Study on the Application of Gatifloxacin-A Novel Fluorescent System

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Abstract. we study the use of gatifloxacin with a novel tricyano-dihydrofuran fluorescent system. Gatifloxacin is a 8-methoxy-fluoroquinolone racemate which, in addition to having a broad spectrum of Gram-negative activity in addition to the activity of positive microorganisms, this product has antibacterial effect, bacterial DNA repair, transcription and replication can be suppressed. Such drugs are widely used at present, so they are getting more and more attention. Therefore, it is very important for these drugs to be tested efficiently. The application of the new type of tricyano-dihydrofuran fluorescence system can detect it efficiently, then we carry out a detailed application of its research.

Introduction

Quinolones, which target bacterial DNA, block DNA gyrase, can cause irreversible damage to bacterial DNA and are effective against bacteria. Quinolone antibiotics can be applied to the human body and can also be applied to animals. The antibacterial agent has the characteristics of wide antibacterial spectrum and strong activity, little side effect, no cross-resistance with other drugs, and has been widely used in animal husbandry and aquaculture Application, can be used for prevention and treatment of diseases[1-5].There is an antibacterial drug called gatifloxacin, is a quinolone drugs, as humans gradually found that quinolones antibacterial effect is particularly good, so with quinolones synthesized more and more new structures, so for quinolones University drug testing methods are more and more important[6-9].

Experimental Methods.

solution configuration，Preparation of new tricyne dihydrofuran standard solution，after the new tricyanohydrofuran synthesized has been vacuum-dried, 35.8 mg of novel tricyano-dihydrofuran was accurately weighed and dissolved in an appropriate amount of chloroform. Fully dissolved, formulated as $1.5x10^{-4}$ mol/L of the mother solution, spare. Preparation of gatifloxacin (GFLX) standard solution, precision weighing 5 mg of dry gatifloxacin (AR≥99.0%) standard, with a certain amount of methanol to be completely dissolved, dissolved, formulated to a concentration of 100mg/L of the mother solution for use in the experiment can be Dilute the stock to the desired concentration.

Results and discussion

Fluorescence excitation and emission spectra

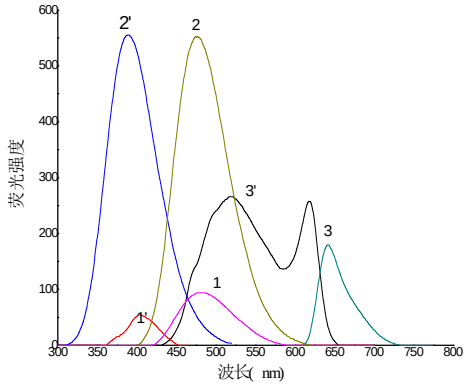

Fig. 1 Fluorescence excitation and emission spectra

1 gatifloxacin emits light 1 'gatifloxacin excitation light; 2 gatifloxacin - a novel tricyclic dihydrofuran emission light, 2' gatifloxacin - a novel tricyclic dihydrofuran excitation light; 3 Novel tricyano-dihydrofuran emission, 3 'new tricyclic dihydrofuran excitation

Take 3ml of new tricyano-dihydrofuran-gatifloxacin charge transfer complexes into the fluorescence excitation and emission spectra, and then separate the gatifloxacin working solution 3ml and new tricyanoid mother liquor 3ml under the same conditions of excitation and emission spectra for control experiments. The results shown in Figure 1, the new tricyano-dihydrofuran solution and gatifloxacin solution monomer has fluorescence characteristics.When the novel tricyano -dihydrofuran complexed with gatifloxacin, a charge-transfer complex was formed. The characteristic peak of the complex did not shift from the gatifloxacin monomer in their excitation and emission spectra The fluorescence intensity of the complex was significantly enhanced, indicating that the novel tricyano-dihydrofuran and gatifloxacin have formed charge-transfer complexes.

pH effect，Take seven dried plugged test tubes were added 0.5ml of new tricyclic dihydrofuran mother liquor and 4ml gatifloxacin working solution, a slight concussion to complete fusion, adding methanol was fixed, the test tube was kept at 40℃ constant temperature Heat the water bath for 30 minutes, control the pH value within the range of 4.5 ~ 7.5, remove it and place it to room temperature. The appropriate amount of 7 mixed solution were scanned at 354nm fluorescence intensity. It can be seen from the figure that when the pH value of the system is controlled within the range of 4.5-5.0, the fluorescence intensity of the system continues to increase. When the pH value is 5.5, the fluorescence intensity reaches the strongest. The peracid will destroy the complex structure. Therefore, pH = 5.5 is the best pH value.

Effect of DCDHF-2-V concentration，Take seven dried plugged test tube, were added 4ml of gatifloxacin and 0.5ml of new tricyclic dihydrofuran mother liquor, a slight concussion to complete fusion, adding anhydrous methanol volume, the tube was placed in a constant temperature water bath The concentration of DCDHF-2-V was determined by heating at room temperature, fixing the concentration of gatifloxacin, keeping the temperature at 40 ℃ in water bath for 30 min and pH = 5.5. The fluorescence intensity of the system at different concentrations of DCDHF-2-V was measured. 1.0×10^{-3}mol.L^{-1}-3.5×10^{-4}mol.L^{-1} system has a strong fluorescence intensity, continue to study.

Gatifloxacin complex for the best screening conditions，Amount of DCDHF-2-V affected，0.1-0.7ml DCDHF-2-V with gradient of 0.1ml and 1.0ml and 2.0ml was added to the dried plugged test tube with 4ml of gatifloxacin respectively, and the volume was fixed with anhydrous methanol, In 40℃ water bath for 30 min, placed at room temperature, measured fluorescence intensity The measured results show that when the amount of DCDHF-2-V 0.5 mL fluorescence enhancement. Low concentrations and high concentrations result in lower fluorescence (low concentration, incomplete reaction; high concentration, low fluorescence).

The amount of gatifloxacin affected，Take 1-8 ml, gradient of 1 g of gatifloxacin solution, were added to 0.5 mLDCDHF-2-V with a plugged dried test tube, add anhydrous methanol volume, shake well, 40℃ water bath temperature 30 min, cooled to room temperature, measured fluorescence intensity The measured results show that when the dosage of gatifloxacin is controlled at 4ml, the fluorescence intensity of the charged complex is the highest, which is the best condition at this time.

Temperature and time effects

Temperature effects，According to the above experimental method, fixed the other experimental conditions remain unchanged, the temperature of the water bath were controlled at 20-60℃, with a plug containing the test tube was heated for 30 minutes, removed and placed to room temperature. The fluorescence intensity of the complex was measured as shown in Figure 2. The data show that the temperature increase helps to increase the fluorescence intensity of the system, but the complex tends to be stable when the temperature reaches 40℃ (warming facilitates the formation of the complex, but the temperature Should not be too high, damage components), so the optimal reaction temperature of the system is 40℃.

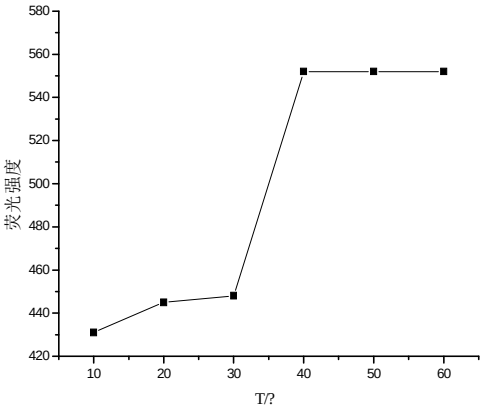

Fig. 2 Temperature effects

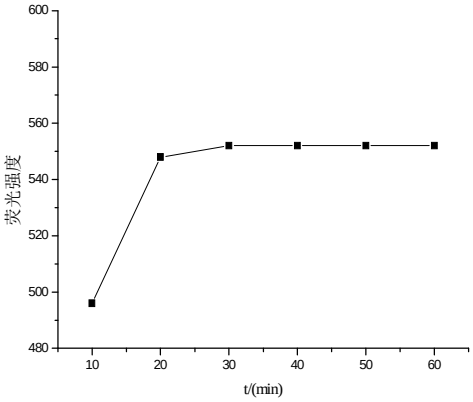

Fig. 3 The impact of time

The impact of time，Fixing the other experimental conditions (single variable method), under the conditions of temperature determination, respectively, in the reaction 10min~60min selected six full time period when detecting the fluorescence intensity of the complex, the results shown in Figure 3, Gatifloxacin andThe new tricyano-dihydrofuran reacted completely at 30 minutes.

Reaction mechanism，Novel tricyano-dihydrofuran has a planar molecular structure (D-π-A type), is a strong electron acceptor material. Gatifloxacin C-7 position even with a lone pair of nitrogen atoms, and the new electron acceptor of a new type of cyanide dihydrofuran under the appropriate conditions to occur in charge-transfer reaction, a new charge-transfer complex Things. The reaction principle is as follows:

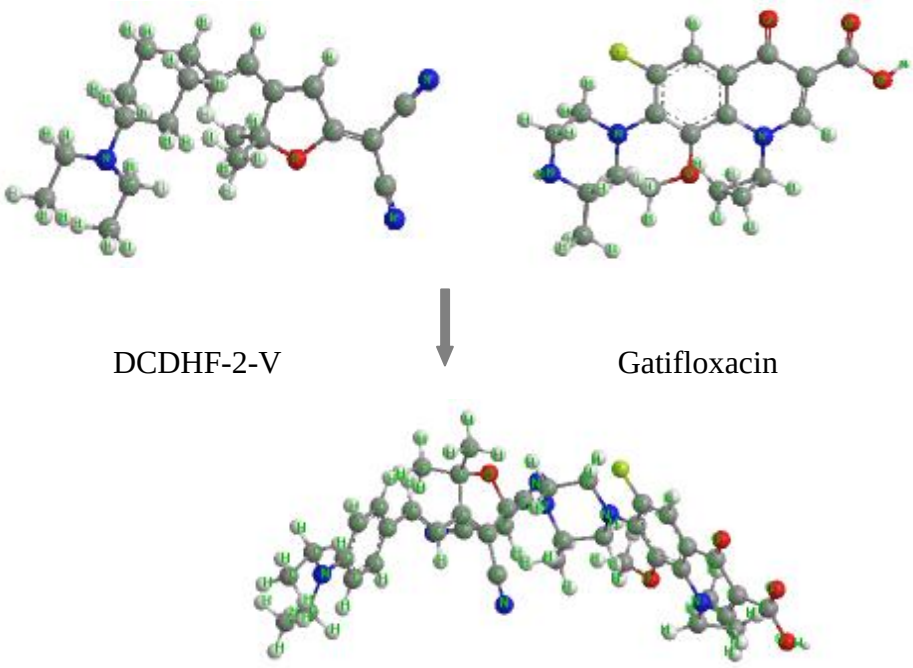

Fig. 4 Reaction mechanism

Sample Determination，5 tablets of gatifloxacin in a certain area , mortar into powder, accurately weighed total amount of grinding 1/5 (equivalent to 100mg drugs), with an appropriate amount of anhydrous methanol to dissolve, microwave ultrasound or glass rod Soluble. To be completely dissolved, anhydrous methanol volume (100ml). Pipette exactly 10 mL sample solution, diluted to 1×10^{-3} mol/L anhydrous methanol, spare. Using a series of optimization conditions explored by the above experimental scheme, the content of norfloxacin in the sample was determined, and six parallel determination results were selected. The results are shown in Table1Experimental data. In order to increase the effectiveness of the results, standard addition method was used to determine the recovery rate of the drug.

Table1. sample measurement results and recovery rate

sample	Add amount (mg)	Measured value (mg)	Recovery rate (%)	RSD(%)
1	9.53	9.55	100.21	1.07
2	9.53	9.61	100.83	1.13

Conclusions

Gatifloxacin In the chloroform and methanol solution system, gatifloxacin can form a stable charge-transfer complex with the novel tricyano-dihydrofuran. The optimum pH value of this system is 5.5, the reaction temperature is 40 ℃ and the reaction time is 30 min. The fluorescence intensity of the system is significantly higher than ofloxacin. Therefore, this method can be used as a new method for the determination of ofloxacin in medicine, and the reaction conditions are mild, high speed and high sensitivity.

Acknowledgements

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References

[1] Hui Wang,Zhikuan Lu,Samuel.J.Modifications of DCDHF single molecule fluorophores to impart water solubility[J].Tetrahedron Letters,2007,48: 3471–3474.

[2] EnriqueFont-Sanchis,,a Raquel E. Galian,b Francisco J.Alkoxy-styryl DCDHF fluorophores[J]. Physical Chemistry Chemical Physics ,2010, 12:768–7771.

[3] Wissam Bentoumi.Concise Multigram-Scale Synthesis of Push–Pull Tricyanofuran-Based Hemicyanines with Giant Second-Order Nonlinearity: An Alternative for Electro-optic Materials [J]. Chem. Soc.Rev,2014, 20: 8909-8913.

[4] Hui Wang, Zhikuan Lu,.The influence of tetrahydroquinoline rings in dicyanomethylene dihydrofuran (DCDHF) single-molecule fluorophores [J].Tetrahedron, 2006, 10: 103-114.

[5] Mingqian He, Thomas M. Leslie, John A. Sinicropi. r-Hydroxy Ketone Precursors Leading to a Novel Class of Electro-optic Acceptors[J]. Chem. Mater.2002, 14: 2393-2400.

[6] Rurack.K., Genger,U. R. Rigidization, preorientation and electronic decoupling-the ‘magic triangle’ for the design of highly efficient fluorescent sensors and switches.[J]. Chem. Soc.Rev. 2002,31:116-127.

[7] Pearce,D.A.,Jotterand,N. Derivatives of 8-Hydroxy-2-methyl quinoline are powerful prototypes for zinc sensors in biological systems, [J]. J.Am. Chem.Soc, 2001, 123(21) 5160-5161.

[8] Sigmund.H.Pfleiderer.W. A new type of labeling of nucleosides and nucleotides, [J]. Helv Chim Acta,2003,86(7):2299-2334.

[9] Adamczyk.M.,Chan.C.,Fino.J. et al.,Synthesis of 5-and 6-hydroxymethyl fluoresce in phosphora inidites.[J]- J.Org.Chem.2000,65(2):596-601.