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A sensitive "turn-on" fluorescent assay for quantification of ceftriaxone based on L-tryptophan-Pd(II) complex fluorophore

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ABSTRACT

Based on L-tryptophan-Pd(II) system, a sensitive and selective fluorimetric assay for the quantification of ceftriaxone (CTRX) had been developed. The experimental results showed that in pH 4.0 Britton–Robinson (BR) buffer medium, the fluorescence of L-tryptophan (L-Trp) ($\lambda_{ex}/\lambda_{em}$ = 276 nm/352 nm) could be efficiently quenched by Pd(II). When CTRX was added to the mixed solution of the L-tryptophan and Pd(II), the fluorescence of L-Trp recovered. The reaction mechanism and the reasons for the fluorescence recovery were also discussed. Pd(II) reacted with L-Trp to form a 1:1 chelate complex, and then, after CTRX was added in L-Try-Pd(II) system, the ligand exchange reaction occurred between L-Trp and CTRX, which resulted in the fluorescence recovery. Under the optimized experimental conditions, the recovered fluorescence intensities at 352 nm showed excellent linear relationship with the concentration of CTRX over the range of 6.0×10^{-8}–2.4×10^{-6} mol L^{-1} (0.040–1.59 μg mL^{-1}). The correlation coefficient (R) was 0.9997 and the detection limit was 1.8×10^{-8} mol L^{-1} (11.9 ng mL^{-1}). Furthermore, the assay had been applied to determine trace amount of CTRX human urine samples with satisfactory results.

1. Introduction

Since the discovery of the first major cephalosporin antibiotic by cephalosporin, in 1948, a multitude of other kinds of cephalosporin antibiotics have been gradually discovered, synthesized and used clinically. Ceftriaxone (CTRX) is a member of semisynthetic third-generation cephalosporin antibiotics (the structure of CTRX is shown in Fig. 1). Compared with the first and second-generation cephalosporin, it shows broader spectrum antimicrobial activity, especially more excellent intrinsic efficacy against Gram-negative and gonorrhoeae pathogens. And now it has enjoyed widespread application as chemotherapeutic agents in the treatments of various clinical infection syndromes, such as meningitis, pneumonia, genital infection, respiratory tract infection, urinary tract infection, and gonorrhea. However, due to its heavy use in clinical practice, many strains of bacteria have become resistant to normal doses of CTRX [1–3]. In addition, adverse reactions and toxicological liabilities in the treatment, in particular neurotoxicity, nephrotoxicity, pseudolithiasis, hemolytic anemia and anaphylactic shock become more obvious [4–8]. Thus, it is of great imperative to establish suitable analytical methods for quantitative detection of trace CTRX in biological fluids to ensure human health.

Up to date, a tremendous number of techniques have been described in the published literatures for CTRX detection. These techniques included HPLC [9,10], spectrophotometry [11,12], capillary electrophoresis [13], chemiluminescence [14] and resonance Rayleigh scattering method [15,16]. Although most of these methods have been shown to be high in accuracy and sensitivity, they often require sophisticated instruments, complicated sample pretreatment and well-trained technical personnel. Thus, it is desirable to develop efficient and simple sensing systems for CTRX assay.

Fluorescence spectroscopy, which displays various advantages such as simplicity, high sensitivity and rapid response time, has become a valuable technique for the detection of trace amount of species. But, so far, only a small number of spectrofluorimetric methods have been published for the determination of CTRX [17–21]. What is more, since CTRX is a non-fluorescence complex in aqueous solution, recently, most of reported fluorimetric methods are limited to the following mechanism: (1) rely on the fluorescence quenching ability of CTRX to a fluorescent probe, (2) based on the alkaline degradation, acidic hydrolysis or some corresponding chemical reactions of CTRX to make CTRX convert into fluorescent products. For example, Lories I. Bebawy established a new fluorimetric method for the assay of four cephalosporin antibiotics. In tris buffer solution, these drugs could form a ternary complex with Tb^{3+}, leading to the fluorescence quenching of terbium. The detection limit for CTRX was 5.09×10^{-7} mol L^{-1} [17]. Qu et al. successfully prepared L-cysteine capped ZnS (L-Cys-ZnS) quantum dots (QDs), which

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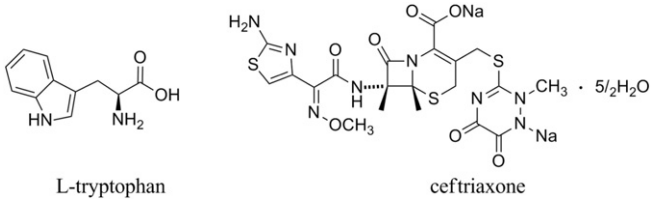

Fig. 1. The structure of L-tryptophan and ceftriaxone.

could be applied as fluorescent probe to determine CTRX based on the fluorescence quenching. The detection limit was 0.05 mg L^{-1} [18]. Mahmoud A. Omar et al. developed a specific kinetic spectrofluorimetric method for the determination of seven cephalosporin antibiotics based on their degradation under an alkaline condition that produced fluorescent substance. The detection limit was 0.3003 μg mL^{-1} [19]. Jasmin Shah et al. reported a fluorimetric method for the quantification of CTRX in bulk powder, pharmaceutical formulations and spiked human plasma. In the method, CTRX was converted into a fluorescent compound by reacting with 0.8 M ethyl acetoacetate and 25% formaldehyde in pH4.2 buffered medium with the detection limit of 1.94×10^{-2} μg mL^{-1} [20]. Two years later, they developed a new and cost-effective method for the determination of CTRX in commercial formulations and spiked human plasma. The method proposed the conversion of ceftriaxone into a fluorescent product by reacting with orthophthalaldehyde in the presence of sulfite. The detection limit was 1.30×10^{-3} μg mL^{-1} [21].

Although these published methods are rapid and specific, they are not sensitive enough. In addition, the mechanism of the methods is limited to the above reaction mechanisms. Herein, we report a "turn-on" fluorescent strategy for the assay of CTRX based on L-tryptophan-Pd(II) complex fluorophore for the first time.

L-Trp (the structure is seen in Fig. 1) is a strong fluorescent compound that can form chelates with some metal ions, such as Cu(II), Co(II), Ni(II), Tb(III), La(III), and Pd(II) [17,22–25]. And some metal ions, including Cu(II), Fe(III), Pd(II)and Ni(II) can react with L-Trp [26,27], resulting in high efficient quenching of fluorescence of L-Trp. Interestingly, recent studies have reported that L-Trp can be deprived of Cu(II) by methionine and adenine, which leads to the fluorescence recovery of L-Trp [27,28]. The recovered fluorescence intensities are linearly proportional to the concentration of these analytes. Herein, our research finds that when CTRX is added into L-Trp-Pd(II) solution, the fluorescence of L-Trp also recovered. This can be attributed to the ligand exchange reaction. As reported, CTRX can react with Pd(II) to form chelate. Since the compound is more stable than L-Trp-Pd(II), L-Trp will be deprived by CTRX. Based on this, a new sensitive "turn-on" spectrofluorimetric method for the assay of CTRX is successfully established. The detection limit is 1.8×10^{-8} mol L^{-1} (11.9 ng mL^{-1}).

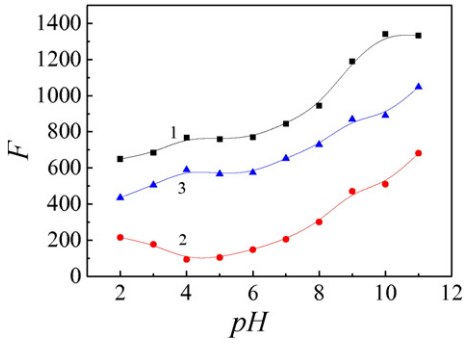

Fig. 3. Effect of acidity. 1 L-Trp, 2 L-Try-Pd(II), 3 L-Try-Pd(II)-CTRX; $c_{L\text{-}Trp} = 4.0 \times 10^{-6}$ mol L^{-1}; $c_{Pd(II)} = 4.0 \times 10^{-6}$ mol L^{-1}; $c_{CTRX} = 2.4 \times 10^{-6}$ mol L^{-1}.

2. Experimental section

2.1. Instruments and reagents

The fluorescence spectra and UV-vis absorption spectroscopy were recorded with a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan) and a UV-2450 spectrophotometer (Shimadzu, Japan) using a 1 cm path length, respectively. The pH value adjustment was conducted on a pHS-3D pH meter (Shanghai Scientific Instruments Company, China).

The stock solution of L-Trp (2.0×10^{-3} mol L^{-1}, Aladdin, Shanghai) was prepared by dissolving 0.0408 g L-Trp with appropriate amount of 0.1 mol L^{-1} NaOH and then diluted it with doubly distilled water. The working solution was obtained by diluting the stock solution to 2.0×10^{-4} mol L^{-1}. The stock solution of CTRX (Hainan Hailing Pharmaceutical Co. Ltd., China) solution was 1.0×10^{-3} mol L^{-1}. The working solution was obtained by diluting the stock solution to 4.0×10^{-5} mol L^{-1}. A stock solution of $PdCl_2$ (2.0×10^{-3} mol L^{-1}, Shanghai Reagent Factory, China) was prepared by dissolving 0.3546 g $PdCl_2$ in 0.5 mL concentrated hydrochloric acid and then diluting to 100 mL with doubly distilled water. The working solution was prepared by diluting the stock solution to 2.0×10^{-4} mol L^{-1}.

Britton–Robinson (BR) buffer solutions were prepared by mixing the mixed acid of 0.04 mol L^{-1} H_3PO_4, H_3BO_3 and CH_3COOH and 0.2 mol L^{-1} NaOH in proportions. The pH values were adjusted with a pH meter.

All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout.

2.2. Procedure

1.0 mL pH 4.0 BR buffer solution, 200 μL 2.0×10^{-4} mol L^{-1} L-Trp, 200 μL 2.0×10^{-4} mol L^{-1} Pd(II) and the predetermined volume of CTRX solution was added in a standard 10 mL volumetric flask in sequence. The mixture was then filled with water to the mark and

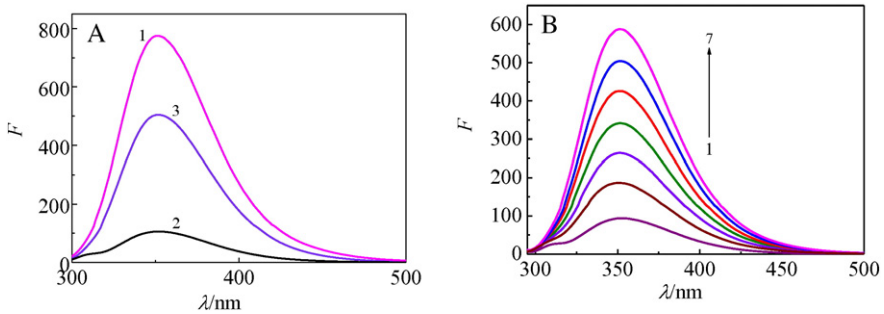

Fig. 2. Fluorescence spectra ($\lambda_{ex} = 276$ nm). A. 1 L-Trp, 2 L-Try-Pd(II), 3 L-Try-Pd(II)-CTRX; $c_{L\text{-}Trp} = 4.0 \times 10^{-6}$ mol L^{-1}; $c_{Pd(II)} = 4.0 \times 10^{-6}$ mol L^{-1}; $c_{CTRX} = 1.6 \times 10^{-6}$ mol L^{-1}; pH = 4.0, B. $c_{L\text{-}Trp} = 4.0 \times 10^{-6}$ mol L^{-1}; $c_{Pd(II)} = 4.0 \times 10^{-6}$ mol L^{-1}; c_{CTRX}: (1–7): 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4($\times 10^{-6}$ mol L^{-1}); pH: 4.0.