Construction and Study of the Application of Novel Norfloxacin Fluorescent Probes

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Abstract. In this experiment, a fluorescent probe of tricyanohydrofuran-norfloxacin was successfully constructed. Fluorescence spectrophotometer was used to detect the fluorescence intensity of the system under the condition of the excitation wavelength was 362nm and the emission wavelength was 448nm. The optimal experimental conditions were optimized (the concentration of the complex, the amount of norfloxacin, pH, temperature and reaction time). The optimal concentration of the complex in the system is in the range of 1.0×10⁻³⁻³⁻⁵×10⁻⁴ mol·L⁻¹, the dosage was 0.6mL, and optimal dosage of norfloxacin was 3.0 ml, the optimum pH of the system was 5.5, under this condition the fluorescence intensity of the system was the strongest. At the same time, the intramolecular charge transfer reaction mechanism of fluorescent probe was studied. And testing the content of norfloxacin capsules, the results were satisfactory. A new testing method of norfloxacin content successfully created.

Keywords: Norfloxacin; Cyano-cyanohydrin; Fluorescence probe; Charge transfer.

1. Introduction
Norfloxacin is the earliest Flhioroquinolones antibacterial drug used in clinical medicine. It has good antibacterial activity against various bacteria in vitro. The antibacterial mechanism is inhibition of DNA gyrase, with broad antibacterial spectrum, widely distributed in the body, strong bactericidal and non-cross-resistance with other antibacterial drugs[1, 2]. Currently, HPLC is more commonly used for the detection of norfloxacin due to the separation efficiency, good selectivity, high detection sensitivity and automation. However, the high cost of analysis, liquid chromatography and routine maintenance, the analysis time is longer. Therefore, it is very necessary to establish a new method to detect the content of norfloxacin.

Fluorescence is a physical property[3], and fluorescent materials are widely used in various industries. For example, in biological systems, fluorescent materials are widely used in biomarkers to study metabolism and other basic processes. Adjusting and optimizing the performance of fluorescent dyes is a hot topic today. In short, the method of fluorescent probe for detecting drugs is widespread attention by schoors with the high sensitivity and good linear relationship. Among them, the electron acceptor reagent needs to have an unsaturated bond structure and can react with the lone pair electron structure to form a charge-transporting complex under certain conditions. The complex has a fluorescence characteristic absorption peak at a specific wavelength, and the concentration of the target drug can be calculated according to the intensity of the absorption peak, so adding electron
acceptor reagent into the Norfloxacin fluorescence analysis method is an effective means. The new type of tricyano-dihydrofuran is an organic material with nonlinear optical activity [9]. This material plays an important role in modern high-tech fields such as laser technology, optical switches, optical communications, optical storage. Novel tricyano-dihydrofuran is a coplanar conjugate structure with strong electron-withdrawing cyano which is a new donor-acceptor fluorophore structure with unsaturated bond structure [4-7]. Therefore, in this experiment, a new type of tricyano-dihydrofuran electron acceptor prophase synthesized by the experimental group was used as the main fluorescent probe part, complexed with norfloxacin, and a novel method for the detection of quinolones was established by the charge-transfer fluorescence emission mechanism.

2. Experimental Part

FormatPreparation of tricyano-dihydrofuran standard solution. Then it was prepared into a solution of $1.5 \times 10^{-4} \text{ mol/L}$, and saved.

Preparation of norfloxacin (CPFX) standard solution: 5 mg of norfloxacin (AR $\geq 99.0\%$) standard was accurately weighed, dissolved with a certain amount of methanol, Then it was prepared a solution of 100 mg/L, saved, diluted to the desired concentration.

Fluorescence analysis experimental method: First, a few 10ml dried stopper tube was prepared, washed with methanol and dried. 3.0ml of tricyano-dihydrofuran solution was accurately measured and transferred into a stopper tube, then the appropriate amount of norfloxacin solution was also transferred into the stopper test tube, then shaked, and the anhydrous methanol was added to the markline. Tube was heated at 20 °C for 40 minutes then transferred to room temperature. 3.0ml of the complex was measured, and added to the test-tube, placed in a fluorescence cell, and the emission spectra of the complex was detected at the condition of the excitation wavelength was 362nm and emission wavelength was 448nm. The blank test was made with the same experimental method as above.

3. Results and Discussion

3.1. Fluorescence Spectra

![Fluorescence Spectra](image)

**Figure 1.** The spectra of the sample solution.

1 Norfloxacin emission spectrum 1' Norfloxacin excitation spectrum; 2 Complex emission spectrum 2' Complex excitation spectrum; 3 Tricyano-dihydrofuran emission spectrum 3' Tricyano-dihydrofuran excitation spectrum
3ml of tricyanohydrofuran-norfloxacin charge-transfer complexes solution, 3ml of norfloxacin solution and 3ml of tricyanohydrofuran solution were took for detecting the fluorescence excitation and emission. The results were showed in Fig. 1. The tricyano-dihydrofuran solution, norfloxacin solution and the charge-transfer complex solution all have fluorescence luminescence properties. Compared with the norfloxacin solution, the position of the excitation and emission peak of the complexes did not shift. When the complex of tricyloxy dihydrofuran and norfloxacin was formed, the fluorescence intensity significantly enhanced.

3.2. The Effect of pH
For Separately, 0.6 ml of tricyano-dihydrofuran solution and 3.0 ml of norfloxacin solution were added to seven dried plugged test tubes, gently agitated to complete fusion, and added with anhydrous methanol to the mark. The test tube was heated in a constant temperature water bath, then pH was controlled from 4.5 to 7.5, and then allowed to stand at room temperature. The fluorescence intensity of 7 mixed solutions with different pH was scanned at 362nm respectively. The result was showed in Fig.2. When pH is 5.5, the intensity is the strongest, and over-acid or over-base will destroy the complex structure. Therefore, 5.5 was chooser as the optimal pH value for the subsequent experiments.

![Figure 2. The effect of different pH value on fluorescence intensity system.](image)

3.3. Effect of DCDHF-2-V Concentration
Separately, 0.6 ml of tricyano-dihydrofuran solution and 3.0 ml of norfloxacin solution were added to seven dried plugged test tubes, gently agitated to complete fusion, and added with anhydrous methanol to the mark. The results showed that when the concentration of DCDHF-2-V was in the range of $1.0 \times 10^{-3} \text{mol.L}^{-1}$-$3.5 \times 10^{-4} \text{mol.L}^{-1}$, the fluorescence intensity of the system was the strongest.

3.4. Effect of the Amount of DCDHF-2-V
3.0 ml of norfloxacin solution were added to nine dried plugged test tubes, and 0.1-0.9ml of DCDHF-2-V were added into test tubes respectively. After shaking and mixing, test tubes were placed in a water bath at 30°C for 40 minutes, and left to room temperature for detection of the fluorescence intensity. The results showed that both the low concentration and the high concentration of DCDHF-2-V could affect the fluorescence intensity. When the amount of DCDHF-2-V was 0.6mL, the fluorescence intensity of the system was the strongest.

3.5. Effect of the Amount of Norfloxacin
0.6 ml of DCDHF-2-V solution were added to eight dried plugged test tubes, and 1-8ml of norfloxacin solution were added into test tubes respectively. After shaking and mixing, test tubes were placed in a water bath at 20°C for 40 minutes and left to room temperature for detection of the intensity. The
results showed that when the amount of norfloxacin is 3.0ml, the highest intensity of complex is obtained, this is optimal amount of norfloxacin.

3.6. Effect of the Reaction Time

Fluorescence intensity of the complex was detected at the reaction time of 10 min, 20 min, 30 min, 40 min, 50 min and 60 min respectively. Tricyanohydrofuran reaction with norfloxacin has been completed at 30 minutes. Further study found that the complex was placed after a period of time, was tested again, the results no change, indicating that the stability of the complex is better.

3.7. Sample Determination

A factory of 5 capsules norfloxacin capsules were ground into powder, after the sample was completely dissolved, anhydrous methanol was added to the mark (100 ml). 10mL of sample solution was accurately transferred and diluted to 1x10⁻³mol/L with anhydrous methanol, and six parallel determination results were selected. The results were showed in Table 1.

| sample | Added amount(mg) | Measured amount(mg) | Recovery rate(%) | RSD(%) |
|--------|-----------------|---------------------|------------------|--------|
| 1      | 10.43           | 10.37               | 99.42            | 1.28   |
| 2      | 10.43           | 10.28               | 99.56            | 1.12   |

4. Conclusion

The experimental results showed that norfloxacin and tricyanohydrofuran successfully established a good optical properties of fluorescent probes for the detection of drug content in the methanol solution system. At same time, the optimum conditions of system were screened, and the optimum concentration range of the tricyanohydrofuran was 1.0×10⁻³-3.5×10⁻⁴mol·L⁻¹, and the optimum concentration was 0.6mL, the optimal dosage of norfloxacin was 3.0ml, under this condition the fluorescence intensity of the system was the strongest. The data showed fluorescence intensity of the probe was significantly enhanced compared to norfloxacin.

Acknowledgments

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