Study on the Synthesis of Charge Transfer Complex of Nifedipine and Tetracyanoethylene

Lu Tian¹, Fanbo Wang², Jiang Wu³ and Yuguang Lv¹, *

¹College of Pharmacy Jiamusi University Heilongjiang, Jiamusi, 154007, China
²First Affiliated Hospital of Jiamusi University Jiamusi, Heilongjiang, 154002, China
³School of Stomatology, Jiamusi University, Jiamusi, P.R. China

*Corresponding author email: yuguanglv@163.com

Abstract. Purposes: To construct a new method for the determination of nifedipine and apply it to the determination of in vitro drugs in order to achieve simple and accurate quantitative detection. Procedures: A charge transfer complex of nifedipine tetracyanoethylene was constructed, and the fluorescence intensity changes of the complex and nifedipine were compared. Screen the effect of drug dosage, reaction time and reaction temperature on the fluorescence intensity of the system. Results: Nifedipine is a substance with its own fluorescence characteristics, but its own fluorescence intensity is not as high as expected. Through the addition of tetracyanoethylene, it is found that the fluorescence intensity of nifedipine has been greatly enhanced, combined with ultraviolet A new absorption peak was found in the spectrum, indicating that the two were successfully complexed. Conclusions: The experiment successfully synthesized the nifedipine charge transfer complex, and constructed a new method for the determination of nifedipine content, which is of great significance for the determination of the content of drugs in vitro.

Keywords: Nifedipine; Tetracyanoethylene; Fluorescence intensity; Content determination.

1. Introduction
Since my country’s entry into a well-off society, the awareness, treatment, and control rates of hypertension have been quite low. Hypertension has seriously threatened the physical and mental health of the human body[1-3]. Therefore, the prevention, diagnosis and treatment of this disease has been widely adopted by the whole society attention. In medicine, hypertension is characterized by the continuous increase of arterial blood pressure, which can be accompanied by the occurrence of organ function or organic damageAccording to whether the cause is clear, it can be divided into two types: primary hypertension and secondary hypertension. Surgical treatment and medical treatment are targeted treatment methods based on the conditions caused by the cause. Usually, antihypertensive drug therapy is the more common treatment.

Nifedipine, as a kind of antihypertensive drug, plays an important role in the treatment of hypertension. Up to now, scientific researchers have worked tirelessly to develop a variety of blood pressure lowering drugs. While these drugs enter the human body to exert their efficacy, they also produce certain toxic side effects that affect health[4]. Therefore, it is of great significance to establish an optimized analytical method for the determination of nifedipine drug content[5-7].

There are many methods to determine the content of nifedipine. This project mainly uses fluorescence analysis to determine the content of nifedipine. As a commonly used analytical method in the laboratory, fluorescence analysis is simple and efficient. It is more widely used than mass
spectrometry, chromatography, spectroscopy and so on. It is mainly due to its own unique advantages and is widely used.

2. Experiment

2.1. Experimental Method
Instruments: 970CRT Fluorescence Spectrophotometer; UV/Vis-265 UV-Visible Spectrophotometer; Electronic balance; Constant temperature water tank. Reagent: Nifedipine; Tetracyanoethylene; Absolute ethanol. Preparation of nifedipine standard solution, and 5×10⁻³ mol/L tetracyanoethylene standard solution.

Accurately measure 2.5 mL of tetracyanoethylene and an appropriate amount of nifedipine solution in a 10 mL colorimetric tube, dilute to volume with absolute ethanol, shake well, heat in a constant temperature water tank at 40°C for 40 minutes, and cool the sample to room temperature. Measure the excitation wavelength at 435 nm, record the fluorescence intensity, and repeat the above operation method for comparison.

2.2. Spectral Analysis of the Charge Transfer Reaction of Nifedipine
Scan the fully reacted nifedipine-tetracyanoethylene charge transfer complex, and measure the fluorescence spectra of nifedipine working solution and tetracyanoethylene working solution under the same conditions as a control group. Place the fully reacted test solution in a quartz cuvette, use distilled water as a calibrated UV spectrophotometer to scan the UV characteristic peaks, and use equal amounts of nifedipine working solution and tetracyanoethylene working solution as controls. Draw the absorption spectra of the three, and determine the location of the strong absorption peak of the charge-transfer complex.

2.3. Single Factor Experiment
The best effect of fluorescence intensity was determined by single factor experiment to investigate the dosage of tetracyanoethylene, the dosage of nifedipine, the reaction temperature and the reaction time.

2.3.1. The influence of the amount of tetracyanoethylene. Add 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0, 2.0 mL of tetracyanoethylene solution to a colorimetric tube containing 4ml of nifedipine solution, and dilute to volume with absolute ethanol. Heat in a constant temperature water bath at °C for 40 minutes, measure the fluorescence intensity at 435 nm, and determine the optimal dosage of tetracyanoethylene solution.

2.3.2. The impact of nifedipine dosage. Take 1-8 mL of nifedipine solution and add it to a colorimetric tube containing 0.5 mL of tetracyanoethylene, make the volume constant with ethanol, shake well, and heat in a water bath at 40°C for 40 min at a constant temperature, and measure at 435 nm. Its fluorescence intensity determines the best dosage of nifedipine.

2.3.3. The influence of reaction temperature. According to the experimental method, under the condition that other experimental conditions are fixed, the fluorescence intensity of the charge transfer reaction between nifedipine and tetracyanoethylene in a water bath at 30°C-60°C is measured to determine the optimal temperature required for the experiment.

2.3.4. Effect of reaction time. Under the determined temperature conditions, different time periods with a reaction time of 10 min-50 min were selected to observe the fluorescence spectrum to determine the best time.

3. Experimental Results and Discussion

3.1. Experimental Results

3.1.1. Fluorescence excitation and emission spectra before and after the charge transfer reaction of nifedipine. Take out the completely reacted nifedipine-tetracyanoethylene charge transfer complex for
fluorescence scanning, and obtain the fluorescence spectra of nifedipine working solution and tetracyanoethylene working solution under the same conditions as a control group. The results are shown in Figure 1. It can be seen from the picture that the nifedipine solution itself has certain fluorescence characteristics. When nifedipine interacts with tetracyanoethylene to form a charge-transfer complex, the fluorescence intensity of the complex is significantly enhanced.

![Figure 1. TCNE(a), Nifelat(b), Nifelat+TCNE(c) emission spectra.](image)

Take out the completely reacted test solution in a quartz cuvette, use distilled water as a calibrated UV spectrophotometer to scan the UV characteristic peaks, and take equal amounts of nifedipine working solution and tetracyanoethylene working solution as controls Group, draw the absorption spectra of the three as shown in Figure 2. Nifedipine has a characteristic absorption around 400 nm, tetracyanoethylene has a characteristic absorption around 250 nm, and the complex has a characteristic absorption around both wavelengths. The characteristic absorption indicates the formation of charge transfer complex.

![Figure 2. TCNE(a), Nifelat(b), Nifelat+TCNE(c) ultraviolet spectrum.](image)

3.1.2. The influence of the amount of tetracyanoethylene. Add 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0, and 2.0 mL of tetracyanoethylene working solution to 9 stopper colorimetric tubes containing 4 mL of nifedipine working solution. Constant volume of water and ethanol, react in a constant temperature water bath at 40°C for 40 minutes, and measure the fluorescence intensity at 435 nm. The results are
shown in Figure 3. According to the curve in the figure, 0.5 mL of tetracyanoethylene solution is the best amount to form a charge transfer complex.

![Figure 3. Effect of tetracyanoethylene dosage on fluorescence intensity.](image)

3.1.3. The impact of nifedipine dosage. Add 1-8 mL of nifedipine working solution to an eight-bracket colorimetric tube containing 0.5 mL of tetracyanoethylene working solution, and dilute to the calibration line with absolute ethanol after slight shaking. Then put a colorimetric tube in a constant temperature water bath with a temperature of 40°C, react for 40 minutes, measure a certain amount of the reaction solution and cool it to room temperature, and measure the fluorescence intensity at 435 nm. The results are shown in Figure 4. According to the curve in the figure, 5 mL of nifedipine solution is the best amount to form a charge transfer complex.

![Figure 4. Effect of nifedipine dosage on fluorescence intensity.](image)

3.1.4. The influence of reaction temperature. Add 0.5 ml of tetracyanoethylene working solution and 5 ml of nifedipine working solution to the colorimetric tube, dilute to volume with absolute ethanol, place them in a constant temperature water bath at 30-60°C, and react for 40 min. Measure the fluorescence intensity at nm, and the result is shown in Figure 5. According to the curve in the figure, 40°C is the best temperature for the charge transfer complex reaction.
3.1.5. Effect of reaction time. Under the above-defined conditions, select different time periods from 10 min to 50 min to observe the fluorescence spectrum, and measure the fluorescence intensity at 435 nm, as shown in Figure 6. The fluorescence intensity of the complex at 30 min Strongest.

![Figure 6](image)

Figure 6. Effect of reaction time on fluorescence intensity.

3.1.6. Establishment of nifedipine standard curve. Under the best experimental conditions, record the experimental data and make the working curve between nifedipine and fluorescence intensity, as shown in Figure 7, nifedipine at 3.2×10⁻⁶-1.0×10⁻⁴ mol·L⁻¹, in the range of L-1, it has a good linear relationship with the fluorescence intensity. Linear equation: If = 2.0018C + 450.37, R = 0.9986, correlation coefficient R² = 0.9972.

![Figure 7](image)

Figure 7. Working curve of nifedipine concentration and system fluorescence intensity.
3.1.7. Determination of nifedipine sample content. Take 10 nifedipine capsules from a specific manufacturer, pour out the contents, mix well, grind, accurately weigh a certain amount (approximately 20 mg nifedipine), and add a certain amount of water to a 50 mL volumetric flask, Put in the weighed nifedipine, dissolve and dilute it, fully shake and filter, measure 2 mL of the additional filtrate, use the same operation method as above, and use it as a sample solution after shaking. Under the best selected experimental conditions, the determination is carried out according to the standard curve method. The labeled amount of this product is 250 mg/capsule, the recovery rate is 92.8%, RSD = 0.72% (n = 5)

4. Conclusion
In this experiment, the fluorescence of some substances is low. By adding some substances, the complex can emit fluorescence, and its content can be determined by fluorescence intensity. Therefore, the addition of fluorescent reagents greatly reduces the experimental difficulty and broadens the research direction of analysis. As a common analytical method in laboratory, fluorescence analysis is simple and efficient. It is more widely used than mass spectrometry, chromatography and spectrometry.

Because of the low fluorescence intensity of nifedipine, tetracyanoethylene is a strong electron acceptor. Nifedipine calcium channel blocker antihypertensive drugs were selected as electron donors to form a 1:1 n - π type charge transfer complex, which enhanced the fluorescence intensity of the system. A new method for the quantitative determination of nifedipine in vitro was established and applied to the determination of nifedipine in vitro.

Acknowledgements
This work was financially supported by the National Science Foundation of China (No.21346006), Department of scientific research project in Heilongjiang province (No. B2017015), Excellent discipline team project of Jiamusi University ((No. JDXKTD-2019007).

References
[1] Mu S, Shimosawa T, Ogura S, et al. Epigenetic modulation of the renal beta-adrenergic-WNK4 pathway in salt-sensitive hypertension[J]. Nat Med, 2011, 17 (5): 573-580.
[2] Lee H, Rezai-Zadeh N, Seto E. Negative regulation of histone deacetylase 8 activity by cyclic AMP-dependent protein kinase A[J]. Mol Cell Biol, 2004, 24(2): 765-773.
[3] Wu Y, Xu M, Wang H, et al. Lercanidipine hydrochloride versus felodipine sustained-release for mild-to-moderate hypertension: a multi-center, randomized clinical trial[J]. Curr Med Res Opin, 2015, 31(1): 171-176.
[4] White WB. The risk of waking-up: impact of the morning surge in blood pressure[J]. Hypertension, 2010, 55 (4): 835-837.
[5] Lee JH, Bae JW, Park JB, et al. Morning hypertension in treated hypertensives: baseline characteristics and clinical implications[J]. Korean Circ J, 2011, 41 (12): 733-743.
[6] Yang D D, Han F, Shi Y F, Huang X Y, Huang D M, Cai Y Q. Chin J Anal Lab, 2019, 38 (7): 828.
[7] Ishihara O, Araki R, Kuwahara A, et al. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: An analysis of 277, 042 single-embryo transfer cycles from 2008 to 2010 in Japan[J]. Fertility and Sterility: Official Journal of the American Fertility Society, Pacific Coast Fertility Society, and the Canadian Fertility and Andrology Society, 2014, 101(01): 128-133.