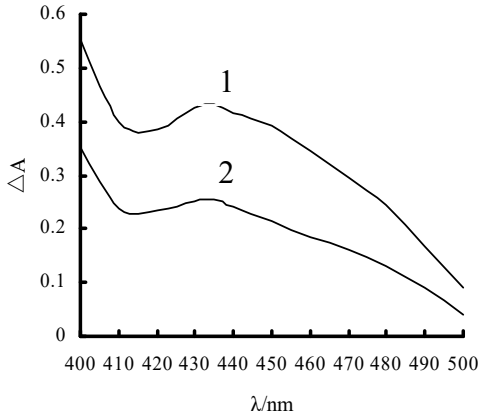

Fig.2 Absorption spectrum
1-the blank solution($K_2Cr_2O_7$+H_2SO_4); 2-the determination solution ($K_2Cr_2O_7$+H_2SO_4+cysteine)

Control conditions: $K_2Cr_2O_7$ solution:5.00 mL; H_2SO_4 solution: 2.00 mL; cysteine standard solution: 5.00 mL; reaction temperature: room temperature; reaction time:5 min.

3.2 Reaction temperature

From table 1, we can see that as the reaction temperature increases, the absorbance(△A) also increases accordingly. The absorbance(△A) reaches maximum value and is remained essentially unchanged when reaction temperature is 85 ℃ ~ 95 ℃. Afterwards, the absorbance(△A) decreases when reaction temperature is 100℃. Thus, 85℃ is used.

Table 1 The effect of reaction temperature

Temperature /℃	30	40	45	50	55	60	65
Absorbance (△A)	0.182	0.197	0.202	0.218	0.230	0.256	0.265
Temperature /℃	70	75	80	85	90	95	100
Absorbance (△A)	0.281	0.290	0.295	0.302	0.299	0.300	0.292

Control conditions: maximum absorption wavelength:435 nm; $K_2Cr_2O_7$ solution:5.00 mL; H_2SO_4 solution: 2.00 mL; cysteine standard solution: 5.00 mL; reaction time:20 min; running water cool time:5 min; placement time:5 min.

3.3 Reaction time

The effect of the reaction time is showed in table 2. It can be seen from table 2 that the absorbance (△A) increases with the reaction time increases, at first. After the reaction time is 20 min, the absorbance(△A) remains essentially unchanged. So, 25 min is chosen.

Table 2 The effect of reaction time

Reaction time /min	5	10	15	20	25	30	35
Absorbance (△A)	0.256	0.274	0.281	0.288	0.287	0.288	0.289
Reaction time /min	40	45	50	60	70	80	90
Absorbance (△A)	0.290	0.291	0.293	0.292	0.294	0.295	0.295

Control conditions: maximum absorption wavelength:435 nm; $K_2Cr_2O_7$ solution:5.00 mL; H_2SO_4 solution: 2.00 mL; cysteine standard solution: 5.00 mL; reaction temperature:85 ℃; running water cool time:5 min; placement time:5 min.

3.4 Placement time

The datas in Table 3 indicate that the placing time had a minimal effect on the absorbance(△A). Therefore, 5 minute is applied.

Table 3 The effect of placement time

Placement time/min	5	10	15	20	25	30
Absorbance (△A)	0.289	0.288	0.291	0.289	0.288	0.287
Placement time/min	35	40	60	90	120	
Absorbance (△A)	0.290	0.289	0.291	0.292	0.291	

Control conditions: maximum absorption wavelength:435 nm; $K_2Cr_2O_7$ solution:5.00 mL; H_2SO_4 solution: 2.00 mL; cysteine standard solution: 5.00 mL; reaction temperature:85 ℃; reaction time:25 min; running water cool time:5 min.

3.5 H_2SO_4 solution dosage

The effect of H_2SO_4 solution dosage can be seen in Figure 3. The results show that the absorbance (△A) increases as H_2SO_4 solution dosage increases, then the absorbance(△A) reaches maximum value and is remained basically unchanged when H_2SO_4 solution dosage is 3.00 ~ 5.00 mL. Whereafter, the absorbance(△A) decreases with the increase of H_2SO_4 solution dosage. Thus, H_2SO_4 solution dosage is selected as 4.00 mL.

3.6 $K_2Cr_2O_7$ solution dosage

The effect of $K_2Cr_2O_7$ solution dosage is shows in Figure 4. Form Figure 4, we can see that the absorbance(△A) increases with $K_2Cr_2O_7$ solution dosage increases in the beginning, and then the absorbance(△A) reaches maximum value when $K_2Cr_2O_7$ solution dosage is 6.00 ~ 9.00 mL. Hence, 7.00 mL $K_2Cr_2O_7$ solution is used.

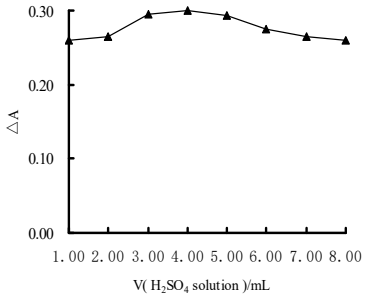

Fig. 3 Effect of H_2SO_4 solution dosage
Control conditions: maximum absorption wavelength: 435 nm; $K_2Cr_2O_7$ solution:5.00 mL; cysteine standard solution: 5.00 mL; reaction temperature:85 ℃; reaction time:25 min; running water cool time:5 min; placement time:5 min.

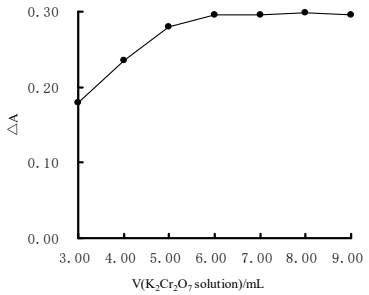

Fig. 4 Effect of $K_2Cr_2O_7$ solution dosage
Control conditions: maximum absorption wavelength:435 nm; H_2SO_4 solution:4.00 mL; cysteine standard solution:5.00 mL; reaction temperature:85 ℃; reaction time:25 min; running water cool time:5 min; placement time:5 min.

3.7 Calibration curve

In the optimum conditions(maximum absorption wavelength:435 nm; $K_2Cr_2O_7$ solution:5.00 mL; H_2SO_4 solution:4.00 mL; reaction temperature: 85 ℃; reaction time:25 min; running water cool time:5 min; placement time:5 min), a series of determination solution(with different cysteine concentration) are prepared, and the blank solution is prepared in the same way. Then the

absorbance(A_1) of the blank solution and the absorbance(A_2) of the determination solution are measured at 435 nm against water, the decrease value of absorbance($\triangle A=A_1-A_2$) is calculated. The calibration curve is showed in Figure 5. The linear equation between the the cysteine mass concentration and the absorbance (△A) is △A=0.0161+2.2861C(mg/mL) in the range of 0.002400~0.1680 mg/mL, and the correlation coefficient is 0.9996.

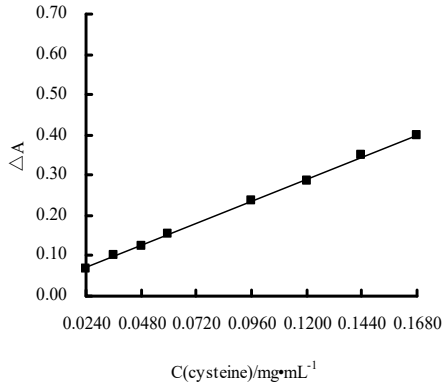

Fig. 5 Calibration curve

3.8 Determination the content of food grade cysteine

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

In the optimum conditions, 0.50 mL food grade cysteine sample solution are added. According to the proposed method(2.2 method), the absorbance(A_1) of the blank solution and the absorbance (A_2) of the determination solution are measured at 435 nm against water, the absorbance($\triangle A=A_1-A_2$) is calculated. The content of food grade cysteine can be calculated based on the absorbance($\triangle A$) and the linear equation, and the recovery rates of standard addition are determined. Meanwhile, the content of the food grade cysteine is determined by standard method. The results as show in Table 4.

Table 4. The determination content of food grade cysteine n = 5

Sample	Proposed method ($mg \cdot g^{-1}$)	RSD (%)	Standard method ($mg \cdot g^{-1}$)	Added ($\mu g \cdot mL^{-1}$)	Recovered ($\mu g \cdot mL^{-1}$)	Recovery rate (%)
Food grade cysteine	989.9	0.4	973.5	24.00	23.71	98.8
				36.00	34.64	96.2

From Table 4, we can see that the content of food grade cysteine is 989.9 $mg \cdot g^{-1}$ by proposed method, and the recovery rates are 96.2% ~ 98.8%. The content of food grade cysteine is 973.5 $mg \cdot g^{-1}$ by standard method.

4. Conclusion

In this paper, a new method for the determination of cysteine by potassium dichromate spectrophotometry is established. Under the optimal conditions, the content of food grade cysteine is determined by proposed method, and the recovery rates of standard addition are 96.2% ~ 98.8%. The determination result by proposed method agree well with the determination result by standard method. This method does not need complicated or expensive equipment, and it does not require expensive reagents also. Therefore, this method has the advantages of simply, rapidness, convenience, low analytical cost and so on. It is obvious that this study had certain practical significance and practical value on establishing a new method for the determination of cysteine.

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