Determination of Trace Iron in Three Kinds of Tonic Herbs by Dual-Indicator Dual-Wavelength and Catalytic Kinetic Spectrophotometric Method

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Abstract: One catalytic discoloring spectrophotometry with double indicators and dual wavelength is proposed in the Thesis to measure the content of trace iron in three kinds of hematinic, artemisia integrifolia and other vegetables. In HCl medium, methyl orange and methylene blue are indicators. At wavelength of 500 nm and 665 nm, the change value ΔA in absorbance of catalytic system and non-catalytic system is measured and thus the iron content is measured. The conclusion is that the Method is characterized by simple operation, high sensitivity, favorable selectivity and reliable results.

1. Introduction

Iron is the essential microelement with the highest content in human body and it plays an important role in regulating metabolism of organism. The proportion of iron-deficiency anemia is very high in our country and almost one out of ten suffers from the iron-deficiency anemia. Iron element is the “core element in human body”, which almost accounts for 0.006% of body weight and which has important physiological function and plays an important role in regulating metabolism of human body. In case the storage volume of iron in human body is insufficient, it will influence heme synthesis of cells and thus give rise to anemia [1]. There are numerous hematinic in traditional Chinese medicine in which white peony root, Angelica sinensis and polygonum multiflorum are the most common. However, people have dead zone in understanding of traditional Chinese medicine and they think hematinic has high content of iron and thus can be used to supplement trace iron in human body and thus cures anemia without being aware of compatibility of traditional Chinese medicine. As for vegetables, eggplant, artemisia integrifolia and spinach etc. are iron-replenishment foods which are very popular among folks and methods used to test iron content in these drugs and vegetables mainly include atomic absorption spectrophotometry and spectrophotometry. The advantages of atomic absorption spectrophotometry include low detection limit, high sensitivity and mature methods. However, instruments used in this method are expensive in price and tedious in operation and this method is not universal for it requires detection by professionals. As for spectrophotometry, the iron is measured mainly by phen spectrophotometry. The technology is mature but low in sensitivity and thus cannot meet requirements of measuring trace iron. At present, there are some reports of measuring trace iron by spectrophotometry by catalytic kinetics [2,3] but most of methods have single wavelength and single indicator [4] and reports about measuring method with double indicators and dual wavelength are rare. To find out content of iron in traditional Chinese medicine and food, a new detection method is established and the spectrophotometry in which hydrogen peroxide catalyst by iron is used to decolor double indicators such as methyl orange and methylene blue is adopted in the Thesis to measure the trace iron in traditional Chinese medicine and food. Part 1 Experimental
1.1. The Instrument and Reagent

T6 ultraviolet spectrophotometer in the new century (Beijing's general instrument co., LTD.); HH - 6 digital constant temperature water-bath water (guohua electric appliance co., LTD.); AA320N CRT atomic absorption spectrophotometer (Shanghai precision scientific instrument co., LTD.); Electronic universal furnace (suzhou jiangdong precision instruments co., LTD.); Kay type flask (zhengzhou zhongtian experimental instrument co., LTD.).

Radix paeoniae alba, angelica, fleece-flower root, all bought in qiqihaer city pharmacy; Iron (III) standard stock solution: including 100 g/mL, when diluted into (including 0.1 g/mL standard solution; Methyl orange solution: 0.001 mol/L; Methylene blue: 0.001 mol/L; Hydrochloric acid: 0.10 mol/L; Sulfate: 0.05 mol/L; Hydrogen peroxide: volume fraction of 15%; Concentrated nitric acid (AR); Perchlorate (AR); NaOH (GR); Secondary distilled water.

1.2. Experiment Method

Take two 25 mL volumetric flask 1 and 2, respectively, in turn, add 0.001 mol/L of methyl orange solution 0.80 mL, 0.001 mol/L 0.60 mL methylene blue solution, 0.10 mol/L 1.50 mL of hydrochloric acid, 15% hydrogen peroxide 1.00 mL. Adding iron to volumetric flask 2 (III) standard solution 0.30 ml (catalytic systems), water volume, shake well. In 95 plus or minus 0.5°C constant temperature water bath pot, heat after 15 min, quickly remove and cooling water for 2 min. With 1 cm dish, with water as A reference, the wavelength of 500 nm and 665 nm, respectively measuring catalytic systems (A500 featured, A665), and the catalytic system (A0500, A0665) absorbance, calculate Δ A = (A0500 - A500 featured) + (A0665 - A665).

2. The Results and Discussion

2.1. Absorption Curve

According to section 1.2 of the experimental method, water as the reference, in 300-700 nm wavelength range to draw the absorption curve of catalysts and catalytic system, the results show that the catalytic system and system at 500 nm and 665 nm are absorbing peak, and iron of methyl orange (absorption peak at 500 nm) and methylene blue (absorption peak at 665 nm) has obvious catalysis, this experiment select measuring wavelength of 500 nm and 665 nm, as shown in figure 1.

![Fig. 1 catalytic systems and the absorption curve of catalytic system](image)

(curve 1: non catalytic system, curve 2: iron catalysts)

2.2. Reaction Media Choices

Other conditions according to section 1.2 of the experimental method, fixed and reagent dosage, respectively to investigate H$_2$SO$_4$ volume (0.05 mol/L ) to the rightness ΔA value, the influence of the experimental results show that: in HCl medium, the determination sensitivity is higher, so choose HCl acid medium.
2.3. The selection of the amount of hydrochloric acid

According to the experimental method of section 1.2, and fixed other condition and the dosage of reagents. Adding hydrochloric acid (0.10mol/L) were investigated as the volume of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00mL for the determination of the value of ΔA. The experimental results show that: when the amount of HCl was 1.50mL, the maximum value in the range of 0.2-0.8 and the stability of ΔA. So the selection of hydrochloric acid is 1.5ml in this experiment. As following, figure 2.

![Fig. 2](image)

Fig. 2 Effects of the amount of hydrochloric acid on the ΔA value.

2.4. The selection of the amount of Hydrogen peroxide

According to the experimental method of section 1.2, and fixed other condition and the dosage of reagents. Adding hydrogen peroxide were investigated as the volume of 0.00, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00mL for the determination of the value of ΔA. The experimental results show that: when the amount of H₂O₂ was 1.50mL, the maximum value in the range of 0.2-0.8 and the stability of ΔA. So the selection of the amount of H₂O₂ is 1.00ml in this experiment. As following, figure 3.

![Fig. 3](image)

Fig. 3 Effects of the amount of H₂O₂ on the ΔA value.

2.5. The selection of the amount of Methyl orange solution

According to the experimental method of section 1.2, and fixed other condition and the dosage of reagents. Adding Methyl orange solution were investigated as the volume from 0 to 1.00mL, and determining the value of ΔA value every 0.10mL. The experimental results show that: when the amount of Methyl orange solution was 0.80mL, the maximum value in the range of 0.2-0.8 and the stability of ΔA value. So the selection of the amount of Methyl orange solution is 0.80ml in this experiment. As following, figure 4.

![Fig. 4](image)

Fig. 4 Effects of the amount of Methyl orange solution on the ΔA value.

2.6. The selection of the amount of Methylene blue

According to the experimental method of section 1.2, and fixed other condition and the dosage of
reagents. Adding Methylene blue were investigated as the volume from 0 to 1.00mL, and determining the value of ΔA every 0.10mL. The experimental results show that: when the amount of Methylene blue was 0.60mL, the maximum value in the range of 0.2-0.8 and the stability of ΔA value. So the selection of the amount of Methylene blue is 0.60ml in this experiment. As following, figure 5.

![Graph](image1)

**Fig. 5 Effects of the amount of Methylene blue on the ΔA value**

2.7. The selection of reaction temperature

According to the experimental method of section 1.2, and fixed other condition and the dosage of reagents. The influences of reaction temperature were investigated respectively from 70℃ to 100℃, and determined ΔA value every 5℃. The experimental results show that: when the reaction temperature was 95℃, the maximum value in the range of 0.2-0.8 and the stability of ΔA value. So this study selected water bath heating and the temperature is 95℃. As following, figure 6.

![Graph](image2)

**Fig. 6 Effects of the reaction temperature on the ΔA value**

2.8. The selection of reaction time.

According to the experimental methods of section 1.2, and fixed other condition and the dosage of reagents. The influences of reaction time were investigated respectively from 10 to 18min, and determined ΔA value every 1min. The experimental results show that: when the reaction time was 15min, the maximum value in the range of 0.2-0.8 and the stability of ΔA value. So this study selected the reaction time is 15min. As following, figure 7.

![Graph](image3)

**Fig. 7 Effects of the reaction time on the ΔA value**

2.9. The working curvy and the detection limit.

Under the optimum experimental conditions. According to the experimental methods of section 1.2, respectively taking iron standard solution of different experiment. The ΔA value were measured at wavelength of 500nm and 665nm. Calculating ΔA value and drawing working curve. By working curves we can know iron (III) mass concentration showed a good linear
relationship with the ΔA in the range of 0~0.02000 g/mL. The linear regression equation is \( \Delta A = 38.971\rho (\mu g/mL) - 0.0064 \), \( r=0.9995 \). By deducted the absorbency of blank value after is 0.01, and the corresponding concentration as the detection limit. The detection limit of this method that was experimentally measured is \( 8.0 \times 10^{-10} \) g/L.

**Fig. 8 Working Curve**

### 2.10. The influence of co-existing ions

According to the above experimental method, when Fe(III) becoming 0.012 µg/mL, testing about 15 kinds of the influence of co-existing ions on the determination results, including the \( K^+, Na^+, Ag^+, Cu^{2+}, CO_3^{2-}, Ca^{2+}, Cl^-, Mg^{2+}, Mn^{2+}, Zn^{2+} \) and so on, this relative error of plus or minus 5% or less, the results showed that in addition to the Cu\(^{2+}\) allowances for 1 times, 1000 times the rest of the sample quantity will not affect the measurement. Therefore, most of the ion of allowances is higher, general samples can be directly determined.

### 2.11. The analysis of sample

Respectively measure 0.5 g of dry and smashed white peony root, Angelica sinensis and polygonum multiflorum as well as mixture of dry and smashed eggplant peel, pulp and stalk, mixture of neck and leaf of artemisia integrifolia and mixture of neck and leaf of spinach, and then add 10 mL perchloric acid and 10 mL concentrated nitric acid to 250 mL Kjeldahl flask and place it for 24 h. And then, it is placed in electronic multi-purpose furnace for digestion until it turns into colorless and transparent solution. Adjust the pH value to be 5 and then set the constant volume to be 100 mL and then take some solution to measure according to experimental method. Compare the result with results measured by atomic absorption spectrophotometry and the experiment results show that results of the method are reliable and its accuracy and recovery rate conform to requirements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>The determination RSD%(n=5)</th>
<th>Atomic absorption value(µg/g) recovery(n=5)</th>
<th>Atomic Absorption Spectrometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPA</td>
<td>11.8</td>
<td>11.2</td>
<td>1.8</td>
</tr>
<tr>
<td>angelica</td>
<td>105.6</td>
<td>102.2</td>
<td>0.9</td>
</tr>
<tr>
<td>FM</td>
<td>187.9</td>
<td>179.4</td>
<td>0.8</td>
</tr>
<tr>
<td>100.1</td>
<td>102.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Table 2  Test results of iron content in vegetable samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>The method value (µg/g)</th>
<th>Atomic absorption spectrophotometry</th>
<th>RSD%(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eggplant</td>
<td>904.4</td>
<td>898.2</td>
<td>1.5</td>
</tr>
<tr>
<td>100.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemisia</td>
<td>113.5</td>
<td>106.2</td>
<td>1.2</td>
</tr>
<tr>
<td>99.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>7.533</td>
<td>8.235</td>
<td>0.9</td>
</tr>
<tr>
<td>101.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Discussion

In HCl medium is presented in this paper, the iron catalyzed hydrogen peroxide oxidation of methyl orange and methylene blue indicator fade new method for determination of trace iron. The two using methylene blue and methyl orange indicator method for the determination of double wavelength has not yet been reported, the method for determining high sensitivity low detection limit good selectivity ,the result is reliable, suitable for the determination of trace concentrations of iron.

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