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RESEARCH ARTICLE

Development of an optical sensor for chlortetracycline detection based on the fluorescence quenching of L-tryptophan

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A simple and selective spectrofluorimetric method for the detection of chlortetracycline (CTC) was studied. In pH 7.4 buffer medium L-tryptophan (L-Trp), applied as the fluorescence probe, interacted with CTC resulting in fluorescence quenching of the probe. CTC was detected with maximum excitation and emission wavelengths at $\lambda_{ex}/\lambda_{em}$ = 275/350 nm. Notably, quenching of fluorescence intensities was positively proportional to the CTC concentration over the range of 0.65–30 μmol L^{-1} and the limit of detection was 0.2 μmol L^{-1}. Effect of temperature shown in Stern–Volmer plots, absorption spectra and fluorescence lifetime determination, indicated that fluorescence quenching of L-Trp by CTC was mainly by static quenching. The proposed study used practical samples analysis satisfactorily.

KEYWORDS

chlortetracycline, detection, fluorescence quenching, L-tryptophan

1 | INTRODUCTION

Chlortetracycline (CTC) belongs to the broad-spectrum antibiotic family of tetracyclines which is widely utilized as veterinary medicine for poultry and swine production in the USA.[1,2] It is also frequently used in the animal industry for controlling, preventing and treating animal health issues and to increase the animal's growth rate.[3] As CTC is not absorbed completely by the animal, there is great concern over potential life-threatening effects such as manure-derived CTC finding its way into biological systems from surface water, or into rivers by ground water runoff. The selection of resistant microorganism, which may cause a serious public health concern for humans is also a concern.[4–6] Antibiotic-resistant organisms must be controlled or removed from water and wastewater to prevent potential problems for the general public.[7–9] International rules and legislation on maximum residue limits and dosages have been set to protect the environment against the risks of related CTC abuse.[10–12] To monitor CTC and study its fate in the environment such as in manure, a fast and simple analytical method to detect chlortetracycline is needed.

Currently, there are many analytical techniques that can be used to detect CTC, these are mainly electrochemical[13–16] plus high-performance liquid chromatography (HPLC),[17–20] ultraviolet visible (UV) spectroscopy,[21,22] enzyme-linked immunosorbent assay (ELISA),[23] and capillary electrophoresis.[24,25] Electrochemical techniques offer simplicity, low cost, high-efficiency, selectivity and mechanical stability, however the production of a functionalized electrode is a lengthy process. Chromatography offers sensitive and specific multi-analytical results, but is time consuming and relies on highly skilled personnel and expensive equipment. Immunosorbent assays are reproducible and cost effective, but demand several complicated procedures to gain the antibody. Capillary electrophoresis is fast and effective, yet lacks reproducibility.[26] Thus there is a great need for a simple, sensitive and cost-efficient technique for CTC detection.

Here, we describe L-Trp as a fluorescent sensor for highly sensitive and selective detection of CTC under optimum conditions through fluorescence quenching methodology. The chemical structures of CTC and L-Trp are shown in Figure 1 and the simple detection strategy is presented in Scheme 1. Fluorescence intensity was proportional to CTC concentration within a certain range and had a low limit of detection. Furthermore, the proposed method was used to determine CTC with favourable results in tap water and milk samples.

Abbreviations used: BR, Britton–Robinson; CTC, chlortetracycline; UV, ultraviolet visible.

2 | EXPERIMENTAL

2.1 | Instruments

All steady-state fluorescence measurements were carried out using a Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) and fluorescence spectra were recorded with the slits (Ex/Em) set at 5.0/5.0 nm. A FL-TCSPC Fluorolog-3 fluorescence spectrometer (Horiba Jobin Yvon Inc., France) was used to measure fluorescence emission decay curves in the systems. In addition, a UV-2450 spectrophotometer (Shimadzu, Japan) was applied to collect the absorption spectrum. An S-3C pH meter (Shanghai Scientific Instrument Company, China) was used to adjust the pH values of the aqueous solutions.

2.2 | Chemicals

The stock solutions of 4.0×10^{-4} mol L^{-1} L-tryptophan (L-Trp) and 1.0×10^{-4} mol L^{-1} chlortetracycline hydrochloride (CTC) were both made up and maintained at 4°C, the working solution was freshly prepared by diluting the stock solution to lower concentrations. All materials were purchased from commercial suppliers and utilized directly without further purification. The Britton–Robinson (BR) buffer solution was used to adjust the acidity in this whole experiment and aqueous solutions were prepared using deionized water (Milli-Q system) throughout the study.

2.3 | Recommended procedure for spectrofluorimetric determination of CTC

To a 10.0 ml colorimetric tube was added BR buffer solution (pH = 7.4, 1 ml), 0.5 ml of 4.0×10^{-4} mol L^{-1} L-Trp solution and a suitable aliquot of CTC, the mixture was diluted to the mark with double-distilled water. The solutions were thoroughly mixed at room temperature 25(± 5)°C and the fluorescence intensity was measured after 10 min, recording at $\lambda_{ex}/\lambda_{em}$ = 275 nm/350 nm. The relative fluorescence intensity (ΔF) of this system was calculated using the equation $\Delta F = F_0 - F$, where F_0 and F were the fluorescence intensities of the reagent blank and the mixture, respectively.

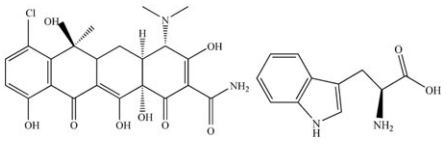


FIGURE 1 The chemical structure of CTC (left) and L-Trp (right)

3 | RESULTS AND DISCUSSION

3.1 | Fluorescence spectra

Figure 2 shows the excitation and emission spectra. L-Trp exhibited quite strong inner fluorescence, and its excitation and emission wavelengths were close at 275 and 350 nm, respectively. L-Trp characteristic spectra remained almost the same when L-Trp was reacted with CTC, but the fluorescence was gradually quenched. Figure 2 shows the noticeable changes in the fluorescence spectra when different concentrations of CTC were added to the solution containing L-Trp. It was noted that when different concentrations of CTC were added to the flask, there was a marked decrease in the sensors' fluorescence intensity. Furthermore, signaling quenching intensity was proportional to the amount of CTC and remained linear, suggesting that the L-Trp could act as a fluorescent probe to quantitatively detect CTC.

3.2 | Effect of pH

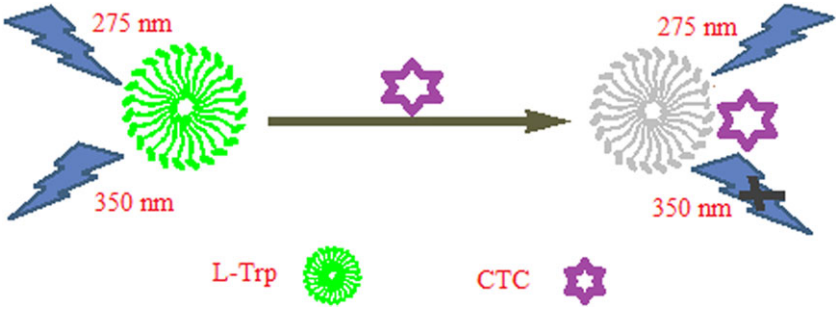
The influence of acidity upon the fluorescence response to the reaction products was investigated by conducting experiments containing a series of different acidity BR buffer solutions. As shown in Figure 3, changes in ΔF were highest within the pH range 7.0–7.8, but fluorescence intensity was maximum at higher pH. Thus a buffer at pH 7.4 was selected as the reaction medium for further experiments.

3.3 | Effect of fluorescence probe concentration

The influence of L-Trp concentration (range $0.5–6 \times 10^{-5}$ mol L^{-1}) was investigated. Results indicated that fluorescence intensity of L-Trp increased with increasing concentration, but there was no maximum ΔF signal in the L-Trp–CTC system. Therefore, the appropriate suitable concentration of L-Trp for analysis was 2×10^{-5} mol L^{-1} was selected in this experiment for the next experiments.

3.4 | Effect of reaction time and stability

Changes in reaction time and stability of the fluorescence intensity were also studied. As shown from the fluorescence spectra, the fluorescence intensity remained almost constant at room temperature



SCHEME 1 Schematic diagram for the detection of CTC by a L-Trp probe

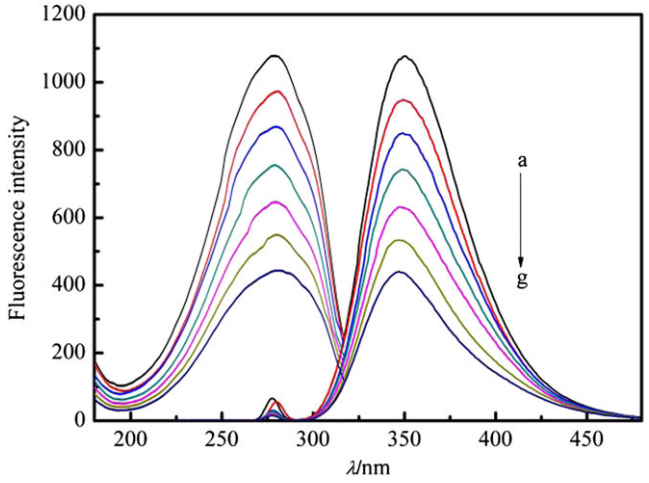


FIGURE 2 Fluorescence excitation and emission spectra. C_{L}-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} (a–g) = (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0) × 10^{-5} mol L^{-1}, respectively, pH = 7.4

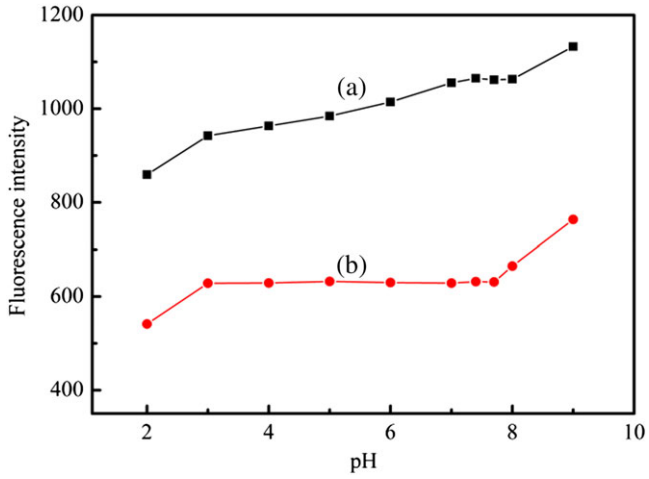


FIGURE 3 Effect of pH on fluorescence intensity in the L-Trp–CTC system. A: L-Trp; b: L-Trp- CTC. C_{L}-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} = 2.0 × 10^{-5} mol L^{-1}, pH = 7.4

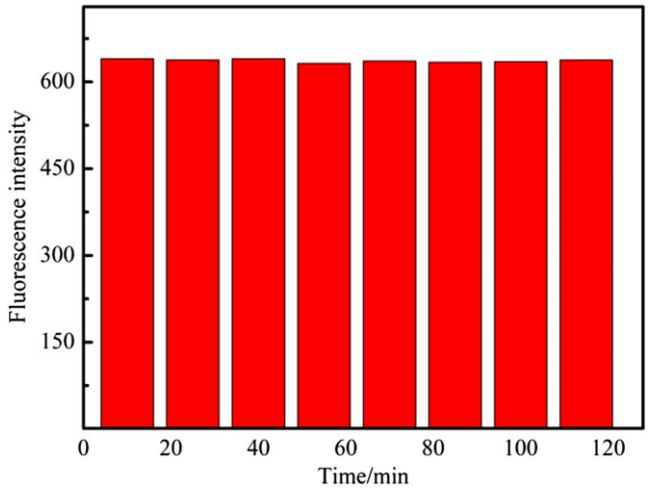


FIGURE 4 Stability of the system. C_{L}-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} = 2.0 × 10^{-5} mol L^{-1}, pH = 7.4

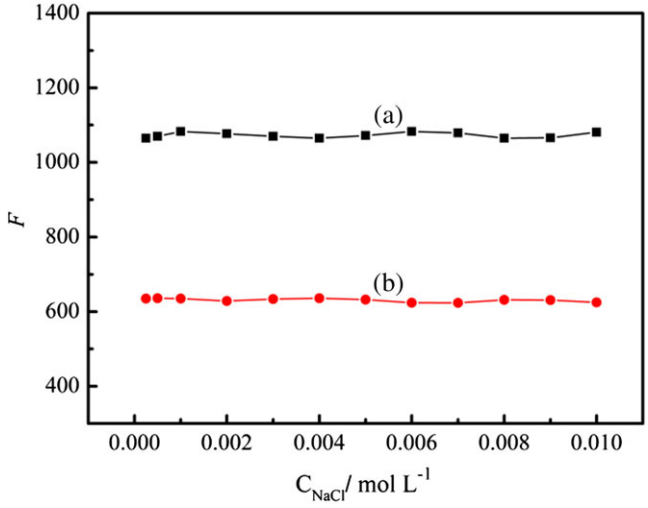


FIGURE 5 Effect of ionic strength. A: L-Trp; b: L-Trp–CTC, C_{L}-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} = 2.0 × 10^{-5} mol L^{-1}, pH = 7.4

15(±5)°C, with uniform mixing and after 10 min. As shown in Figure 4, the fluorescence signals remained relatively stable at least for 2 h. Therefore to obtain the induced results, the spectra were recorded within 10 min.

3.5 | Effect of ionic strength

The effect of ionic strength on L-Trp–CTC was investigated by adding different concentrations of NaCl solution. As can be seen in Figure 5, Δ*F* varied little and stayed relatively stable with increase in NaCl concentration from 0.00 to 0.01 mol L^{-1}; relative error was less than ±5%. Hence, ionic strength had almost no effect on the fluorescence intensity of the system.

3.6 | Selectivity of the method

To study selectivity of the probe for CTC determination, the effects of foreign coexisting substances were studied by premixing 2.0 × 10^{-5} mol L^{-1} CTC with other substances; the results are displayed in Table 1. As it depicted, some sugars, amino acids, drugs and common ions produced little interference, apart from several high valency cations (such as Cu^{2+} Fe^{3+}), on CTC determination with a relative error of less than ±5%. Moreover, many coexisting objects could be tolerated even at relatively high concentration by the fluorescence intensity of the system. However, some metal ions may influence detection and should be avoided in these processes.

3.7 | Calibration curve

As shown above, the calibration curve was constructed under optimum conditions via a similar set of experiments but by varying the CTC concentration. The corresponding results are listed in Figure 6, and show that there was good linearity of CTC concentration with a correlation coefficient of R^2 = 0.9991. In addition, based on the IUPAC definition, the limit of detection (3σ/k) was 0.2 μmol L^{-1} and the limit of quantification (10σ/k) was 0.65–30 μmol L^{-1} for the L-Trp–CTC

TABLE 1 Effects of foreign coexisting substances (C_{CTC} = 2.0 × 10^{-5} mol L^{-1} = 9.6 μg ml^{-1})

Species	Concentration (μg ml^{-1})	Relative error (%)	Species	Concentration (μg ml^{-1})	Relative error (%)
$ZnSO_4$	455.4	1.6	Ascorbic acid	70.4	3.6
NaF	672	−2.1	L-Aspartic acid	266.2	2.3
$NaNO_2$	166	−4.8	Citric acid	192.1	−1.8
KI	664	−0.9	L-Threonine	238.2	−3.1
$CaCl_2$	87.2	−1.7	D-Sucrose	1949	−2.6
$KAl(SO_4)_2$	228	1.6	Glucose	891	2.1
$NH_4Fe(SO_4)_2$	26.6	3.5	L-Serine	189.2	1.6
$MnSO_4$	111	−4.3	L-Arginine	733.7	1.8
$CuSO_4$	10.9	4.9	Neomycin	80	2.5
NH_4Cl	85.6	−1.8	Ceftriaxone sodium	10.8	−4.9
$MgCl_2$	190	−3.4	Moroxydine	100	3.2
Na_2HPO_4	142	−2.9	Diphenhydramine	160	−2.8

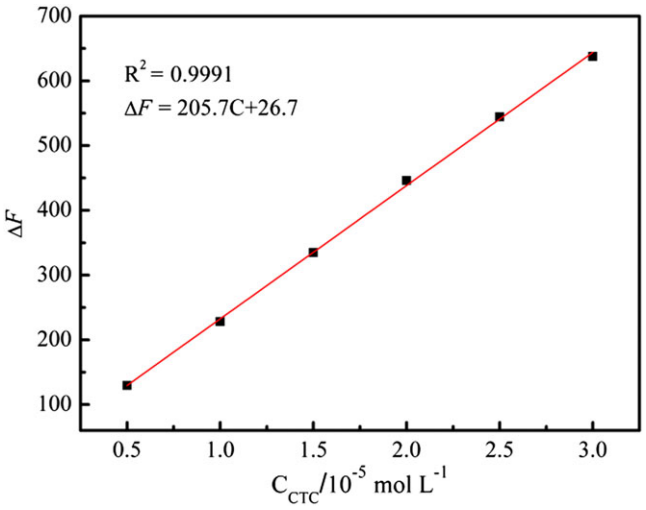


FIGURE 6 Linear correlations of fluorescence intensity towards CTC concentration. CL-T_{rp} = 2.0 × 10^{-5} mol L^{-1}

system. An analytical features comparison of several previous methods with this proposed method for CTC determination is given in Table 2. These other methods had their own merits such as a quite low limit detection, but each method also had some drawbacks such as troublesome pre-preparation processes, high cost and being time consuming. The approach detailed here is simple, rapid, low cost and time efficient and therefore can be widely applied to social need.

3.8 | Proposed mechanism

3.8.1 | Effect of temperature on the quenching constants

Fluorescence quenching behaviours include dynamic and static quenching.[29,30] The former is due to collision between quenching agent and the fluorophor, while the latter results from the formation of a non-luminous complex. Therefore, the quenching mechanism was investigated using the Stern–Volmer equation[31,32]:

$$F_0/F = k_q\tau_0\,[Q] = 1 + K_{SV}\,[Q]$$

where F and F_0 are the fluorescence intensities of L-Trp in the presence or absence of quencher, respectively; k_q is the quenching rate constant, K_{SV} is the Stern–Volmer quenching constant, $k_q = K_{SV}/\tau_0$; τ_0 is the average fluorescence lifetime in the absence of quencher, and $[Q]$ is the concentration of the quencher.[33] There are marked characteristics in quenching mechanisms that can be distinguished by their dependence on temperature. The quenching constant K_{SV} increases distinctly with increased temperature for dynamic quenching, whereas the reverse trend is true for static quenching. Figure 7 shows the Stern–Volmer plots of F_0/F versus $[Q]$, which exhibited a straight line at different temperatures of the system, therefore the L-Trp–CTC interaction was via static quenching.

TABLE 2 Analytical features of several typical methods

Methods	Reagents	Detection limit (μmol L^{-1})	Remarks	Ref.
Electrochemical	Carbon sphere@MnO_2	0.26	Simple, good stability, acceptable repeatability, highly sensitivity and selectivity	[16]
HPLC	Pig faeces	NG	Simple, sensitive, highly selective and suitable for determination	[18]
ELISA/ICA	Monoclonal antibody	0.12	Simple, reproducible, sensitivity, fast, portable and easy-operated	[24]
TRFIA	Europium samarium	0.06	Practical, ultrasensitive, highly selective and cost effective	[27]
Adsorption	Pinewood biochar	NG	Feasibility, reasonable capital scale-up capability and capital and operational costs	[28]
Fluorescence quenching	L-Tryptophan	0.2	Simple, rapid, sensitive, low cost and no need for organic solvent	This work

ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; ICA, immunochromatographic assay; NG, data not given; TRFIA, dual-label time-resolved fluoroimmunoassay.

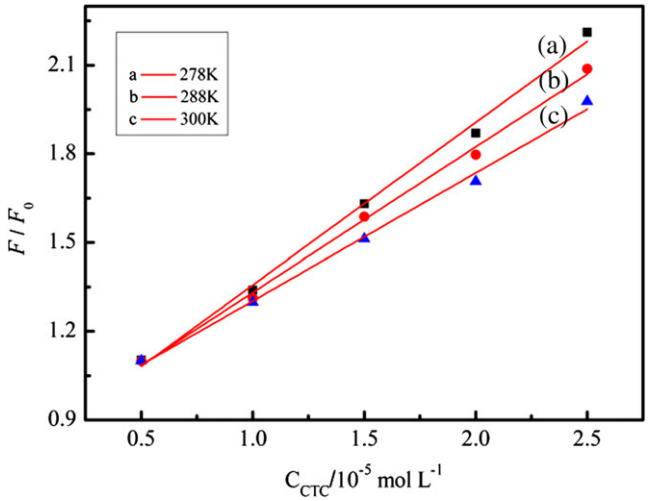


FIGURE 7 Stern–Volmer plots for the system at different temperatures. CL-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} = (0.5, 1.0, 1.5, 2.0, 2.5) × 10^{-5} mol L^{-1}, respectively, pH = 7.4

3.8.2 | The absorption spectra

The absorption spectra were also investigated. As seen from Figure 8, L-Trp (a) had absorbance near 220 nm, and 275 nm; CTC (b) had a clear absorbance at 275 nm and 375 nm and a minor absorption peak at 227 nm. When L-Trp and CTC were mixed together the absorption peak of L-Trp–CTC (c) occurred only at 220 nm, 275 nm and 375 nm, while the minor absorption near at 227 nm had disappeared. Therefore, fluorescence quenching of the L-Trp–CTC system was due to production of a complex.

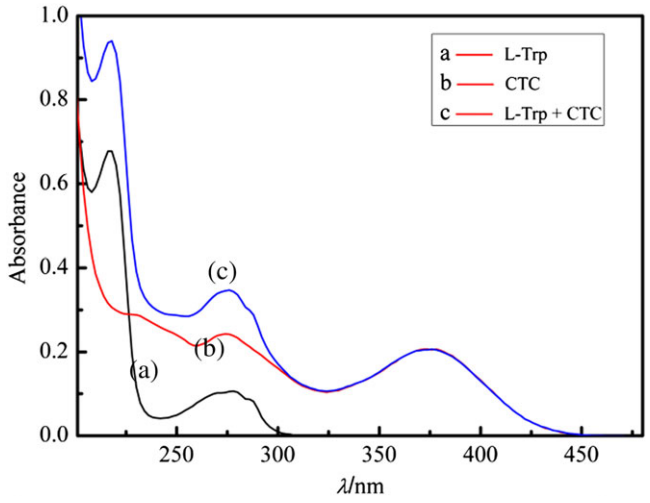


FIGURE 8 Absorption spectra. L-Trp (a), CTC (b), L-Trp–CTC (c), CL-T_{rp} = 2.0 × 10^{-5} mol L^{-1}, C_{CTC} = 1.0 × 10^{-5} mol L^{-1}, pH = 7.4

3.8.3 | The detection of fluorescence lifetime

In addition, to further verify the results, we measured fluorescence lifetime as the most definitive method to distinguish static and dynamic quenching. The lifetime (τ_0) of the fluorescence molecule in its excited state changes little in the presence of quencher during static quenching. Conversely, τ_0 is shorter if dynamic quenching occurs. In static quenching, τ_0/τ = 1, as the quenching medium cannot change the fluorescence lifetime of the excitation state of a fluorescent molecule; whereas $\tau_0/\tau = F_0/F$ of fluorescence lifetime can be reduced during dynamic quenching.[34,35] Fluorescence emission decay curves of L-Trp and L-Trp–CTC system are shown in Figure 9. The fluorescence lifetime of L-Trp (3.15 nsec) and L-Trp–CTC (3.12 nsec) was τ_0/τ = 1, therefore the system underwent static quenching.

3.9 | Determination of CTC in tap water and milk samples

To test the practicality of this method in real samples, recovery tests using tap water and milk samples were performed. Water samples were collected by a routine technique from our laboratory and the obtained samples were stored in 0.5 L polyethylene bottles. Milk samples (Yili milk, 250 ml) were brought from a local supermarket and centrifuged at high speed (12 000 rpm/min) for 10 min in order to remove any insoluble particulates, then diluted 20 times with double-distilled water prior to detection. Both samples were directly determined and the standard addition method was exploited to evaluate the credibility of the proposed method; the results are listed in Table 3. In tap water and milk samples, the range of average recovery was 99.0–100.2% and 98.4–99.9% for CTC determination, respectively. Therefore, this method was feasible for determining CTC in real sample analyses of water and milk.

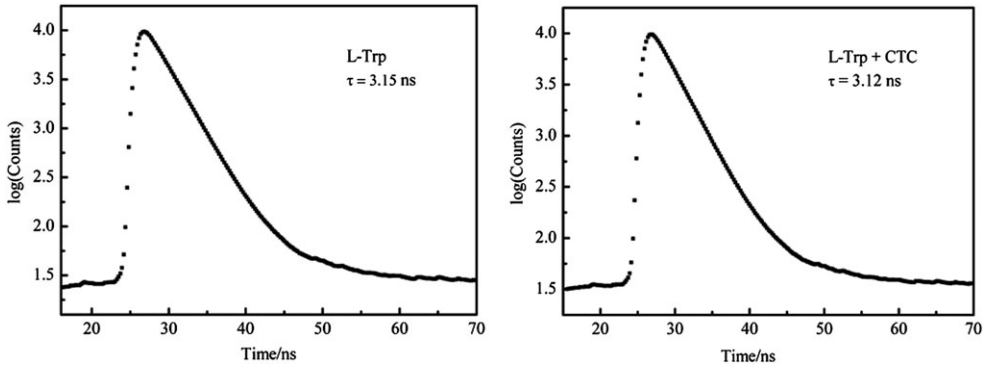


FIGURE 9 Fluorescence emission decay curves of L-Trp and L-Trp–CTC. CL-T_{rp} = 6.0 × 10^{-5} mol L^{-1}, C_{CTC} = 2.0 × 10^{-5} mol L^{-1}, pH = 7.4

TABLE 3 Determination of CTC in tap water and milk samples

Samples	Found (10^{-5} mol L^{-1})	CTC added (10^{-5} mol L^{-1})	Found (n = 5) (10^{-5} mol L^{-1})	Recovery (%)	RSD (%)
Tap water 1	ND	1.0	0.99, 0.97, 0.97, 1.00, 1.02	99.0	2.1
Tap water 2	ND	1.5	1.49, 1.48, 1.51, 1.52, 1.51	100.1	1.6
Tap water 3	ND	2.5	2.51, 2.49, 2.51, 2.52, 2.49	100.2	1.3
Milk 1	ND	1.0	1.01, 1.01, 0.97, 0.99, 1.01	99.8	1.8
Milk 2	ND	1.5	1.47, 1.50, 1.48, 1.47, 1.46	98.4	1.5
Milk 3	ND	2.5	2.49, 2.51, 2.48, 2.53, 2.48	99.9	2.2

ND, not detected; RSD, relative standard deviation.

4 | CONCLUSIONS

A simple, sensitive sensor L-Trp was used to detect the CTC with a significant results. In this approach, L-Trp acted as a fluorescence probe that could be quenched by CTC. There was a good linear relationship with CTC concentration over a certain range. The described strategy could be applied for the parallel detection of CTC in tap water and milk samples. Our study verified the development of a useful protocol for the rapid and efficient determination of CTC in food and aquatic environments.

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