The Determination of Thiourea Dioxide Residue in Pasta Products by LVFX-AuNPs Fluorescence Resonance Energy Transfer Spectrometry

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Abstract. Fluorescence resonance energy transfer occurs between levofloxacin (LVFX) and gold nanoparticles (AuNPs), which leads to fluorescence quenching of levofloxacin. Due to the strong Au-S bond formed between thiourea dioxide and gold nanoparticles, levofloxacin is released from the surface of gold nanoparticles and fluorescence of the system is recovered. Based on this, a fluorescence spectrometry method for determination of thiourea dioxide was established. This method was used to determine the content of thiourea dioxide in pasta products with RSD less than 3.5% and recovery rate of 93.3~103.3%.

1. Introduction
Thiourea dioxide (CH₄N₂SO₂) is a banned food preservative [1]. As thiourea dioxide is added into food, the food can be preserved and bleached. In recent years, many manufacturers have added thiourea dioxide to pasta products to attract consumers by improving their appearance and taste, so the determination of thiourea dioxide in food is a very important task. At present, the determination methods of thiourea dioxide in food mainly include liquid chromatography[2], fluorescence method[3] and chemiluminescence method[4]. There is no application of fluorescence resonance energy transfer method in the determination of thiourea dioxide in food.

In this paper, the fluorescence resonance energy transfer between levofloxacin( LVFX) and gold nanoparticles( AuNPs ) was used to quench the fluorescence of levofloxacin, while thiourea dioxide could destroy the energy transfer between levofloxacin and gold nanoparticles and restore the fluorescence of levofloxacin. On this basis, a new method for the direct determination of thiourea dioxide in pasta products by fluorescence resonance energy transfer spectroscopy was established.

2. Experimental
2.1. Apparatus and Reagents
CaryEclipse fluorescence visible spectrophotometer (American Agilent Technology Co., Ltd.); Leici PHSJ- 5 acidiometer (Shanghai Yidiang Scientific instrument Co., Ltd.); Cary60 UV-vis spectrophotometer (American Agilent Technology Co., Ltd.).

The stock solution of levofloxacin(3.0×10⁻⁵mol·L⁻¹): 1.1mg levofloxacin standard was weighed accurately and dissolved in appropriate amount of water to make 3.0×10⁻⁵mol·L⁻¹ stock solution.

The stock solution of thiourea dioxide (1.0×10⁻⁵mol·L⁻¹) : 10.8mg thiourea dioxide standard was weighed accurately and dissolved in appropriate amount of water to make 1.0×10⁻⁵mol·L⁻¹ stock solution. All the reagents used are analytical pure and water is doubly distilled water.
2.2. Experimental Methods

2.2.1 Preparation of gold nanoparticles[5]. The 50 mL mol L\(^{-1}\) chlorotonic acid solution was added to the 100 mL round bottom flask and heated to reflux, and then 5 mL 38.8 m mol·L\(^{-1}\) trisodium citrate solution was added to the solution. The color of solution quickly changed from light yellow to colorless to atropurpureus, and finally turned to wine red. The mixture was heated and refluxed for 15 mins. After the solution was completely cooled, it was transferred to the 50 mL brown Volumetric flask, and then the mixture was diluted with water to obtain 1.0 × 10\(^{-3}\) mol·L\(^{-1}\) AuNPs solution and was stored at 4°C.

2.2.2 Determination methods. 300 μL 3.0×10\(^{-7}\)mol·L\(^{-1}\) levofloxacin and 0.6mL 1.0×10\(^{-3}\) mol·L\(^{-1}\) AuNPs solution were mixed. Then 1 mL of sample solution diluted 150 times and 1 mL of Na\(_2\)HPO\(_4\)-NaH\(_2\)PO\(_4\) buffer solution (pH=5.8) were taken, and diluted to 10 mL then oscillated and shaken. At room temperature (25 °C), the fluorescence intensity of the system was measured at excitation wavelength 287 nm, emission wavelength 474 nm (slit width 10 nm).

3. Results and Discussion

3.1. Spectral Properties of AuNPs and LVFX.

The UV-vis absorption spectra and fluorescence spectra of AuNPs, 8.0 × 10\(^{-6}\) mol ·L\(^{-1}\) AuNPs solution prepared by trisodium citrate reduction were shown in figure 1. From Fig. 1 it can be seen that the maximum absorption peak of AuNPs was at 522 nm, and the AuNPs had fluorescence properties with an excitation wavelength of 313 nm and a maximum emission peak at 416 nm.

Fig. 2 was a fluorescence spectrum of 3.0 × 10\(^{-7}\) mol ·L\(^{-1}\) levofloxacin solution with an excitation wavelength of 287 nm, and an emission wavelength of 474 nm.

![Figure 1.](image)

Figure 1. Ultraviolet-visible absorption spectrum and fluorescence spectrum of AuNPs solution (8.0×10\(^{-6}\) mol L\(^{-1}\) AuNPs).

3.2. The Fluorescence Quenching Effect of AuNPs on LVFX and Its Mechanism.

300 μL 3.0×10\(^{-7}\) mol ·L\(^{-1}\) levofloxacin solution was transferred to 10 mL colorimetric tube, then different volumes of 1.0 × 10\(^{-3}\) mol ·L\(^{-1}\) AuNPs solution and 1mL Na\(_2\)HPO\(_4\)-NaH\(_2\)PO\(_4\) buffer solution (pH=5.8) were added in turn. The mixture was diluted with water to a certain volume, and thoroughly mixed. The fluorescence spectra of the solutions were scanned at room temperature at excitation wavelength 287 nm, emission wavelength 474 nm, as shown in figure 3.
Figure 2. fluorescence spectrum of levofloxacin solution (3.0×10^{-7} mol L^{-1}).

The volume of AuNPs solution corresponding to curves 1-7 is 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mL respectively.

Figure 3. fluorescence quenching spectrum of levofloxacin by AuNPs.

Figure 3 showed that AuNPs had obvious quenching effect on the fluorescence of levofloxacin. The volume of AuNPs solution in 0.1~0.5mL was linearly related to the fluorescence intensity of levofloxacin, and the linear regression equation was Y = 657.8 -958.4 V (r = 0.9989).

The fluorescence quenching effect of AuNPs on levofloxacin was caused by fluorescence resonance energy transfer, in which levofloxacin was the energy donor and AuNPs were the energy receptors. According to the spectral characteristics, AuNPs had the maximum absorption at the wavelength of 522nm, levofloxacin had the maximum emission peak at the wavelength 474nm. The emission spectrum of levofloxacin and the absorption spectrum of AuNPs overlapped to a great extent, which provides a necessary condition for the transfer of fluorescence resonance energy between them. Through the dosage test of AuNPs solution, the volume of AuNPs solution was linear with the fluorescence intensity of levofloxacin in the range of 0.1-0.5mL. It was determined that the volume of AuNPs solution added to quenching levofloxacin was 0.5mL.

3.3. Fluorescence Recovery of LVFX-AuNPs System by Thiourea Dioxide

300 μL 3.0 × 10^{-7} mol ·L^{-1}LVFX solution was transferred to 10 mL colorimetric tube, then 0.5 mL 1.0 × 10^{-3} mol ·L^{-1}AuNPs solution, different volumes of thiourea dioxide solution, 1mL Na2HPO4- NaH2PO4 buffer solution (pH=5.8) were added in turn, The mixture was diluted with water to a certain volume, and thoroughly mixed. The fluorescence spectrum of each solution was scanned at room temperature at excitation wavelength 287 nm, emission wavelength 474 nm, as shown in Figure 4.

The concentration of thiourea corresponding to curve 1-7 is 0, 0.4, 0.6, 0.8, 1.0, 3.0 and 5.0 μ mol ·L^{-1}, respectively.

Figure 4. Effect of thiourea concentration on fluorescence intensity of the system.
Figure 4 showed that when thiourea dioxide was added to LVFX-AuNPs system, the fluorescence of thiourea dioxide could be restored. The reason was that the S atoms on thiourea dioxide mercapto formed stable Au-S bonds with AuNPs. The stable complexes formed by the S atoms destroy the energy transfer of fluorescence resonance between LVFX and AuNPs. The fluorescence of LVFX was released and restored. Moreover, the intensity of fluorescence recovery was linearly related to the amount of thiourea dioxide added.

3.4. Systematic Study on Fluorescence Resonance Energy Transfer Characteristics of LVFX-AuNPs
The effect of acidity
The acidity of solution greatly affected the interaction between LVFX-AuNPs and thiourea dioxide-LVFX-AuNPs. B-R buffer solution, phosphate buffer solution and borax buffer solution were used as reaction media to measure the fluorescence intensity of the system. The results showed that the fluorescence intensity of the system changed the most and was stable in phosphate buffer solution. According to the test method other test conditions were fixed, the effects of phosphate buffer solutions of different pH on the system were tested. The results showed that the fluorescence recovery rate of thiourea dioxide to LVFX-AuNPs increased at first and then decreased with the increase of pH, then increases and then decreases, showing a broken line relationship. The fluorescence recovery rate reached its maximum at pH=6.00. In this experiment, phosphate buffer solution with pH=6.00 was selected to control the acidity of the reaction.

The effect of reaction time According to the test method, The effects of AuNPs on the fluorescence quenching rate of LVFX and the effect of thiourea dioxide on the fluorescence recovery rate of LVFX at different reaction time were determined by fixing other test conditions. The results showed that the reaction time had little effect on the fluorescence quenching rate and fluorescence recovery rate.

The effect of reaction temperature According to the test method, other test conditions were fixed, and the influence of AuNPs on the fluorescence quenching rate of LVFX and the effect of thiourea on the fluorescence recovery rate of LVFX were determined when the reaction temperature was 15-50 °C. The experiment showed that the fluorescence quenching rate was the highest when the reaction temperature was 15 °C, but the fluorescence recovery rate was lower. The fluorescence recovery rate was the highest when the reaction temperature was 25 °C, but the fluorescence quenching rate was lower. In addition, the fluorescence intensity of levofloxacin was continuously weakened with the increase of temperature. The comprehensive test results showed that the reaction temperature of 25°C is the best, so in this experiment the reaction temperature is 25 °C.

3.5. Interference Test
According to the test method, the effects of common ions and organic substances such as starch on the determination results were investigated when the concentration of thiourea dioxide in the system was 1.0×10⁻⁶ mol·L⁻¹. The results showed that when the relative error was within ±5%, the maximum allowable amount of coexisting substances (measured by thiourea dioxide times) was: glucose (1000), Mn²⁺, Cd²⁺ (1000), starch (500), fructose, Ca⁵⁺, Na⁺, Zn²⁺, Cr³⁺, Mg²⁺, K⁺ (100), Al³⁺, Ni²⁺(10), vitamin C (0.5).

3.6. The Working Curve and the Detection Limit
According to the experimental method, there is a linear relationship between the fluorescence intensity of LVFX-AuNPs system and the concentration of thiourea dioxide in the range of 4.0 × 10⁻⁷~5.0 × 10⁻⁶ mol·L⁻¹. The linear regression equation is Y = 1.865 × 10⁸ C + 1.350 × 10² (r = 0.998 9), and the detection limit is 1.5 × 10⁻⁷mol·L⁻¹.

3.7. Discussion on Reaction Mechanism
The surface of AuNPs obtained by reduction of chlorogenic acid with sodium citrate is negatively charged, which is stabilized by electrostatic repulsion. After mixed with AuNPs, levofloxacin can be adsorbed on the surface of AuNPs by electrostatic interaction. fluorescence resonance energy transfer occurred between the AuNPS and levofloxacin to weaken the fluorescence of the system. and after
thiourea dioxide is added, thiourea dioxide and nano gold formed a stable compound to replace levofloxacin, so that the fluorescence of levofloxacin is recovered. Based on this, thiourea dioxide can be quantitatively determined by measuring the fluorescence intensity of LVFX-AuNPs system after adding thiourea dioxide.

4. Sample Determination

4.1. Sample Treatment
10g pasta was ultrasonically treated with 20 mL of ethanol (8+2) solution for 15mins, the supernatant was collected, the residue was ultrasonically extracted twice with 20 mL of ethanol (8+2) solution, the extracts were combined, 10 mL of the extract was centrifuged at 3500 r min\(^{-1}\) for 15 mins, 6 mL of the supernatant was removed, and the sample solution was obtained after filtration with a filter membrane and constant volume with water to 25 mL.

4.2. Sample Determination
The content of thiourea dioxide in noodle and flour samples was determined respectively according to the test method. Each sample was determined 5 times in parallel. The relative standard deviation of the measured value was calculated and the standard recovery test was carried out. At the same time, the samples were analyzed by liquid chromatography. The results are shown in Table 1.

From Table 1, it can be seen that the measured values of thiourea dioxide in noodles and flour samples were basically consistent with the results determined by liquid chromatography, with RSD less than 3.5%, the standard addition recovery rate of noodles samples was 93.3~102.2%, and the standard addition recovery rate of flour samples was 96.7~103.3%.

Table 1. Analytical results of samples (n=5).

| sample   | Measured value of this method \(c/(\mu\text{mol} \cdot \text{L}^{-1})\) | RSD/\% | Measured value by liquid chromatography \(c/(\mu\text{mol} \cdot \text{L}^{-1})\) | Scalar addition \(c/(\mu\text{mol} \cdot \text{L}^{-1})\) | Measured total \(c/(\mu\text{mol} \cdot \text{L}^{-1})\) | Recovery rate /\% |
|----------|-------------------------------------------------|--------|-------------------------------------------------|-----------------|---------------------------------|------------------|
| noodle   | 0.16                                            | 2.7    | 0.14                                            | 0.3             | 0.44                            | 93.3             |
|          | 0.6                                            | 0.72   | 0.9                                             | 0.6             | 1.08                            | 102.2            |
|          | 0.9                                            | 0.68   | 0.3                                             | 0.9             | 0.68                            | 103.3            |
| flour    | 0.37                                           | 3.1    | 0.39                                            | 0.3             | 0.68                            | 103.3            |
|          | 0.6                                            | 0.95   | 0.9                                             | 0.9             | 1.25                            | 97.8             |

5. Acknowledgement
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6. References
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