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# The Synthesis, Characterization and Application of Ciprofloxacin Complexes and Its Coordination with Copper, Manganese and Zirconium Ions

**Zhengde Tan[1], Fengjiao Tan[2], Li Zhao[2], Junyong Li[1]**

[1]Department of Chemistry and Chemical Engineering, Hunan Institute of Engineering, Xiangtan, China; [2]Xiangtan County People's Hospital, Xiangtan, China.
Email: tzd0517@126.com

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## ABSTRACT

In our work, ciprofloxacin was extracted from the raw material ciprofloxacin hydrochloride and coordinated with the metal ions of copper, manganese and zirconium. The procedures include the comparison of the autoclave method with the solvothermal method, synthesizing the corresponding complexes and conducting antibacterial test on nearly 20 kinds of bacteria. It is shown that under the condition of PH 1 and 110˚C - 116˚C, the autoclave method and solvothermal method can be utilized to obtain the ciprofloxacin complexes with $Cu^{2+}$, $Mn^{2+}$ and $Zr^{2+}$ as the metal ligands after reacting for 8 hours. The raw material, ligands and monocrystals were characterized by IR, DSC, UV and fluorescence spectrum. Meanwhile a great number of antibacterial tests were carried out, revealing favorable bacteriocidal properties of the resulting complexes.

**Keywords:** Ciprofloxacin; Ligand; Characterization; Antibacterial

## 1. Introduction

Quinolone antibacterial drugs [1] has many characteristics, such as broad antibacterial spectrum [2-4], high bactericidal activity, low toxicity and unique mechanism. As one of the most important antibiotics, it is widely used in various infectious diseases in clinical treatment. Among the first generation, the most improtant antibiotics are nalidixic acid and piromidic acid; they have a remarkable influence on most gram-negative bacteria, but almost no influence on gram-positive bacteria and pseudomonas aeruginosa. On behalf of the second generation Quinolone antibacterial drugs, Cinoxacin and pipemidic acid inhibits gram-negative bacteria while improving bactericidal activity compared with the first generation [5,6]. Norfloxacin, enoxacin, ciprofloxacin and ofloxacin are the representatives of the third generation. The third generation quinolone has wide antimicrobial spectrum, except for having strong bacteriostasis for gram-negative bacteria, it also shows the strong activity for gram-positive bacteria. However, there are still many problems and challenges have not been settled regarding drug function mechanism, drug resistance, further development and etc. Because of the exceptional position in medicine and beneficial biological performance of metal ions, it has great theoretical and practical significance for the development and application of quinolones and new metallic anticancer compounds as well as promote the complexation of drug ligands or synthesis of metallic compounds in terms of investigation on the mechanism and inorganic medicine [7].

Currently, most research focus on the structure of quinolone-metal complexes, however, the research on the nature of complexes is neither comprehensive nor deep [8-11]. The synthesis, structure and physicochemical properties of quinolone-transition metal complexes are fully studied and there are also reports on the biological properties. But these researches mainly target at the norfloxacin and cinoxacin metal complexes. So far little material is available on transition metal complexes of ciprofloxacin (CPFX). Although much initial work has been done to deal with the subject, researches in this field are far from intensive and systematic, not to mention that no pronounced progress has been made. Therefore, it is absolutely necessary to further study this kind of promising antibiotics. Herein, we report the synthesis and crystal structure of the title complexes, researching the coordination way of ciprofloxacin and metal ions and obtaining the new material and new use from activity test *in-vitro*.

In this paper, autoclave method, hydrothermal synthesis and direct mixing with the solvent were utilized to

 

synthesize all kinds of complexes, with ciprofloxacin deeply associated with physiological function as the transition metals. The coordination chemistry of the physiochemical properties of the complexes are investigated by IR, fluorescence spectrum UV-Visable, DSC and etc. And the subsequent bacteriostasis tests indicate the exceptional performance of the metal complexes.

## 2. Experimental Section

### 2.1. Main Reagents and Equipment

Capsule of ciprofloxacin hydrochloride, obtained by HPGC Group General Pharmaceutical Factory. Acetic acid (AR), provided by Shantou Xilong Chemical Co., Ltd. Copper sulfate dihydrate (AR) and zirconium oxychloride (AR), purchased from the Development Center of Tianjin Kermel Reagent Co., Ltd. Manganese chloride(AR), offered by Tianjin Tianyida chemical Co., LTD. Ethanol (AR), offered by Tianjin Damao Reagent Factory, acetone (AR) by Tianjin Fuyu Fine Chemical Industry Co., Ltd and hydrochloride (AR) by Hengyang Xunyuan Chemical Reagent Co., Ltd.

Magnetic agitator with thermostat heater, DF-101S, Gongyi Yuhua Instrument Factory high-pressure reaction kettle, KF-20 ml, Xi'an Taikang Biological Science and Technology Co., Ltd; vacuum pump with circulating water system, SHZ-D(III), Gongyi Yuhua Instrument Co., Ltd; vacuum drying oven, DZF-250, Zhengzhou Greatwall Scientific Industrial and Trading Co., Ltd; fluorospectro photometer, 960MC, Shanghai Precision and Scientific Instrument Co., Ltd; Fourier infrared spectrometer, AVATAR370, Thermo Nicolet Corp in America.; differential scanning calorimeter, DSC 200 F3, Netzsch Scientific Instruments Co., Ltd; Ultraviolet spectrometer, UV8100, Shanghai Jiashi Scientific Instrument Co., Ltd.

### 2.2. Experimental Procedures

#### 2.2.1. Extraction of Ciprofloxacin

The raw material, ciprofloxacin hydrochloride, was deprived of hydrochloride and went through other reactions to obtain pure ciprofloxacin. The reaction is **Figure 1**.

After grinding the chemical granules, ciprofloxacin hydrochloride (45.963 g) was put into a beaker (250 ml). At the temperature of 50˚C, ammonia water (1 mol/L) was slowly added till the solution became neutral and the solution was stirred for 15 minutes. The stirred solution was filtered and put into the vacuum drying oven for 6 hours after removal of filter cake. Finally, the dried ciprofloxacin product was grinded into powder, weighing 46.27 g, ready for the following synthesis.

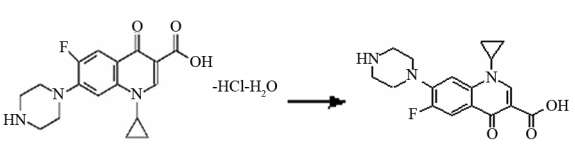


**Figure 1. Preparation of ciprofloxacin.**

#### 2.2.2. Synthesis of Ciprofloxacin Copper

Add a certain amount of hydrochloric acid (1 mol/L) into a beaker with ciprofloxacin (2.5 g). Fully stirring was conducted to make ciprofloxacin dissolve. When PH value was kept around 1, copper sulfate dehydrate was added (AR, 0.77 g) into the solution, then stirred it for half an hour in a 75˚C water bath. The operation will be done in two ways. The first is to pour the solution into the autoclave in the oil bath and oxygen-proof reaction was conducted for 8 hours at 115˚C. Then the solution cooled to room temperature with heaters off, and was kept overnight. The solid was filtered off, washed with water and placed in the vacuum oven to obtain the product. The second is to pour the resulting solution in a three-necked round-bottom flask and install the condensation reflux unit. Likewise, the reaction was conducted for 8 hours at 115˚C and then cooled to room temperature. The solution with white and flocculent upper side was filtered, washed and dried in the vacuum oven to obtain the resulting chemicals.

#### 2.2.3. Synthesis of Ciprofloxacin Zirconic

Sodium hydroxide (1 mol/L) was added to ciprofloxacin (2.5 g) and the mixture was stirred to make ciprofloxacin dissolve in the solution. Zirconium oxychloride (1.61 g) was added at PH value of 12 and stirred continuously in the 75˚C water bath for another half an hour. The solution turned white and pasty and poured it into a three-necked round-bottom flask (250 ml). After installation of the condensation reflux unit, the solution was reacted at 110˚C for 8 to 10 hours, then cooled to room temperature with colorless solution in the upper side and white sediment in the lower side. The solid was filtered off and washed with water, dried in the vacuum oven and grinded into the resulting powder.

#### 2.2.4. Synthesis of Ciprofloxacin Manganese

A certain amount of Hydrochloric acid (1 mol/L) was added into ciprofloxacin (2.5 g) and the mixture was stirred to make ciprofloxacin dissolve in the solution. 1.57 g $MnCl_2$ was added at PH value of 1 and stirring was done in the 75˚C water bath for half an hour. The solution turned white and pasty and was poured into a three-necked round- bottom flask. After installation of the reflux unit, the solution was reacted at 110˚C for 8 to 10 hours, (the solution begins to turn yellow during 20 min reaction) then cooled to room temperature with colorless solution in the upper side and tan lotion in the lower side. The solid was filtered off and washed with

 

the water, then dried in the vacuum oven and grinded into the resulting powder.

## 2.3. Characterization of the Complexes

### 2.3.1. Testing Analysis of Infrared Spectrometer (IR)

The samples (raw materials and synthesized complexes) were grinded with potassium bromide in the proportion of 1:100. The mixture was then pressed into plates, which was tested by means of Fourier infrared spectrometer. The spectra were collected and available in the Result and Discussion section.

### 2.3.2. Testing Analysis of Differential Scanning Calorimeter (DSC)

The calorimetric analysis of as-prepared 0.0050 g samples (raw materials or synthesized complexes) which measured by the electronic balance, accurate to 0.0001 g, it was detected in NETZSCH DSC 200 F3 calorimeter (with nitrogen as the shielding gas, gas speed of 260 ml/min, sampling rate of 200.00 pts/min and heating rate of 20.000 K/min). Refer to the Result and Discussion for the spectrogram.

### 2.3.3. Testing Analysis of Ultraviolet Spectrometer (UV)

The as-prepared samples (raw materials and synthesized complexes) were dissolved in disodium hydrogen phosphate (0.5 mol/l) and sodium dihydrogen phosphate (0.5 mol/l). Acetic acid was also added to assist the dissolution and keep a constant volume of 50 ml. According to the literature, the scanning spectrum was determined between 200 nm and 400 nm, which can be seen in the Result and Discussion section.

### 2.3.4 Testing Analysis of Fluorescence Spectrometer

The as-prepared samples (raw materials and the resulting complexes) were dissolved in disodium hydrogen phosphate (0.5 mol/l) and sodium dihydrogen phosphate (0.5 mol/l). Acetic acid was also added to assist the dissolution and to keep a constant volume of 50 ml. Fluorescence spectra were collected using a 960 MC fluorospectro photometer for the whole wavelength. The scanning spectrum of the complexes was thus determined from the range of 370 nm to 450 nm, hereby find out the maximum absorption wavelength. Refer to the Result and Discussion for the details.

# 3. Results and Discussion

## 3.1. Analysis of the Main Influence Parameter

### 3.1.1. Effect of the pH on the Solubility

The pH value can be adjusted by changing the solvent as shown in **Table 1**. It is concluded from **Table 1** that copper and manganese can well dissolve in hydrochloric acid when pH value is 1, while zirconium can be well dissolved in hydrochloric acid when pH value is 12.

### 3.1.2. Effect of Temperature

It is summarized from **Tables 2** and **3** that the best reflux temperature for aqueous solution is 115˚C, while the most appropriate reaction temperature for autoclave method is around 110˚C. Therefore, the optimum temperature should be controlled at 115˚C.

**Table 1. Data tables of pH value and medium.**

| pH | Metal ions | Medium (1 mol/l) | Results show |
|---|---|---|---|
| 10 | Copper sulfate dihydrate | Sodium hydroxide | Poorly dissolved, less product |
| 1 | Copper sulfate dihydrate | Hydrochloric acid | Well dissolved, good product |
| 4 | Copper sulfate dihydrate | acetic acid Sodium | Poorly dissolved,less product, inconsistent properties |
| 11 | Manganese chloride | hydroxide | Almost undissolved,less product |
| 1 | Manganese chloride | Hydrochloric acid | Well dissolved,good product |
| 4 | Manganese chloride | acetic acid | Almost undissolved,less product |
| 12 | Zirconium oxychloride | Sodium hydroxide | Well dissolved,good product |
| 1 | Zirconium oxychloride | Hydrochloric acid | Poorly dissolved,less product |
| 4 | Zirconium xychloride | acetic acid | Almost undissolved,less product |

**Table 2. Aqueous solution reflux temperature control.**

| Temperature (˚C) | 90 | 100 | 105 | 115 |
|---|---|---|---|---|
| Ciprofloxacin copper | Invalid | Less and worse product | worse product | More and better product |
| Ciprofloxacin manganese | Invalid | Less and worse product | worse product | More and better product |
| Ciprofloxacin zirconium | Invalid | Less and worse product | worse product | More and better product |

**Table 3. Exploration of high pressure reactor method temperature control table.**

| Temperature (˚C) | 100 | 110 | 120 |
|---|---|---|---|
| Ciprofloxacin copper | Less and worse product | More and better product | Less and worse product |
| Ciprofloxacin manganese | Less and worse product | More and better product | Less but better product |
| Ciprofloxacin zirconium | Less and worse product | More and better product | Less but better product |

 

### 3.2. IR Spectrum

The infrared spectra of ciprofloxacin, ciprofloxacin copper, ciprofloxacin manganese and ciprofloxacin zirconium are shown in **Figures 2-4**, respectively.

Among the ciprofloxacin vibrations, those at 1628.81, 1507.34 and 1473.82 $cm^{-1}$ are assigned to vibration absorption of the $CH_2$ on the benzene ring. The 1721 and 3403 $cm^{-1}$ stretching vibrations are attributed to carbonyl and hydroxyl in a carboxyl group, respectively. The 1396 and 941 $cm^{-1}$ vibrations correspond to the bending of O-H,and around 740 $cm^{-1}$ indicates the absorption peak of secondary amine. Overall, the recorded spectrum is indicative of ciprofloxacin, since it is similar to standard spectrum of ciprofloxacin containing hydrogen chloride but no aggregates.

It can be observed in **Figure 3** that the peak in 1700 $cm^{-1}$ disappears while peak in 3400 $cm^{-1}$ remains compared with the standard spectrum, which accounts for the association effect between copper and carbonyl.

From **Figure 4**, two absorption peaks of N-H appear at around 3400 $cm^{-1}$, indicating of the association between manganese and carbonyl.

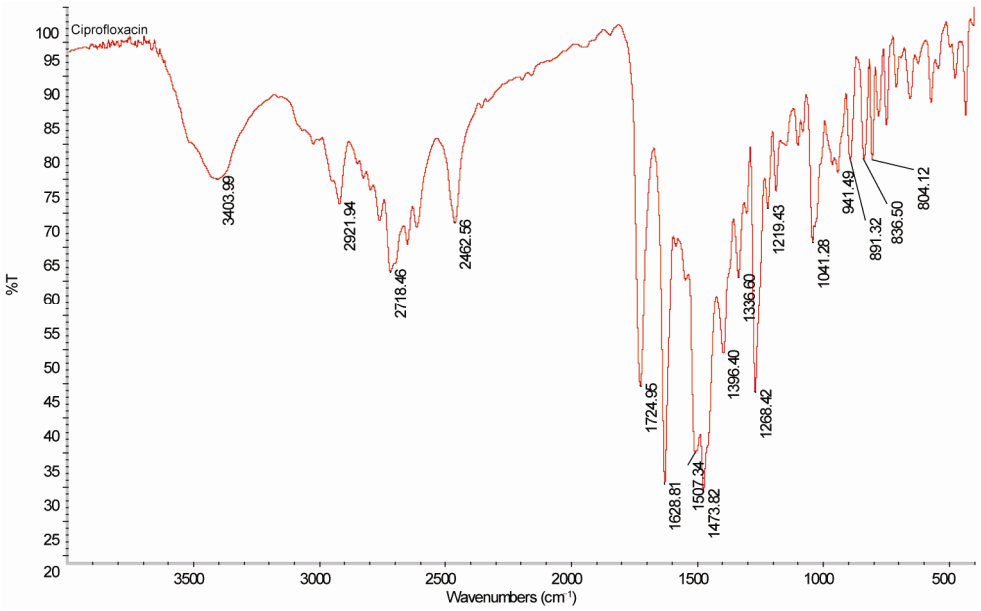


**Figure 2. Infrared spectrum of ciprofloxacin.**

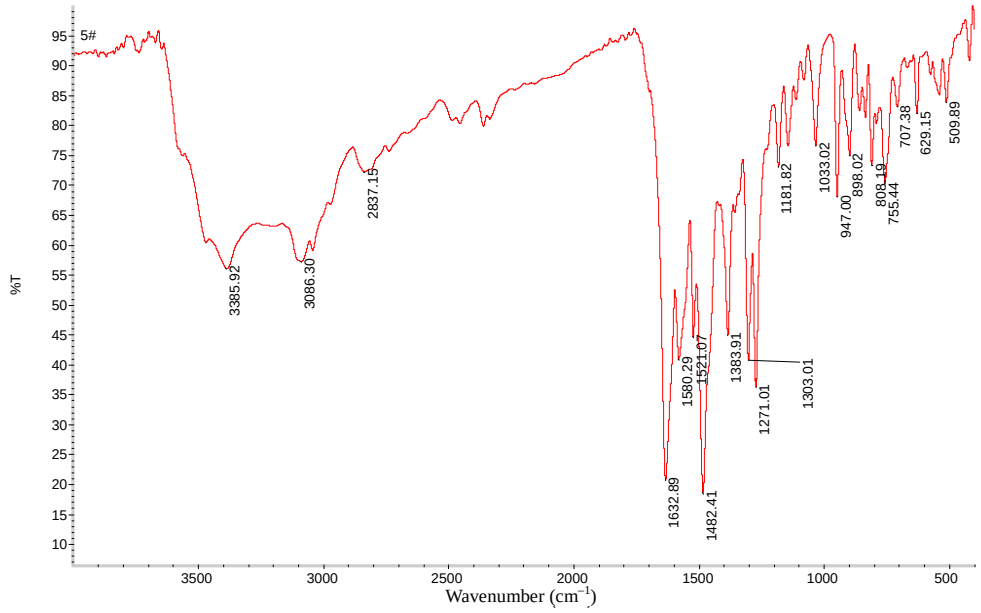


**Figure 3. Infrared spectra of ciprofloxacin copper.**

 

Compared with standard spectra, From **Figure 5**, the peak in 1700 $cm^{-1}$ disappears while the peak in 3400 $cm^{-1}$ remains, which may be assigned to the association between zirconium and carbonyl, besides, the absence of vibration peaks of C-H between 2500 and 3000 $cm^{-1}$ is associated with the association of zirconium.

### 3.3. DSC

The weight of as-prepared samples is listed in **Tables 4** and **5**.

From **Figure 6**, the endothermic peak of ciprofloxacin may account for the intramolecular change while the exothermic peak is due to the formation of new intermolecular or intramolecular bonds

From **Figure 7**, the strong exothermic peak at 345.4˚C is associated with the formation of new intermolecular hydrogen bonds and several spikes appear at the peak because of instability of synthetic compound.

From **Figure 8**, the 331.5˚C peak is associated with melting process of metal complex, and the existence of metal ions leads to increased melting point. The 363.7˚C exothermic peak demonstrates the formation of new intermolecular bonds in the melt. Therefore, metal ions were coordinated onto ciprofloxacin during this process.

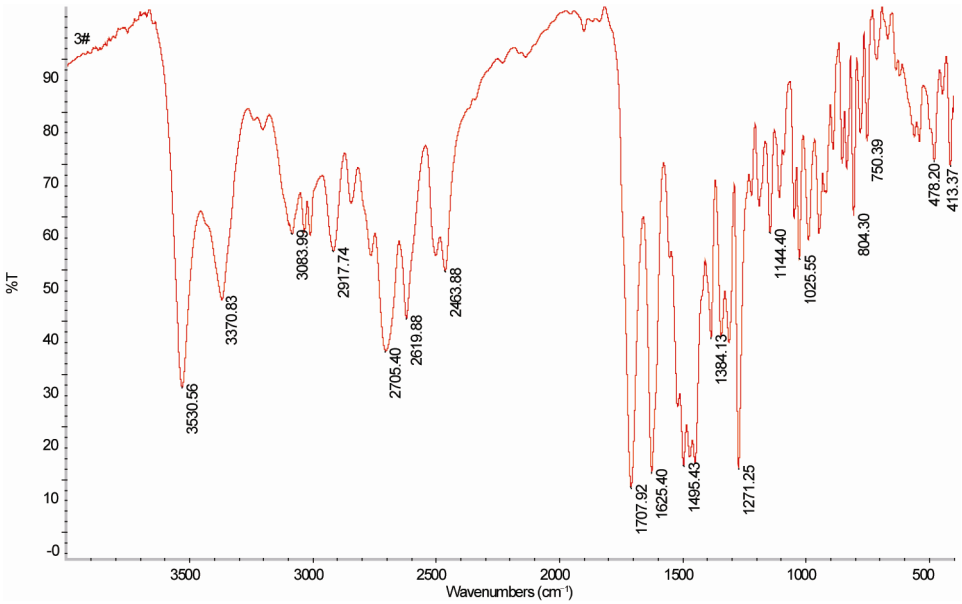


**Figure 4. Infrared spectra of ciprofloxacin manganese.**

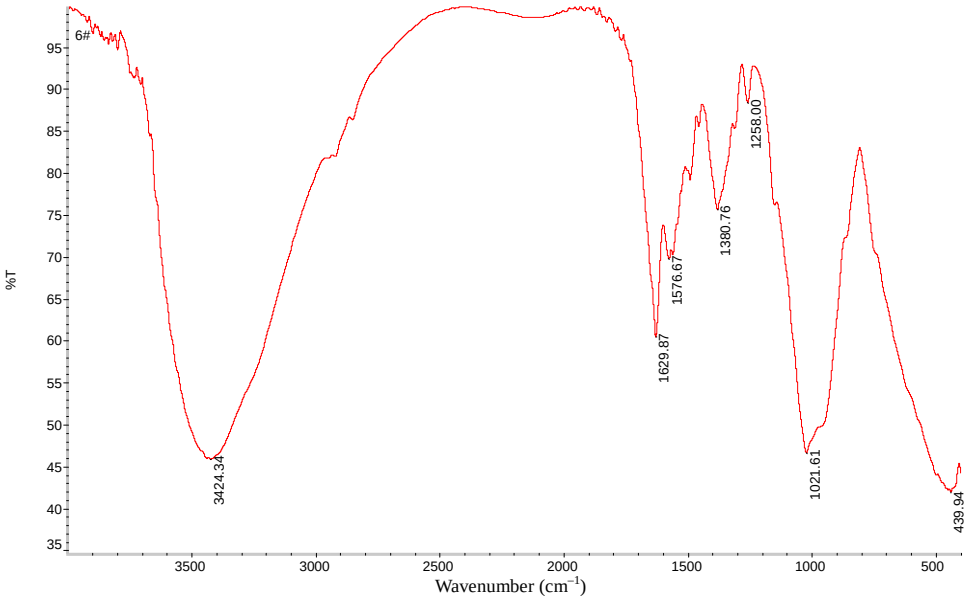


**Figure 5. Infrared spectra of ciprofloxacin zirconium.**

 

**Table 4. Sample quality.**

| Designatin | Ciprofloxacin | Ciprofloxacin copper | Ciprofloxacin manganese | Ciprofloxacin zirconium |
|---|---|---|---|---|
| Weight (g) | 0.0050 | 0.0051 | 0.0050 | 0.0049 |

**Table 5. Temperature list.**

| Mode | Temp (˚C) | Heating rate (K/min) | Sampling rate (pts/min) | Time (hh:mm) | STC | P2 | PG | Co |
|---|---|---|---|---|---|---|---|---|
| Waiting mode (heating) | 20.0 | 40.000 | | | 1 | 1 | 1 | 1 |
| Waiting mode (stabilizing) | 20.0 | | | 02:00 | 1 | 1 | 1 | 1 |
| Dynamic | 450.0 | 20.000 | 200.00 | 00:21 | 1 | 1 | 1 | 1 |
| Urgent | 460.0 | | | | | 1 | 1 | 1 |
| End of waiting mode (heating) | 20.0 | 40.000 | | 00:10 | 1 | 1 | 1 | 1 |
| End of waiting mode (stabilizing) | 20.0 | | | 02:00 | 1 | 1 | 1 | 0 |

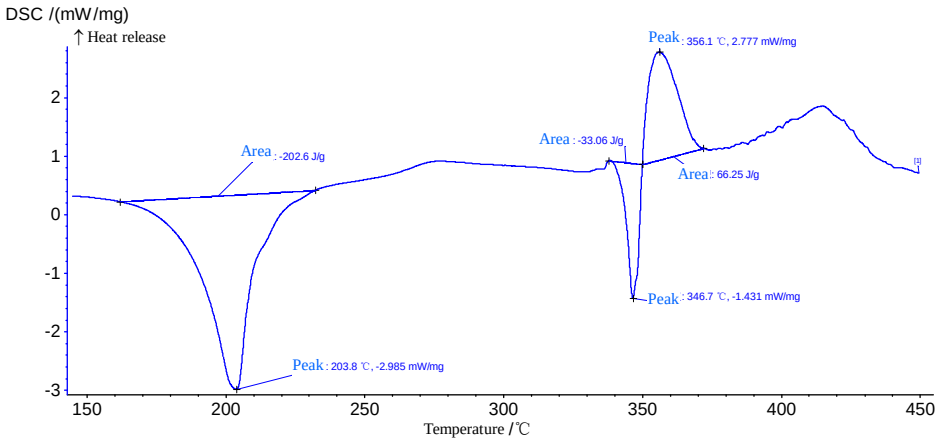


**Figure 6. DSC diagram of ciprofloxacin.**

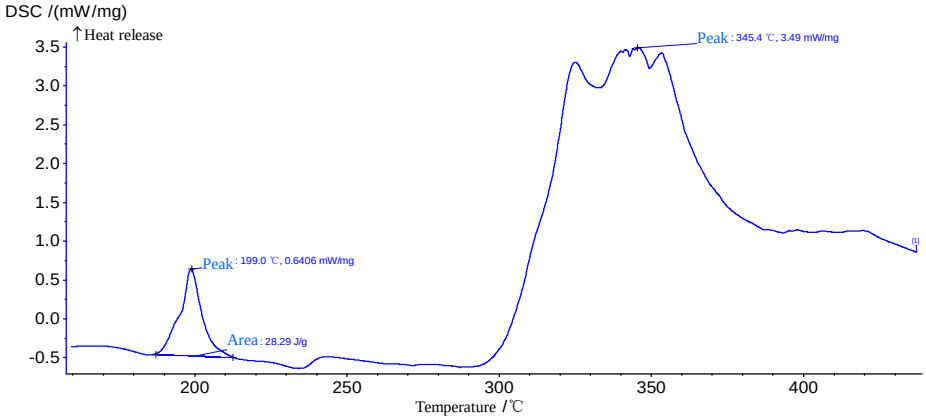


**Figure 7. DSC diagram of ciprofloxacin copper.**

From **Figure 9**, the front is a water peak, it is to be ignored, and then there is no peak, so can't analyse it.

## 3.4. UV Analysis

As **Figures 10-13** show, it can be concluded from the three graphs above that the peaks in the UV spectra of the three complexes show a red shift with varying degree, compared with that of the pure ciprofloxacin. In three metal complexes, both dispersive charge and metallic tracks contribute to the convenient transfer of the electrons, leading to weakened absorption of ultraviolet light and obvious red shift. So conclusion can be drawn that metal ions have an influence on the UV absorption and an indirect reaction occurs between metal ions and ciprofloxacin, resulting in red shift.

 

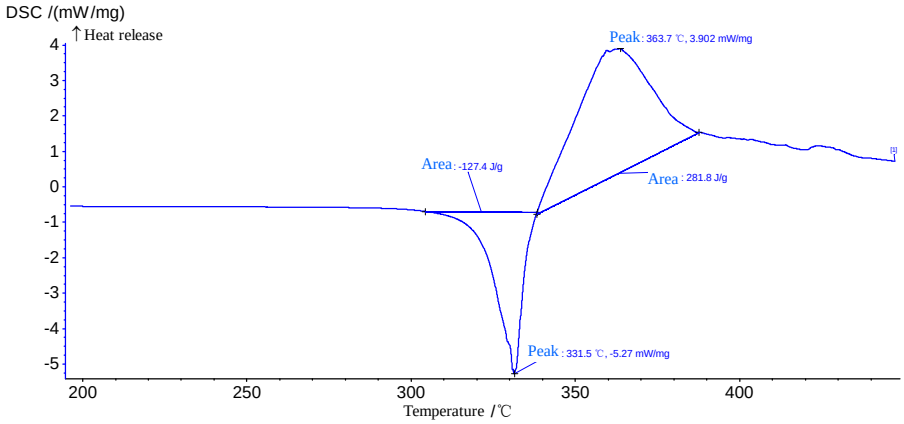


**Figure 8. DSC graph of ciprofloxacin manganese.**

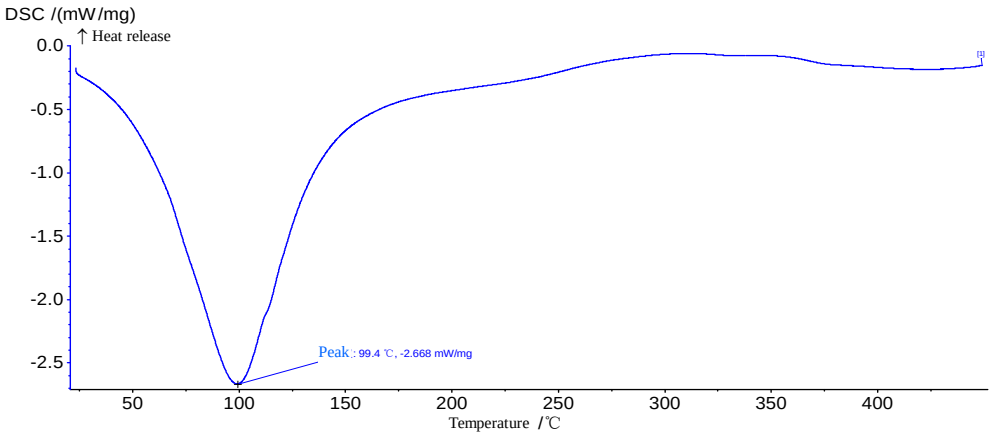


**Figure 9. DSC graph of ciprofloxacin zirconium.**

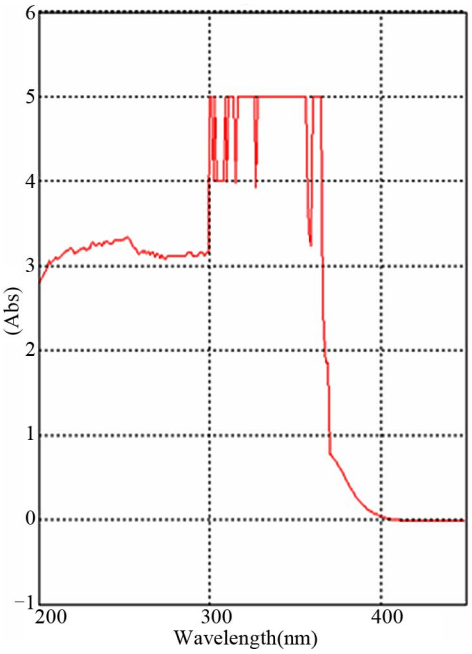


**Figure 10. UV-spectrum of ciprofloxacin.**

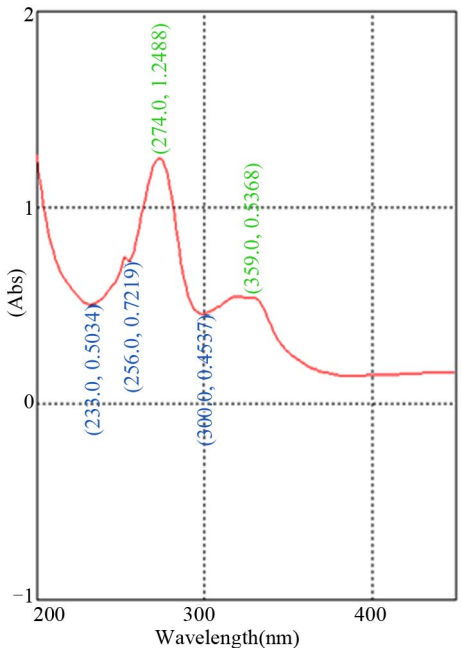


**Figure 11. UV-spectrum of ciprofloxacin copper.**

 

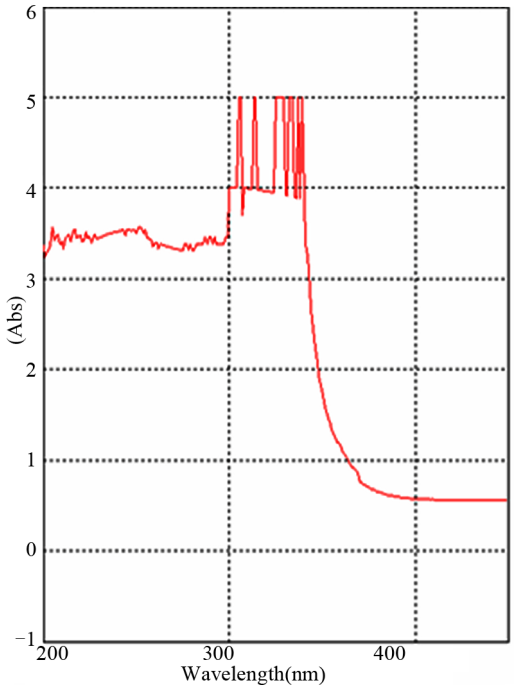


**Figure 12. UV-spectrum of ciprofloxacin manganese.**

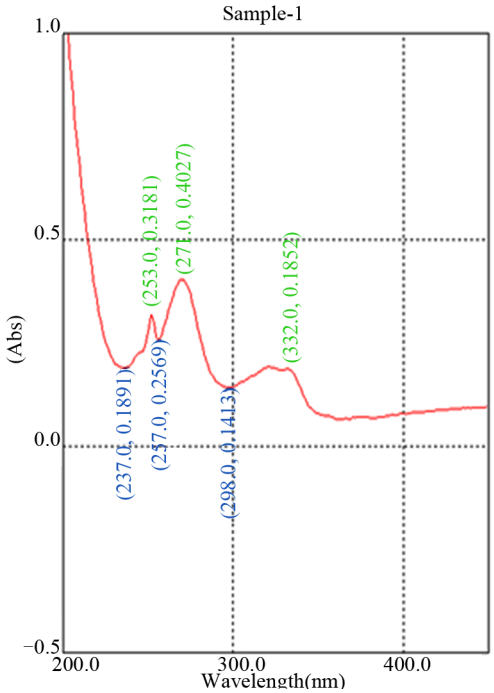


**Figure 13. UV-spectrum of ciprofloxacin zirconium.**

## 3.5. Fluorescence Spectral Analysis

It is shown in **Table 6** that red shift in fluorescence spectral of complex was observed due to existence of metal ions. It can be concluded from this phenomenon that metal ions were successfully coordinated to ciprofloxacin despite of fluctuated fluorescence intensity caused by the impurity. Overall, metal ions make it more apparent for the fluorescence to quench.

## 3.6. *In Vitro* Antimicrobial Activity Test

Parameters of MIC (minimal inhibitory concentration) [12] in the antibacterial test are presented in the **Table 7**.

It can be concluded from **Table 7** that the three complexes have an inhibiting effect on escherichia coli, staphylococcus aureus and staphylococcus epidermidis at low concentration, but remain inactive towards streptococcus pneumoniae and klebsiella pneumoniae. In general, metal complexes of ciprofloxacin exhibit exceptional antimicrobial properties [13,14].

# 4. Conclusion

As one of the third-generation antimicrobial quinolones, ciprofloxacin, possessing broad-spectrum antimicrobial activity, has wide application in clinical treatment of infections and diseases caused by gram-negative bacteria and gram-positive bacterial. In our work, ciprofloxacin was extracted from the raw material ciprofloxacin hydrochloride and coordinated with the metal ions of copper, manganese and zirconium. The procedures include

**Table 6. Data sheet of fluorescence spectral.**

| Type | PH | SENS | Y | $\lambda_{max}$ (nm) | Fluorescence intensity |
|---|---|---|---|---|---|
| HN | 4.0 | 1 | 2 | 445 | 52.1 |
| H-Cu | 6.2 | 1 | 2 | 380 | 76.5 |
| H-Mn | 6.4 | 1 | 1 | 375 | 56.4 |
| H-Zr | 6.2 | 1 | 2 | 375 | 90.6 |

**Table 7. *In vitro* antibacterial activity data sheet.**

| Strains | Copper (MIC) | Manganese (MIC) | Zirconium (MIC) |
|---|---|---|---|
| Streptococcus pneumoniae 70 | >128 | >128 | >128 |
| Streptococcus pneumoniae 9798 | >128 | >128 | >128 |
| Streptococcus pyogenes A12 | >128 | 32 | >128 |
| Staphylococcus aureus 9616 | 16 | 4 | 16 |
| Staphylococcus epidermidis 9726 | 16 | 16 | 16 |
| Escherichia coli ATCC25922 | 16 | 16 | 16 |
| Escherichia coli 834 | 16 | 8 | 8 |
| Pseudomonas aeruginosa ATCC27853 | 32 | >128 | >128 |
| Klebsiella pneumoniae 14 | >128 | >128 | >128 |
| Salmonella typhi H901 | 16 | 16 | 16 |

 

comparison the autoclave method with the solvothermal method, synthesize the corresponding complexes, and conduct antibacterial test on nearly 20 kinds of bacteria. It is shown that under the condition of PH 1 and 116˚C, the autoclave and solvothermal method can be utilized to obtain the ciprofloxacin single crystal after reacting for 8 hours. Under PH 1, 115˚C and PH 12, 110˚C respectively, the test combined corresponding manganese and zirconium coordination compound and adopted solvent volatilization method to obtain corresponding ciprofloxacin single crystal. The raw materials and ligands were characterized by IR, DSC, UV-Visable and fluorescence spectrum. Meanwhile a great number of antibacterial tests were carried out. Three kinds of ciprofloxacin metal ion coordination compound can effectively inhibit the growth of bacteria, among them ciprofloxacin manganese has the most effective antibacterial properties.

## 5. Acknowledgements

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