Talanta 107 (2013) 396–401

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Visual detection of tetracycline antibiotics with the turned on fluorescence induced by a metal–organic coordination polymer

Fei Leng [a], Xi Juan Zhao [a], Jian Wang [b], Yuan Fang Li [a,*]

[a] Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, School of Chemistry and Chemical Engineering, Southwest University, Tiansheng Road 2, Chongqing 400715, China
[b] College of Pharmaceutical Sciences, Southwest University, Tiansheng Road 1, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 23 November 2012
Received in revised form
17 January 2013
Accepted 23 January 2013
Available online 29 January 2013

Keywords:
Tetracycline antibiotics
Fluorescence
Metal–organic coordination polymer
1,4-Bis(imidazol-1-yl-methyl)benzene

ABSTRACT

Here, a rapid, simple and sensitive method for visual detection of tetracycline antibiotics (TCs) was developed based on the turned on fluorescence induced by a metal–organic coordination polymer of Zn(bix) [bix=1,4-bis(imidazol-1-ylmethyl)benzene]. Tetracycline antibiotics, such as tetracycline hydrochloride (TC), doxycycline hyclate (DC), chlortetracycline hydrochloride (CTC) and oxytetracyline hydrochloride (OTC), showed very weak fluorescence in Tris–HCl buffer of pH 8.68–9.40. However, the fluorescence intensities of these TCs were greatly enhanced in the presence of Zn(bix) and were found to be proportional to the concentrations of TCs. Accordingly, TCs could be quantitatively determined with the detection limits of 12.0, 6.0, 19.0 and 19.0 nM for TC, DC, CTC and OTC, respectively. Furthermore, this fluorimetric method was successfully applied to the determination of DC contents in doxycycline hyclate tablets from three factories with satisfactory results compared with the HPLC method. Also, the interaction mechanism between TCs and Zn(bix) was investigated by the absorption spectra, fluorescence spectra and circular dichroism spectra. The results showed that TCs could interact with Zn(bix) to form TCs–Zn(bix) ternary complexes and the turned on fluorescence was mainly ascribed to the restricted conformational rotation of TCs molecules through coordination with Zn(II) from the polymeric networks of Zn(bix).

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Tetracycline antibiotics (TCs, Fig. 1) as broad spectrum antibiotics against Gram-positive and -negative bacteria are produced by *Streptomyces*, which are derived from a common hydronaphthacene nucleus containing four fused rings and have similar chemical structures [1]. TCs are especially effective against some bacteria including *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Gonococcus*, *Cholera*, *Dysentery bacillis*, *Pertussis*, *Rickettsia*, *Chlamydia* and *Mycoplasma*. After being actively transported into the cells of susceptible bacteria, TCs can bind to the 30S ribosomal subparticle, and then produce a bacteriostatic effect by inhibiting protein biosynthesis [2]. However, the widespread use of these drugs has brought serious problems because they can act as toxic substances causing direct toxicity or allergic reactions in some hypersensitive individuals. The problems about the spread of drug-resistant microorganisms can also be caused by low-level doses of antibiotics [3]. In this case, it is important to analyze tetracycline antibiotics.

To our knowledge, tetracycline antibiotics are usually determined by capillary electrophoresis (CE) [4], high-performance liquid chromatography (HPLC) [5,6], capillary electrochromatography (CEC) [7], microbiological analysis (MA) [8–10], electrochemical analysis [11–13], immune assay (IA) [14,15] and the fluorimetric method [16–19], among which, the fluorimetric method is mainly based on the coordination interactions of TCs with metal ions [16,17]. However, there are very few examples involved with the metal–organic coordination polymers.

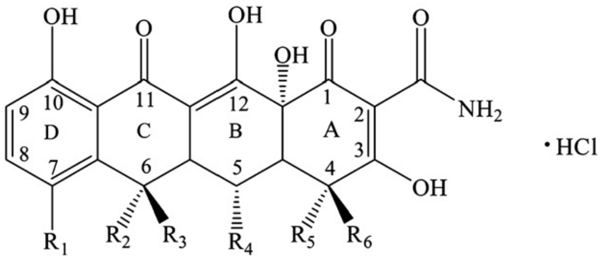
Metal–organic coordination polymers (MOCPs), formed from metal ions and organic ligands, are an emerging materials with high porosity and highly desirable frameworks [20]. MOCPs are constructed through coordination interaction and intermolecular interactions such as hydrogen bonding interaction, π–π stacking interaction, dipole–dipole interaction and van der Waals interaction [21]. Owing to the very large surface area, tunable pore sizes, high thermal stability, as well as tempting magnetic, electrical, optical, and catalytic properties, MOCPs attract considerable attention and show potential applications in gas purification and separation [22,23], catalysis [24,25], sensing [26,27] and drug delivery/release [28,29].

It has been reported that the imidazole ligand of bix [bix=1,4-bis(imidazol-1-ylmethyl)benzene] can react with divalent zinc ion to form amorphous coordination polymers [30], and some studies have been devoted to the synthesis and applications of Zn(bix) coordination polymers [31–35]. For example, Hoskins et al. have reported the structure of $[Zn(bix)_2(NO_3)_2]\cdot 4.5H_2O$,

---

[*] Corresponding author. Tel.: +86 23 68254659; fax: +86 23 68367257.
E-mail addresses: liyf@swu.edu.cn, liyfswu@yahoo.com.cn (Y.F. Li).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.01.039



TCs	R₁	R₂	R₃	R₄	R₅	R₆
TC	H	CH_3	OH	H	$N(CH_3)_2$	H
CTC	Cl	CH_3	OH	H	$N(CH_3)_2$	H
OTC	H	CH_3	OH	OH	$N(CH_3)_2$	H
DC	H	CH_3	H	OH	$N(CH_3)_2$	H

Fig. 1. The structure of tetracycline antibiotics.

which, like the silver derivative, has a 2D polyrotaxane structure [33]. Imaz et al. connected Zn(II) ions through bix organic ligands with 1:5 M ratio to form coordination polymer particles as potential drug delivery systems [32]. Liang et al. constructed a ratiometric fluorescence recognition of guanosine triphosphate based on Zn(II) complex of 1,4-bis (imidazol-1-ylmethyl) benzene [35]. We recently reported a method for selective visual recognition of naringenin and morin with Zn(bix) [36]. In this research, Zn(bix) coordination polymers have been found to turn on the fluorescence of TCs as a result of the formation of TCs–Zn(bix) ternary complexes. The optimal conditions and the mechanism of the interaction between TCs and Zn(bix) coordination polymers have been discussed. The calibration curves of fluorescence intensities against the concentrations of tetracycline antibiotics were constructed by measuring the fluorescence intensities of the four TCs in the presence of Zn(bix), and then quantitative detection of TCs was achieved. In addition, this fluorimetric method was successfully applied to the determination of DC in doxycycline hyclate tablets from three factories with satisfactory results compared with the HPLC method.

2. Materials and methods

2.1. Apparatus

A Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan) was used for recording and measuring the fluorescence spectra at a given wavelength. A U-3010 spectrophotometer (Tokyo, Japan) was used for recording the absorption spectra. A pH 510 precision pH meter (California, USA) was used to measure the pH values. The HH-2 digital display constant temperature water bath (Jintan, China) was used to control the reaction temperature. An IRPrestige-21 Fourier transform infrared spectroscopy (Shimadzu, Japan) was used for recording the infrared spectra. A J-810 circular dichroism spectrometer (Tokyo, Japan) was used for recording the circular dichroism spectra. A Hitachi L-2000 high performance liquid chromatography unit(Tokyo, Japan) was used for determination of doxycycline hyclate. A 300-MHz Bruker Advance Spectrometer (Fallanden, Switzerland) was used for recording the ^{1}H NMR spectra. An S-4800 scanning electron microscope (SEM, Hitachi, Japan) was used to scan the SEM images.

2.2. Chemicals and materials

Tetracycline hydrochloride standard was purchased from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). Chlortetracycline hydrochloride standard, oxytetracyline hydrochloride standard and doxycycline hyclate standard were all purchased from Sino-American Biotechnology Co., Ltd. (Luoyang, China). Seven hydrated zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was purchased from Hunan Institute of Geological Experiment (Xiangzhong, China). Imidazole and α,α′-dichloro-*p*-xylene were purchased from Sigma-Aldrich Co. LLC (Missouri, USA). Tris(hydroxymethyl)aminomethane was purchased from the Okawa Fine Chemical Co., Ltd. (Ningbo, China). Four antibiotic standards were biochemical reagents and the other reagents were of analytical grade. Milli-Q water (18.2 MΩ at 25 °C) was used throughout the experiment.

The stock solutions of 2.0×10^{-3} M tetracycline hydrochloride (TC), doxycycline hyclate (DC), chlortetracycline hydrochloride (CTC) and oxytetracyline hydrochloride (OTC) were prepared by dissolving TC, DC, CTC and OTC with double distilled water, respectively. The 4.0×10^{-5} M TC, DC, CTC and OTC solutions were prepared by diluting the above stock solutions with double distilled water.

Tris–HCl buffer solutions with different pH values were prepared by mixing the 0.1 M tris(hydroxymethyl) aminomethane solution with 0.1 M HCl solution by a certain percentage and the pH values were adjusted with an acidimeter.

2.3. Preparation of Zn(bix) coordination polymer

Bix dihydrate was synthesized following a method according to the published procedure [37]. Imidazole (3.16 g, 46.4 mmol) and α, α′-dichloro-*p*-xylene (0.78 g, 4.46 mmol) were added into methanol (50 mL), and then the methanol solutions was refluxed for 18 h. The methanol was removed by evaporation and some yellow sirup was obtained, followed by crystallization in aqueous solution of K_2CO_3 (6.13 g, 100 mL). The yielded crystalline bix was further recrystallized with double distilled water.

Bix dihydrate was characterized by the infrared spectroscopy and ^{1}H NMR spectra (see Figs. S1 and S2), which was consistent with those reported [38,39]. 0.01 M bix solution was obtained by dissolving a certain amount of bix dihydrate with ethanol.

According to reference [35], Zn(bix) coordination polymer could be easily obtained by mixing the aqueous solution of zinc ions and the ethanol solution of bix in a molar ratio of 1:1. The infrared spectra of Zn(bix) was consistent with the above reference (see Fig. S3). The shape of Zn(bix) coordination polymer was observed by SEM images (see Fig. S4).

2.4. Fluorescence spectra measurement

Into a 1.5-mL EP tube, 0.05 mL of Tris–HCl buffer solutions, 0.05 mL of NaCl solution, 0.05 mL of antibiotic solution, 0.05 mL of 0.01 M bix solution and 0.05 mL of 0.01 M $ZnSO_4$ solution were added. The pH values of Tris–HCl buffer solutions are 9.00, 9.25, 8.68, and 9.40, and concentrations of NaCl solutions are 0.120 M, 0.020 M, 0.050 M, and 0.016 M for TC, DC, CTC and OTC, respectively. Afterwards, the mixture was diluted to a final volume of 0.5 mL with double distilled water. After being mixed thoroughly and put in a water bath for 5 min, the fluorescence spectra were measured for these interacting systems. In addition, the fluorescence intensities of TCs and Zn(bix) were measured alone. The excitation and emission wavelengths in the four systems of TC-Zn(bix), DC-Zn(bix), CTC-Zn(bix) and OTC-Zn(bix) were 364/505, 382/502, 369/513 and 338/495 nm, respectively.

2.5. Determination of doxycycline hyclate contents in pharmaceutical tablets

The contents of commercial doxycycline hyclate tablets from three factories with the same labeled amount were determined. Ten doxycycline hyclate tablets from a random sampling were added to the mortar and pestled sufficiently. After weighing an appropriate amount of powder to put it into a 25-mL test tube, it was dissolved with double distilled water. Afterwards, the solution was moved to a 100-mL volumetric flask and diluted to the mark with double distilled water. After the solution was filtered and diluted with double distilled water to obtain the solution containing 4.0×10^{-5} M doxycycline hyclate, the fluorescence intensity of the solution was measured according to the experimental methods shown in 2.4.

As a contrast, the HPLC method was performed. An appropriate amount of powder was weighed into a 100-mL volumetric flask and dissolved to the mark with 0.01 M HCl solution; after filtering the solution, 5 mL continued filtrate was added into a 50-mL volumetric flask and diluted to the mark with 0.01 M HCl solution to obtain the solution containing 0.1 mg mL^{-1} doxycycline hyclate. Finally, the obtained solution was measured according to the HPLC method [40].

3. Results and discussion

3.1. Fluorescence spectra of the system

As shown in Fig. 2, the fluorescence intensities of tetracycline antibiotics and the polymer of Zn(bix) alone were very weak, and the emission peaks of TC, DC, CTC and OTC were located at 507, 511, 421 and 504 nm, respectively. However, the fluorescence intensities of TCs were significantly enhanced after the interaction with Zn(bix) with their fluorescence peaks shifted to 505, 502, 513 and 495 nm, correspondingly. Furthermore, the fluorescence intensities of the four systems were increased proportionally with the concentrations of TCs in a certain range, indicating that Zn(bix) could be employed for the determination of TCs. The insets of Fig. 2 displayed that the green fluorescence of TCs–Zn(bix) ternary complexes was visible under 365 nm UV lamp light, and hence, this method could also be used for visual detection of TCs.

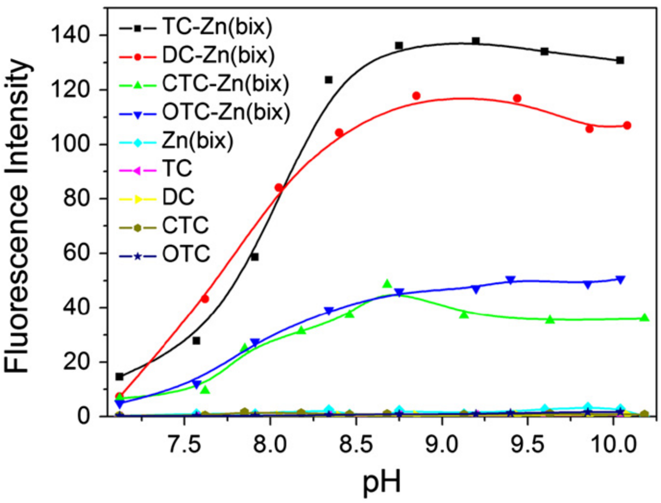


Fig. 3. Effect of acidity on the fluorescence intensities of the four systems. c_{TCs}, 4.0×10^{-6} M.

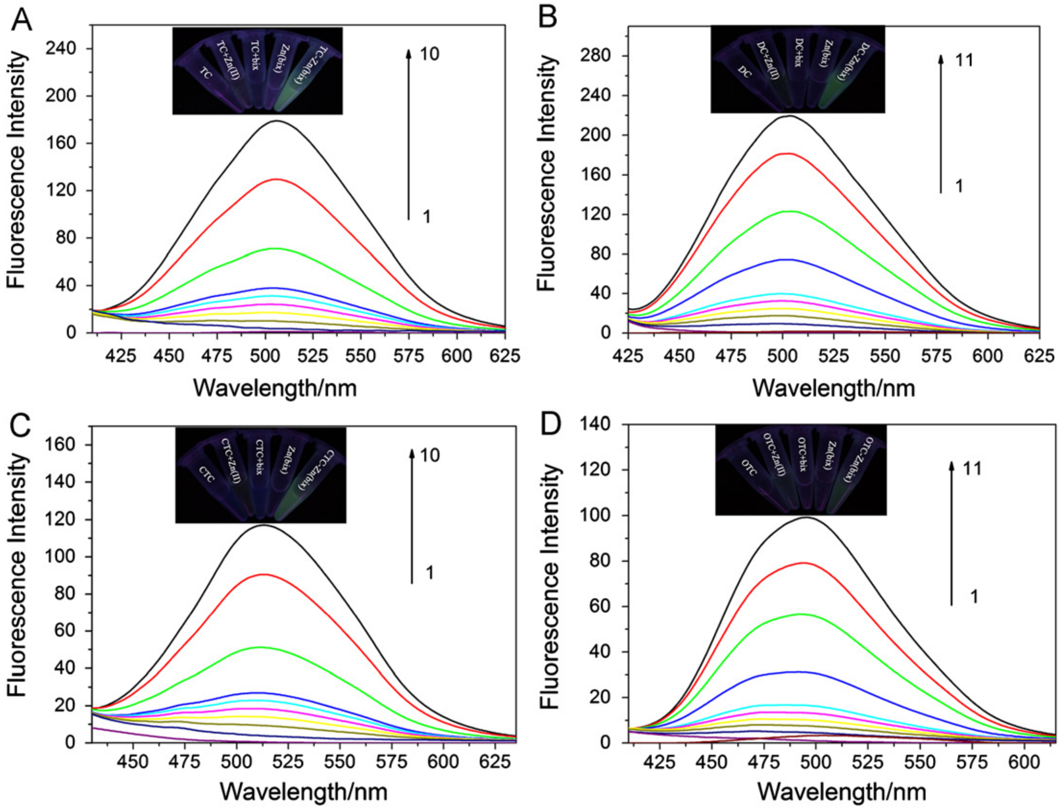


Fig. 2. Fluorescence spectra of the TC–Zn(bix) (A), DC–Zn(bix) (B), CTC–Zn(bix) (C) and OTC–Zn(bix) (D) systems. (A) TC–Zn(bix), curve 1: TC (6.0 μM); curve 2: Zn(bix); curves 3–10: TC–Zn(bix), c_{TC}(3–10) μM^{-1}: 3. 0.2; 4. 0.4; 5. 0.6; 6. 0.8; 7. 1.0; 8. 2.0; 9. 4.0; 10. 6.0. pH 9.00 (Tris–HCl); c_{NaCl}, 0.120 M. (B) DC–Zn(bix), curve 1: DC (8.0 μM); curve 2: Zn(bix); curves 3–11: DC–Zn(bix), c_{DC}(3–11) μM^{-1}: 3. 0.2; 4. 0.4; 5. 0.6; 6. 0.8; 7. 1.0; 8. 2.0; 9. 4.0; 10. 6.0; 11. 8.0. pH 9.25 (Tris–HCl); c_{NaCl}, 0.020 M. (C) CTC–Zn(bix), curve 1: CTC (6.0 μM); curve 2: Zn(bix); curves 3–10: CTC–Zn(bix), c_{CTC}(3–10)μM^{-1}: 3. 0.2; 4. 0.4; 5. 0.6; 6. 0.8; 7. 1.0; 8. 2.0; 9. 4.0; 10. 6.0. pH 8.68 (Tris–HCl); c_{NaCl}, 0.050 M. (D) OTC–Zn(bix), curve 1: OTC (8.0 μM); curve 2: Zn(bix); curves 3–11: OTC–Zn(bix), c_{OTC} (3–11) μM^{-1}: 3. 0.2; 4. 0.4; 5. 0.6; 6. 0.8; 7. 1.0; 8. 2.0; 9. 4.0; 10. 6.0; 11. 8.0. pH 9.40 (Tris–HCl); c_{NaCl}, 0.016 M. The inset shows fluorescence pictures of TCs, TCs–Zn(II), TCs–bix, Zn(bix) and TCs–Zn(bix) from left to right under 365 nm UV light irradiation. c_{TCs}, 4.0×10^{-6} M.

3.2. Optimum reaction conditions

3.2.1. Effect of acidity

We carried out the reaction in the Tris–HCl buffer solution and the effect of acidity on the fluorescence intensities of the four systems was tested under the same ionic strength and the results are shown in Fig. 3. The fluorescence intensities of the four systems reached the strongest and remained stable in the range of pH 8.5–10.0. This may be due to the fact that TCs produced strong fluorescence under basic conditions [17]. In order to obtain high fluorescence intensities with a lower detection limit for TCs, the optimum pH values for determining TC, DC, CTC and OTC were set at 9.00, 9.25, 8.68 and 9.40, respectively for further experiments.

3.2.2. Effect of ionic strength

The influence of ionic strength on the fluorescence intensities was studied by adding the aqueous solution of NaCl to the systems (as shown in Fig. 4) and the experimental results indicated that ionic strength affected the fluorescence intensities of the four systems obviously. As is known, bix can react with Zn(II) to form Zn(bix) coordination polymers [30], and Cl^- at a certain concentration neutralized partial charges on the Zn(bix) coordination polymers, which made Zn(bix) aggregate in a certain degree. The aggregations of Zn(bix) increased the microenvironment viscosity of the systems, and then inhibited the free rotations of TCs, leading to the enhancement of the fluorescence intensities. So, 0.120, 0.020, 0.016 and 0.050 M were chosen as the optimum NaCl concentrations for the TC–Zn(bix), DC–Zn(bix), CTC–Zn(bix) and OTC–Zn(bix) systems, respectively.

3.2.3. Effect of temperature

As shown in Fig. 5, high reaction temperature decreased the fluorescence intensities of the four systems, which was ascribed to the fact that high temperature destroyed the structure of TCs–Zn(bix) complexes, leading to the dissociation of the TCs–Zn(bix) complexes. Hence, the reaction temperatures of the TC–Zn(bix), DC–Zn(bix), CTC–Zn(bix) and OTC–Zn(bix) systems were 25, 30, 40 and 20 °C, respectively.

3.2.4. Effect of incubation time

It was found that the fluorescence intensities of the TCs–Zn(bix) systems reached the maximum rapidly, and could be very stable within 60 min. Accordingly, 5 min was enough to meet the needs of the experiment.

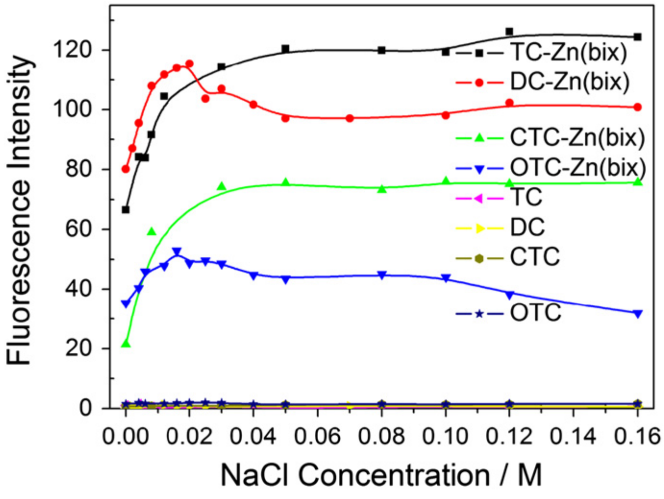


Fig. 4. Effect of ionic strength on the fluorescence intensities of the four systems. c_{TCs}, 4.0×10^{-6} M, the buffer solutions of TC–Zn(bix), DC–Zn(bix), CTC–Zn(bix) and OTC–Zn(bix) systems are Tris–HCl pH 9.00, pH 9.25, pH 8.68 and pH 9.40, respectively.

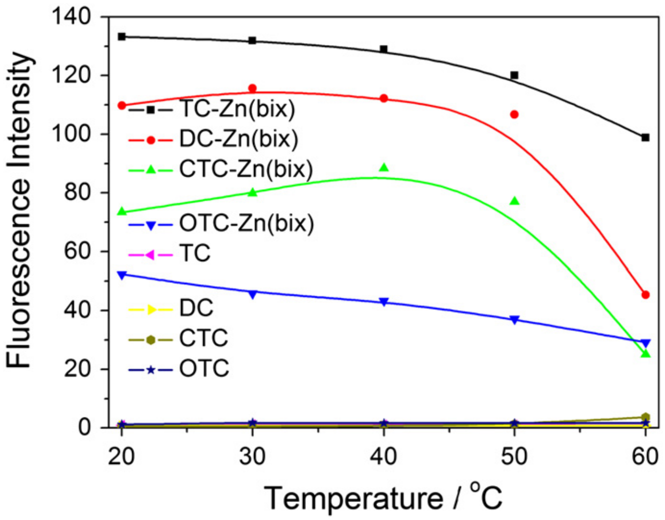


Fig. 5. Effect of temperature on the fluorescence intensities of the four systems: TC–Zn(bix), pH 9.00 (Tris–HCl), c_{NaCl}, 0.120 M; DC–Zn(bix), pH 9.25 (Tris–HCl), c_{NaCl}, 0.020 M; CTC–Zn(bix), pH 8.68 (Tris–HCl), c_{NaCl}, 0.050 M; and OTC–Zn(bix), pH 9.40 (Tris–HCl), c_{NaCl}, 0.016 M. c_{TCs}, 4.0×10^{-6} M.

3.3. Relationship between the fluorescence intensities and the TCs concentrations

Under optimum conditions, the fluorescence intensities of the four TCs in the presence of Zn(bix) were measured, and then the calibration curves of fluorescence intensities against the concentrations of tetracycline antibiotics are constructed in Table 1. It showed that this method for the determination of the four TCs had a very high sensitivity. The sensitivity of DC was the highest and its detection limit was 6.0 nM. Therefore, the method was very suitable for the determination of trace amounts of tetracycline antibiotics.

3.4. Mechanism of the interaction between TCs and Zn(bix) coordination polymer

Owing to similar structures of four tetracycline antibiotics, we took tetracycline hydrochloride (TC) as an example to study the interaction between TCs and Zn(bix) coordination polymer.

The absorption band of TC was centered at 372 nm (Fig. 6), and its weak fluorescence emission was at 507 nm (Fig. 7). It was obvious that bix had a negligible effect on the absorption spectra and fluorescence spectra of TC. In contrast, the interaction between TC and Zn(II) caused a red shift of the maximum absorption peak of TC from 372 to 386 nm and certain enhancements of the absorbance. At the same time, the fluorescence emission peak of TC moved from 507 to 514 nm and the fluorescence intensity was significantly enhanced owing to the formation of the Zn(II)–TC complex [41,42]. However, when TC reacted with Zn(bix), on one hand, the maximum absorption peak of the system moved from 386 to 383 nm and the peak shape changed from flat to sharp, whereas on the other hand, the fluorescence intensity was further enhanced and the fluorescence emission peak moved from 514 nm to 505 nm. Neither the absorption spectra nor the fluorescence spectra were recovered to those of TC alone, indicating that a ternary complex of TC–Zn(bix) was formed, not just a competition between TC and bix for Zn(II).

In addition, in order to further study the interaction between TC and Zn(bix), circular dichroism spectra of the TC–Zn(bix) system were investigated (Fig. 8). The BCD-ring chromophores of TC were

Table 1
Correlation coefficients and linear ranges of the calibration graphs and the detection limits for antibiotics.

System	Excitation/emission wavelength (nm)	Linear regression equation (μM)	Detection limits $3\sigma/k$ (nM)	Linear range (μM)	Correlation coefficient r
TC–Zn(bix)	$\lambda_{ex}/\lambda_{em}=364/505$	$F=3.84+34.00c$	12.0	0.2–6.0	0.9980
CTC–Zn(bix)	$\lambda_{ex}/\lambda_{em}=369/513$	$F=4.88+22.30c$	19.0	0.2–6.0	0.9990
DC–Zn(bix)	$\lambda_{ex}/\lambda_{em}=382/502$	$F=2.94+31.94c$	6.0	0.2–8.0	0.9932
OTC–Zn(bix)	$\lambda_{ex}/\lambda_{em}=338/495$	$F=2.26+13.31c$	19.0	0.2–8.0	0.9975

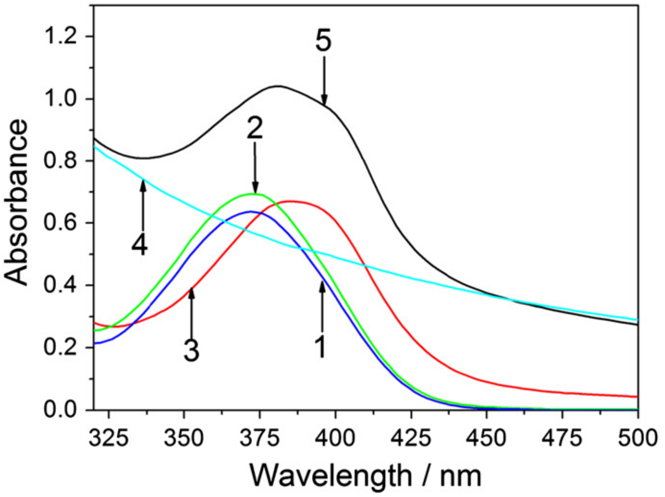


Fig. 6. Absorption spectra of the TC–Zn(bix) system. Curve 1: TC; curve 2: TC–bix; curve 3: TC–Zn(II); curve 4: Zn(bix); and curve 5: TC–Zn(bix). c_{TC}, 1.0×10^{-4} M; c_{bix}, 1.0×10^{-3} M; $c_{Zn(II)}$, 1.0×10^{-3} M; pH 9.00 (Tris–HCl).

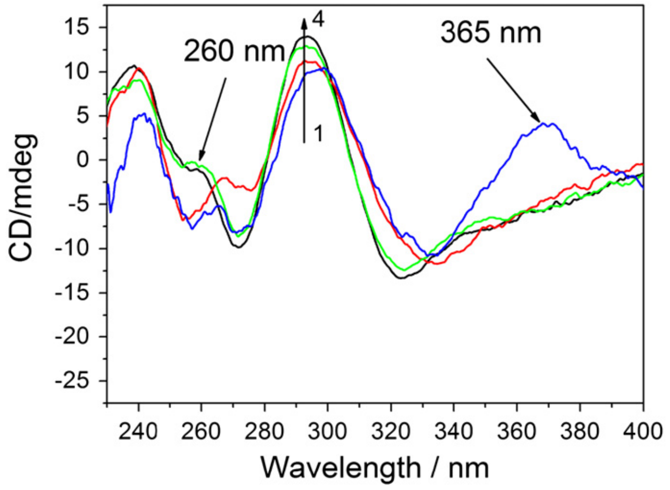


Fig. 8. Circular dichroism spectra of the TC–Zn(bix) system. Curve 1: TC–Zn(bix); curve 2: TC–Zn(II); curve 3: TC–bix; and curve 4: TC. c_{TC}, 1.0×10^{-4} M; c_{bix}, 1.0×10^{-3} M; $c_{Zn(II)}$, 1.0×10^{-3} M; pH 9.00 (Tris–HCl).

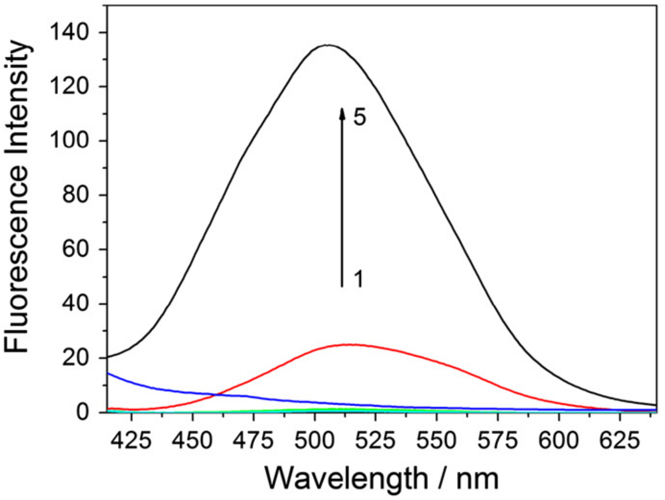


Fig. 7. Fluorescence spectra of the TC–Zn(bix) system. Curve 1 TC; curve 2 TC-bix; curve 3 Zn(bix); curve 4 TC–Zn(II); curve 5 TC–Zn(bix). c_{TC}, 4.0×10^{-6} M; c_{bix}, 1.0×10^{-3} M; $c_{Zn(II)}$, 1.0×10^{-3} M; pH 9.00 (Tris–HCl); c_{NaCl}, 0.120 M. Ex=364 nm.

Table 2
Effect of co-existing substance.

Foreign substances	Allowed multiple	Relative error (%)	Foreign substances	Allowed multiple	Relative error(%)
Amylum	100	−1.95	Arginine	50	−3.40
Saccharose	100	2.54	Serine	50	4.04
Dextrin	100	−7.24	Ca^{2+} (Cl^{2-})	50	−6.71
Glucose	100	6.64	Na^{+} (Cl^{-})	100	3.63
Lactose	100	−1.86	K^{+} (Cl^{-})	100	5.20
Sodium dodecylsulfate	100	7.77	Mg^{2+} (Cl^{-})	25	3.61
Tartaric acid	100	11.18	Ba^{2+} (Cl^{-})	25	−10.80
Threonine	50	2.11	Al^{3+} (SO_4^{2-})	25	−10.84
Histidine	50	4.78	Cu^{2+} (Cl^{-})	5	−10.87
Serine	50	1.65	Zn^{2+} (SO_4^{2-})	100	−12.98
Phenylalanine	50	3.49	Fe^{3+} (Cl^{-})	1	−21.11
Glutamic acid	50	3.95	Mn^{2+} (SO_4^{2-})	1	−62.88
Arginine	50	7.54			

$c_{DC}=4.0\times10^{-6}$ M.

responsible for the bands at 360, 320, 285, and 225 nm, while the A-ring chromophore contributed to the 262 nm band [43]. For the case of TC, the peak at 365 nm was not obvious and the signal peak at 260 nm was attributable to the A-ring chromophore. Bix could not cause an obvious change on the circular dichroism spectra of TC, suggesting that bix did not affect the conformation of TC. Nevertheless, the peak of TC at 260 nm became negative in the presence of Zn(II), which confirmed that Zn(II) could bind to the A-ring of TC leading to the striking conformation change of TC. Interestingly, when TC was mixed with Zn(bix), besides the negative band at 260 nm, a new positive peak appeared at 365 nm, indicating that the interaction between TC and Zn(bix) also occurred at the BCD-ring groups. The obvious change of the peak at 260 nm and 365 nm suggested that the conformation of the TC was changed, which also indicated that the binding of Zn(II) in Zn(bix) to TC was located at both the A-ring and BCD-ring. As a consequence, the conformational rotation of TC molecules was restricted, resulting in significant enhancement of the fluorescence intensity of TC.

3.5. *Analytical application*

Taking DC as an example, the effects of some common metal ions, amino acids and sugars on the determination of DC were

Table 3
Determination results of doxycycline hyclate tablets.

Samples	Labeled amount (mg/ tablet)	Determined (mg/tablet)		RSD (%)	
		HPLC method (n=3)	This method (n=5)	HPLC method	This method
1	100	120.5, 120.6, 121.1	101.3, 98.2, 98.2, 100.9, 96.7	0.23	2.01
2	100	115.7, 115.6, 115.7	95.6, 99.3, 106.7, 99.2, 98.6	0.01	4.08
3	100	110.7, 110.6, 110.6	103.0, 98.6, 108.2, 102.6, 103.5	0.06	3.29

investigated (Table 2). It can be seen that amino acids, sugars, Na^+, K^+, Mg^{2+}, Ba^{2+}, Al^{3+}, Zn^{2+} and Ca^{2+} resulted in no characteristic change in the fluorescence spectra even at a concentration of 25 equivalent of DC. Meanwhile, the fluorescence intensities of the system decreased obviously in the presence of Mn^{2+} and Fe^{3+}, which may be attributed to the coordination of TCs with them [42,44], decreasing the interaction between TCs and Zn(bix). Since the contents of Mn^{2+} and Fe^{3+} were very low in doxycycline hyclate tablets, their effects on the determination of DC could be ignored. So, the coordination polymer of Zn(bix) could be used for determination of doxycycline hyclate tablets.

In order to evaluate the application of the proposed method to real samples, we measured the content of DC in commercial doxycycline hyclate tablets from three factories, together with the results obtained by HPLC method according to “Pharmacopoeia of the People’s Republic of China” (2010 edition). The determined results listed in Table 3 showed high accuracy and good repeatability of this method.

4. Conclusions

In summary, we had successfully employed a metal–organic coordination polymer of Zn(bix) to visually detect tetracycline antibiotics (TCs). Briefly, by adding the coordination polymer of Zn(bix) to the solutions of TCs, the fluorescence of the TCs was turned on, and thus, a new fluorimetric method for the determination of tetracycline antibiotics was developed. This method was simple, fast and sensitive. Furthermore, it had been used for the determination of doxycycline hyclate in doxycycline hyclate tablets with satisfactory results. In our opinion, more efforts can be taken to design and construct metal–organic coordination polymers and to find their potential applications in the fields of analysis and sensing.

Acknowledgments

The authors are grateful to the National Natural Science Foundation of China (NSFC) (No. 21175109) and the special fund of Chongqing key laboratory (CSTC) for the financial support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.01.039.

References

[1] A. Onal, Food Chem. 127 (2011) 197–203.
[2] H. Oka, Y. Ito, H. Matsumoto, J. Chromatogr. A 882 (2000) 109–133.
[3] P. Masawat, J.M. Slater, Sens. Actuators B-Chem. 124 (2007) 127–132.
[4] M.F. Tavares, V.L. McGuffin, J. Chromatogr. A 686 (1994) 129–142.
[5] P. Vinas, N. Balsalobre, C.L. Erroz, M.H. Cordoba, J. Chromatogr. A 1022 (2004) 125–129.
[6] K. Granelli, C. Elgerud, A. Lundstrom, A. Ohlsson, P. Sjoberg, Anal. Chim. Acta 637 (2009) 87–91.
[7] J.L. Chen, K.H. Hsieh, Electrophoresis 31 (2010) 3937–3948.
[8] M.G. Pikkemaat, Anal. Bioanal. Chem. 395 (2009) 893–905.
[9] A.L. Myllyniemi, R. Rannikko, E. Lindfors, A. Niemi, C. Backman, Food Addit. Contam. 17 (2000) 991–1000.
[10] A.L. Myllyniemi, L. Nuotio, E. Lindfors, R. Rannikko, A. Niemi, C. Backman, Analyst 126 (2001) 641–646.
[11] Y.J. Kim, Y.S. Kim, J.H. Niazi, M.B. Gu, Bioprocess Biosyst. Eng. 33 (2010) 31–37.
[12] L. Zhou, D.J. Li, L. Gai, J.P. Wang, Y.B. Li, Sensors Actuators B–Chem. 162 (2012) 201–208.
[13] H.T. Wang, H.M. Zhao, X. Quan, S. Chen, Electroanalysis 23 (2011) 1863–1869.
[14] T. Le, H. Yu, Z.W. Zhao, W. Wei, Anal. Lett. 45 (2012) 386–394.
[15] A. Javier, F. Fatima, S.B. Francisco, M.M. Pilar, J. Agric. Food Chem. 60 (2012) 3837–3846.
[16] K.W. Kohn, Anal. Chem. 33 (1961) 862–866.
[17] H. Poiger, C. Schlatter, Analyst 101 (1976) 808–814.
[18] L.M. Shen, M.L. Chen, X.W. Chen, Talanta 85 (2011) 1285–1290.
[19] S.M. Zhao, S.Q. Han, J. Chin. Chem. Soc. 57 (2010) 1353–1360.
[20] Y. Fu, P. Li, L. Bu, T. Wang, Q. Xie, J. Chen, S. Yao, Anal. Chem. 83 (2011) 6511–6517.
[21] H. Kim, M. Yoon, G.H. Lee, D.H. Lee, J. Shim, K. Kim, G. Park, Inorg. Chem. Commun. 21 (2012) 137–141.
[22] E. Barea, G. Tagliabue, W.G. Wang, M. Perez-Mendoza, L. Mendez-Linan, F.J. Lopez-Garzon, S. Galli, N. Masciocchi, J.A.R. Navarro, Chem. Eur. J. 16 (2010) 931–937.
[23] A. Comotti, S. Bracco, P. Sozzani, S. Horike, R. Matsuda, J. Chen, M. Takata, Y. Kubota, S. Kitagawa, J. Am. Chem. Soc. 130 (2008) 13664–13672.
[24] S.Y. Zhang, Q.H. Liu, M. Shen, B.W. Hu, Q. Chen, H.X. Li, J.P. Amoureux, Dalton Trans. 41 (2012) 4692–4698.
[25] T.J. Ni, F.F. Xing, M. Shao, Y.M. Zhao, S.R. Zhu, M.X. Li, Cryst. Growth Des. 11 (2011) 2999–3012.
[26] C. Zhang, Y. Che, Z. Zhang, X. Yang, L. Zang, Chem. Commun. 47 (2011) 2336–2338.
[27] Z. Xie, L. Ma, K.E. deKrafft, A. Jin, W. Lin, J. Am. Chem. Soc. 132 (2010) 922–923.
[28] W.J. Rieter, K.M. Pott, K.M.L. Taylor, W. Lin, J. Am. Chem. Soc. 130 (2008) 11584–11585.
[29] R.C. Huxford, K.E. deKrafft, W.S. Boyle, D. Liu, W. Lin, Chem. Sci. 3 (2012) 198–204.
[30] I. Imaz, J. Hernando, D.R. Molina, D. Maspoch, Angew. Chem. Int. Ed. 48 (2009) 2325–2329.
[31] L. Carlucci, G. Ciani, D.M. Proserpio, Cryst. Growth Des. 5 (2005) 37–39.
[32] I. Imaz, D.R. Molina, J. Hernando, V. Puntesa, D. Maspoch, Chem. Commun. 46 (2010) 4737–4739.
[33] B.F. Hoskins, R. Robson, D.A. Slizys, Angew. Chem. Int. Ed. 36 (1997) 2336–2338.
[34] S.K. Chawla, B.K. Gill, Polyhedron 16 (1997) 1315–1322.
[35] L.J. Liang, S.J. Zhen, X.J. Zhao, C.Z. Huang, Analyst 137 (2012) 5291–5296.
[36] X.J. Zhao, H.J. Wang, L.J. Liang, Y.F. Li, Spectrochim. Acta Part A 103 (2013) 68–72.
[37] B.F. Hoskins, R. Robson, D.A. Slizys, J. Am. Chem. Soc. 119 (1997) 2952–2953.
[38] P.K. Dhal, F.H. Arnold, Macromolecules 25 (1992) 7051–7059.
[39] I. Imaz, D. Maspoch, C.R. Blanco, J.M.P. Falc, J. Campo, D.R. Molina, Angew. Chem. Int. Ed. 120 (2008) 1883–1886.
[40] C.P. Commission, in: W. Wang, P.L. Gao, H.M. He, L.P. Cui, H.L. Song (Eds.), Pharmacopoeia of the People’s Republic of China (b), China Medicine Science, Beijing, 2010, pp. 691–692.
[41] J.Y. Lee, G.W. Everett, J. Am. Chem. Soc. 103 (1981) 5221–5225.
[42] T. Sakaguchi, M. Toma, T. Yoshida, H. Omura, H. Takasu, Chem. Pharm. Bull. 6 (1958) 1–9.
[43] L.A. Mitscher, A.C. Bonacci, T.D. Sokolosk, Tetrahedron Lett. 9 (1968) 5361–5364.
[44] M.A. Ghandour, H.A. Azab, A. Hassan, A.M. Ali, Monatsh. Chem. 123 (1992) 51–58.