**Spectrophotometric Determination of Acetylcysteine by Cu(I) –Neocuproine**

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**Abstract.** Spectrophotometric determination of acetylcysteine by Cu(I)–neocuproine is studied. The hydrosulfuryl(-SH) in acetylcysteine can reduce Cu(II) to Cu(I), then the chromogenic reagent of neocuproine react with Cu(I) to form the yellow complex. The yellow complex has the maximum absorption at 453 nm, and the amount of yellow complex is proportional to the amount of acetylcysteine in a certain range. Therefore, the content of acetylcysteine in medicine can be determined by measuring the absorbance of the yellow complex.

1. **Introduction**

Acetylcysteine (Figure 1) is an antioxidant containing sulfhydryl groups(-SH). It has been used for the treatment of the acute bronchitis, chronic bronchitis and pulmonary diseases. At present, spectrophotometry¹, flow injection analysis², fluorometry³, HPLC method⁴, electrochemical analysis⁶⁷, etc have already been applied for the determination of acetylcysteine.

![Figure 1. The structure of acetylcysteine](image)

Spectrophotometric determination of acetylcysteine by Cu(I)–neocuproine is reported. The hydrosulfuryl(-SH) in acetylcysteine can reduce Cu(II) to Cu(I), then the chromogenic reagent of neocuproine react with Cu(I) to form the yellow complex. The yellow complex has the maximum absorption at 453 nm, and the amount of yellow complex is proportional to the amount of acetylcysteine in a certain range. Therefore, the content of acetylcysteine in medicine can be determined by measuring the absorbance of the yellow complex, and the results are satisfactory.

2. **Experimental**

2.1 **Equipment and reagents**

UV-2401 UV-visible spectrophotometer (The Shimadzu Corporation, Japan) and 723S spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd) are used.
Acetylcysteine (AC) standard solution: 0.2500 mg·mL⁻¹ is stored at 4°C, shielding from light; CuSO₄ solution: 0.5000 mg·mL⁻¹; Neocuproine solution: 0.01000 mol·L⁻¹; pH 4.8 buffer solution: HAc-NaAc; Buffer solutions of different pH was prepared as references.

Analytical reagent grade reagents and bidistilled water are used.

2.2 Method

The mixed solution (3.50 mL CuSO₄ solution + 1.50 mL neocuproine solution + 3.00 mL pH 4.8 HAc-NaAc buffer solution + appropriate amount acetylcysteine standard solution or acetylcysteine sample solution) is added into a 25 mL comparison tube and is diluted to graduation and mixed well, this is measured solution.

The another mixed solution (3.50 mL CuSO₄ solution + 1.50 mL neocuproine solution + 3.00 mL pH 4.8 HAc-NaAc buffer solution) is added into another 25 mL comparison tube and is diluted to graduation and mixed well, this is reagent blank solution.

The measured solution and the reagent blank solution react for 30 min at 40°C in water both and cooled back to room temperature, then the absorbance is measured using 1.0 cm cuvette at 453 nm against the reagent blank after placing 10 min.

3. Results and discussion

3.1. Reaction mechanism

The hydrosulfuryl(-SH) in acetylcysteine reduce Cu(II) to Cu(I), then Cu(I) react with the chromogenic reagent of neocuproine to form the yellow complex which its maximum absorption at 453 nm. According to the amount of yellow complex is proportional to the amount of acetylcysteine in a certain range, the content of acetylcysteine is determinated indirectly through determinating the absorbance of the yellow complex.

3.2. Maximum absorption wavelength

In the range of 400–500 nm, the absorption spectrum of yellow complex formed from Cu(I) and neocuproine is shown in Figure 2. It shows that the maximum absorption wavelength of yellow complex is at 453 nm. Thus, the maximum absorption wavelength is chosen as 453 nm.

![Figure 2. Absorption spectrum](image)

CuSO₄ solution: 3.50 mL; neocuproine solution: 1.50 mL; AC: 1.00 mL; pH 4.8 HAc-NaAc solution: 3.00 mL; reaction temperature: 40°C; reaction time: 30 min; placing time: 30 min.

3.3. The reaction temperature, reaction time and placing time

In accordance with the experimental method, CuSO₄ solution (3.50 mL), neocuproine solution (1.50 mL), pH 4.8 HAc-NaAc buffer solution (3.00 mL) and acetylcysteine standard solution (1.00 mL) are added. The effect of the reaction temperature is explored. The results show that the absorbance reaches
maximal value and remains constant when the temperature is 35–50℃. Consequently, 40℃ is selected.

Keep other conditions unchanged, when the reaction temperature is 40℃, the effect of the reaction time is probed. The results show that the absorbance remains constant when the reaction time is 20–120 min. Hence, 30 min is chosen.

Keep other conditions unchanged, when the reaction temperature is 40℃ and the reaction time is 30 min, the experimental results of the placing time indicate that the absorbance remains unchanged when the placing time is 10–120 min. Therefore, 10 min is employed.

3.4. pH buffer solution and its dosage
According to the experimental method, CuSO$_4$ solution (3.50 mL), neocuproine solution (1.50 mL) and acetylcysteine standard solution (1.00 mL) are added. When the reaction temperature is 40℃, the reaction time is 30 min and the placing time is 10 min, the effect of pH buffer solution on absorbance is investigated. The results make clear that the absorbance is larger and basically stable when pH buffer solution is 4.0–5.0. Thus, pH 4.8 buffer solution is applied.

Fixed other conditions, the effects of the pH 4.8 buffer solution dosage on absorbance show that the absorbance reaches maximum and remains constant when the amount of pH buffer solution is 3.00 mL–6.00 mL. So, the dosage of pH 4.8 buffer solution is identified as 3.00 mL.

3.5. CuSO$_4$ solution dosage
The effect of CuSO$_4$ solution dosage on absorbance is shown in Figure 3. Figure 3 show that the absorbance has its maximum value and basically unchanged when the CuSO$_4$ solution dosage is 3.50 mL–4.00 mL. Therefore, 3.50 mL of CuSO$_4$ solution is chosen.

3.6. The neocuproine solution dosage
The effect of neocuproine solution dosage on absorbance can be seen in Figure 4. The experimental results show that the absorbance reaches a larger value and basically unchanged when neocuproine solution dosage from 1.00 mL to 2.00 mL. So, 1.50 mL neocuproine solution has been applied.
3.7. Calibration curve

A series of acetylcysteine standard solutions of different concentrations and reagent blank solution are prepared. According to the experimental method, the absorbance is measured at 453 nm against the reagent blank, then draw the calibration curve using absorbance as vertical coordinate and acetylcysteine concentration as horizontal coordinate (Figure. 5). The linear regression equation is A = -0.0016 + 43.154C (mg/mL), the correlation coefficient is 0.9994.

![Figure 5. Calibration curve](image)

3.8. Determination the content of acetylcysteine

10 granules of acetylcysteine capsule were taked, the quality is 3.0350 g after removal of capsule shell, they are ground and blended. Then 2.8695 g powder of acetylcysteine capsule is weighed precisely and dissolved in distilled water and is transferred into a 200 mL volumetric flask, the solution is diluted to the mark and mixed well. The solution is preserved without light at 4°C.

In accordance with the experimental method, the content of acetylcysteine in acetylcysteine capsule is determined. At the same time, the content of acetylcysteine in acetylcysteine capsule is determined by pharmacopoeia method, and the recovery tests of standard addition are performed. The results are shown in Table 1.

| Sample         | Proposed method (mg·g⁻¹) | RSD (%) | Pharmacopoeia Method[9] (mg·g⁻¹) | Added (µg/mL) | Recovered (µg/mL) | Recovery (%) |
|----------------|--------------------------|---------|---------------------------------|--------------|-------------------|---------------|
| Acetylcysteine capsule | 195.2                     | 0.3     | 196.1                           | 2.000        | 2.030             | 101.5         |
|                 |                           |         |                                 | 4.000        | 4.008             | 100.2         |

We can see from Table 1 that the content of acetylcysteine in acetylcysteine capsule is 195.2 mg·g⁻¹ by this proposed method, and the result of pharmacopoeial method is 196.1 mg·g⁻¹. The recoveries are 101.5 and 100.2.

4. Conclusion

Spectrophotometric determination of acetylcysteine by Cu(I)–neocuproine is established. The content of acetylcysteine has been successfully determined using this established method, and the results obtained is compared with those obtain by pharmacopoeia method. The results of recovery test of standard addition are satisfactory. Obviously, this method can be used for the determination of acetylcysteine.
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