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Label-free fluorescence turn on detection of tiopronin with tunable dynamic range based on the ensemble of Alizarin Red S/copper ion

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ABSTRACT

In this study, a new type of rapid, label-free fluorescence turn-on assay for detection of tiopronin using Alizarin Red S (ARS)/copper ion ensemble is developed. ARS is high fluorescence in BR buffer solution. But, the fluorescence of ARS can be significantly quenched by copper ions due to ground-state complexation. However, in the presence of tiopronin, copper ions were released from the ARS and thus restored the fluorescence of ARS. The assay has several important features. First, the system is simple in design, fast in operation and is more convenient and promising than other methods. Second, the proposed assay eliminated the separation process and sophisticated instrumentations. Third, the detection process can be seen with the naked eye and can be easily adapted to automated high-throughput screening. At last, the assay has high sensitivity and selectivity for tiopronin and the detection limit is 0.8 ng/mL which is lower than or at least comparable to the previous methods. Moreover, the dynamic range of the sensor can be tuned simply by adjusting the concentration of copper ions. Importantly, the protocol offers high selectivity for the determination of tiopronin in pharmaceutical tablets, injection and biological samples with satisfactory results. Thus, the assay shows great potential applications in the fields of pharmaceuticals and clinical analysis.

1. Introduction

Tiopronin is a synthetic antioxidant used in clinical applications. It is a weak acidy compound for the treatment of cystinuria, rheumatoid arthritis, hepatic disorders as well as an antidote to heavy metal poisoning [1–3]. Recent studies have shown that it may act as a free radical scavenger because of its thiol group [4]. It can also increase intracellular concentration of GSH and other non-protein sulphydryl group and thereby increase intracellular defense against oxygen free radicals [5]. However, because of its relatively high frequency of side effects, it is dose-related. Therefore, the sensitive determination of tiopronin in biological matrices and pharmaceutical preparation is highly desirable. A range of methods including spectrometric [6], chromatographic [7–11] and amperometry [12] had been proposed for its determination. In most cases, several drawbacks, such as time-consuming and low sensitivity, limit their practical applications. In the past few years, the selectivity of chemiluminescence (CL) [3,13] and fluorimetry [6,12] has attracted much attention to the development of analytical methods for tiopronin. With the development of analytical technique, some assays have been used for the detection of tiopronin [13–15]. For example, Lu et al. [13] reported a CL method for the determination of tiopronin in a pharmaceutical formulation, which was based on the fact that tiopronin could enhance CL between H_2O_2 and luminal in a basic alkaline solution; Xu et al. [14] measured tiopronin based on inhibition of multienzyme redox system. Recently, Hu et al. [15] reported a method for the determination of tiopronin based on the fluorescence quenching of CdTe QDs.

Recently, a wide range of highly sensitive and selective biosensors utilizing the exceptional affinity and specificity of biorecognition schemes have been developed for large-size biomolecules [16–21]. Moreover, other mechanisms leading to high sensor selectivity have to be explored, since the biorecognition principle based on weak multiple-point interaction becomes less viable for smaller analyte molecules [22]. These are based either on strong affinity interactions [23–26] or on a highly specific covalent binding [26], intercalation [27,28], coordination complexes [29], or other supramolecular binding [30]. In this work, we present a new strategy for the highly sensitive and selective label-free detection of tiopronin with tunable dynamic range by exploring a dye/copper ion ensemble.

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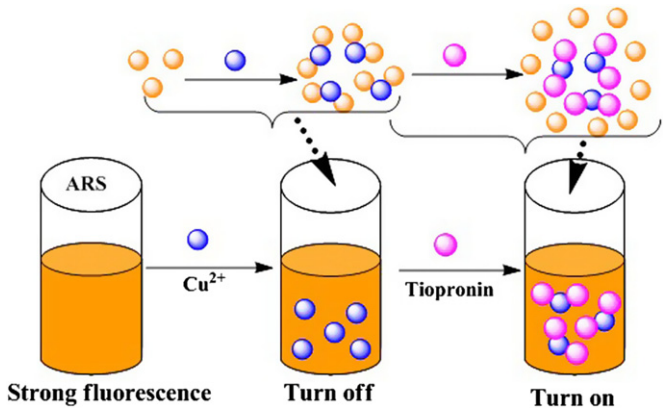

Scheme 1. Illustration of the design rationale for the label-free fluorescence turn-on assay for tiopronin with the ensemble of ARS and Cu^{2+}.

The construction rationale for this fluorometric assay based on ARS/Cu^{2+} ensemble is schematically illustrated in Scheme 1. ARS is an efficient photochemistry and electrochemistry reagent. Moreover, it has great potential application in biological systems. It is virtually nonfluorescent in aqueous solution but shows intense fluorescence in BR buffer solution due to the interaction with boron acid [31]. Copper ion is one of the heavy metal ions and it is a well-known fluorescence quencher. The assay for tiopronin is based on the ensemble of ARS and Cu^{2+}, and the design rationale is explained as follows: (1) It is anticipated that Cu^{2+} in the solution will interact with ARS, mainly because of coordinate interaction between carboxyl groups of ARS and the Cu^{2+}, to form a ARS–Cu^{2+} (2:1) complex. Accordingly, the fluorescence of ARS will be quenched by Cu^{2+} because of the ground-state complexation. (2) Tiopronin molecules have thiol groups which have higher affinity to Cu^{2+} than carboxyl groups in ARS. Thus, it is expected that the ARS–Cu^{2+} complex will be dissociated when tiopronin is added to the solution. As a result, the fluorescence from the ARS will be restored. Therefore, a new label-free fluorescence turn-on assay for tiopronin can be established with the ensemble of ARS/Cu^{2+}.

2. Materials and methods

2.1. Materials and reagents

Alizarin Red S (ARS) was purchased from Shanghai Chemical Corp. (Shanghai, China), and used without further purification. Tiopronin (purity: 99.0%) was purchased from Tianjian Yifang Technology Corp. (Tianjin, China). Tiopronin tablets and injection were obtained from Xinyi Medicine Company (Henan, China). Stock solution of 1.00 mg/mL tiopronin was prepared by dissolving in double distilled water and stored at 4 °C. Working solutions were obtained by serially diluting the stocking solution immediately prior to use. Cu^{2+} was prepared by dissolving some $CuSO_4$ in doubly distilled water. All chemicals used for investigation were of analytical grade purity and doubly distilled water was used throughout. The Britton–Robinson (BR) buffer solution was used to control the acidity of the solution, which was made up of 0.04 mol/L phosphoric acid, 0.04 mol/L acetic acid, 0.04 mol/L boric acid, and 0.2 mol/L sodium hydroxide.

2.2. Apparatus

The fluorescence spectra were measured on an LS-55 spectrophotometer (PerkinElmer, USA) equipped with 150-W Xenon lamp excitation source, using a quartz cell of 1.0 cm path length. The UV–vis spectra were recorded using a Lambda950 UV/vis spectrophotometer (PerkinElmer, USA) equipped with 1.0 cm quartz cells. The wavelength range was 450–600 nm. The photographs were taken with a Canon SX230 digital camera. All pH measurements were made with a DELTA 320-S acidity meter (Mettler-Toledo Instruments Co. Ltd., Shanghai, China).

2.3. Experimental procedure

2.3.1. Fluorescence quenching measurements

First, 2.0 ml BR (pH 6.5) buffer solution and appropriate amount of ARS were added into 10 ml colorimetric tube, and then different amounts of copper ions were added into the above prepared solutions to give solutions whose concentrations of Cu^{2+} were as follows: 0.0, 40.0, 80.0, 120.0, 160.0, 200.0, 240.0, 280.0, 320.0, and 360.0 nM. Each solution was allowed to react for 5 min at room temperature. In all cases, samples were excited at 450 nm, and emission spectra were collected from 460 to 800 nm at a 1000 nm/min scan rate using a LS-55 spectrophotometer (PerkinElmer, USA). Both the excitation and emission slit widths were kept at 10.0 nm.

2.3.2. Fluorescence restoration of ARS/Cu^{2+} system with tiopronin

Solutions of ARS/Cu^{2+} ([ARS]$=6.0\times10^{-7}$ M,[Cu^{2+}]$=300$ nM) were prepared in 2.0 ml pH 6.5 BR buffer solution and placed in colorimetric tube. Different amounts of tiopronin were added into the above solutions to give solutions whose concentrations of tiopronin varied from 0.0 to 64.0 ng/mL. Each solution was allowed to react for 5 min at room temperature, followed by recording the fluorescence spectrum of each solution.

2.3.3. Sample treatment

For analysis of tablets, twenty tiopronin tablets were weighed and powdered in a mortar and the average weight of one tablet was calculated. Then, accurate amount of powdered tablets was dissolved in doubly distilled water and then the solution was filtered into a 100 ml calibrated flask to obtain a concentration of 1 mg/mL tiopronin. Blood samples were prepared by collecting venous blood samples from healthy people (Medical College, Shantou University (Shantou, China)). Appropriate amount of blood sample was kept at 37 °C in a water bath for about 30 min and the blood coagulation was preconditioned. Then, the sample without blood clot was deproteinized by adding approximate acetonitrile and centrifuging at room temperature. Then the acetonitrile was removed under reduced pressure on a rotary evaporator in a 60 °C water bath [32]. The human serum without protein was diluted with BR buffer solution for further experiment.

3. Results and discussion

3.1. Fluorescence quenching of ARS by Cu^{2+}

First, we examined the fluorescence quenching behavior of ARS by Cu^{2+}. Fig. 1a shows the gradual fluorescence decrease of ARS after the addition of Cu^{2+} to the solution. For instance, the solution of ARS in the absence of Cu^{2+} shows a strong fluorescence emission (see Fig. 1a, curve 1). However, after the addition of Cu^{2+} (360 nM), up to 85% quenching of the fluorescence was observed (see Fig. 1a, curve 10). Such a fluorescence change can be distinguished by the naked eye, as depicted in the inset of Fig. 1a, where the photographs of two BR buffer solutions of ARS in the absence and presence of Cu^{2+} are displayed. Additionally, such a sharp decrease of fluorescence intensity caused by Cu^{2+} ensured the determination of tiopronin with high sensitivity. And the quenching of ARS by Cu^{2+} was relatively independent of the incubation time, as it was found that the ARS fluorescence

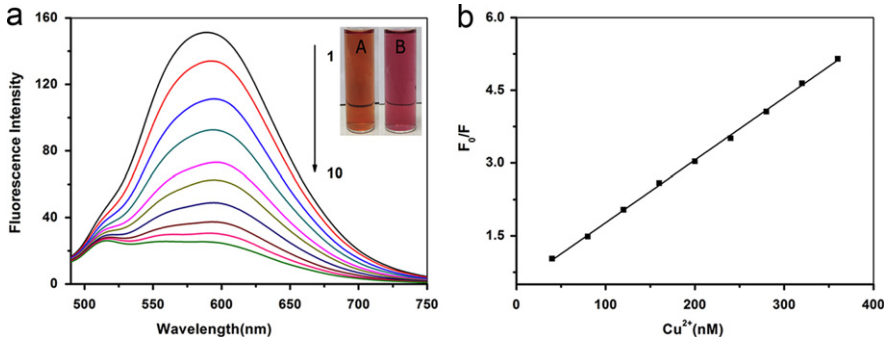

Fig. 1. (a) The fluorescence spectra of ARS (6.0×10^{-7} M) after the addition of different amounts of Cu^{2+}(1 −1 0): 0.0, 40.0, 80.0, 120.0, 160.0, 200.0, 240.0, 280.0, 320.0, 360.0 nM. The inset shows photographs of the solutions of (A) ARS and (B) ARS+Cu^{2+}. (b) The Stern–Volmer plots representing the quenching effect of Cu^{2+} on the ARS fluorescence intensity. All samples were prepared with a BR (pH 6.5) buffer solution, and the excitation wavelength was 450 nm. The measurement was repeated in quintuplicate. The data represent the average.

intensity decreased from 151 to 25 within 2 min after addition of 360 nM Cu^{2+} (see Fig. S1, Supporting Information).

The corresponding Stern–Volmer plot [33] (F_0/F at 589 nm versus [Cu^{2+}], where F_0 is the initial fluorescence intensity of ARS and F is the fluorescence intensity of ARS after the addition of Cu^{2+}) is shown in Fig. 1b. The corresponding Stern–Volmer constant (K_{SV}) was estimated to be 1.29×10^{7} M^{-1} for the quenching of ARS by Cu^{2+}. Additionally, the fact that the Stern–Volmer plot exhibits an upward-curving feature may indicate the formation of a static complex between ARS and Cu^{2+} [34]. Thus, the fluorescence quenching process was attributed to the formation of complex between ARS and Cu^{2+}. From the results above, we can conclude that the high sensitivity of ARS fluorescence emission to Cu^{2+} and the quantitative analysis ability of Cu^{2+} by ARS made the system a good sensor for tiopronin.

3.2. Fluorescence restoration of the ARS–Cu^{2+} system by tiopronin

The experiments were carried out to examine whether the ensemble of ARS and Cu^{2+} can be employed to establish a fluorometric assay for tiopronin. As mentioned above, the fluorescence of the ARS–Cu^{2+} complex was very weak (85% quenching of ARS by Cu^{2+}); moreover, the fluorescence of the complex in a BR buffer solution did not change with time (see Figure S1 in the Supporting Information), indicating that the complex of ARS and Cu^{2+} was stable. Tiopronin had almost no effect on the fluorescence of the ARS according to our observation. However, Cu^{2+} can be chelated by tiopronin, and as a result, the complex of ARS and Cu^{2+} will be dissociated, leading to fluorescence enhancement as discussed above. As expected, the experimental results were shown in Fig. S2 (Supporting Information), the initial BR buffer solution of ARS (6.0×10^{-7} M) and Cu^{2+} (300 nM) showed very weak fluorescence. However, the fluorescence of the ensemble was greatly increased after the introduction of tiopronin into the solution. More importantly, the fluorescence enhancement was also detected by the naked eye (see the inset of Fig. S2, the photographs of two BR buffer solutions of ARS/Cu^{2+} ([ARS]=6.0×10^{-7} M, [Cu^{2+}]=300 nM) in the absence and presence of tiopronin are displayed). As seen in the figure, the color of the solution changed from red to yellow again, which strongly demonstrated that the Cu^{2+} ions were released from ARS after adding tiopronin into the system. Thus, the obtained results clearly confirmed that the ARS/Cu^{2+} system could display the presence of tiopronin, and the fluorescence recovery could be used to establish a calibration curve to afford a quantitative measurement. More significantly, the label-free and turn-on sensing mode offered exceptional advantages here. As known, the major limitation of turn-off probes is that variations in sample environment may be problematic for utilization in quantization measurement, while the turn-on response is able to efficiently decrease background noise and increase detection sensitivity. Moreover, without any separation and modification the assay is simple and fast compared with the other methods.

3.3. Parameter optimization for tiopronin analysis

To obtain better performance of the restoration for probing tiopronin, the effect of pH and other factors were also investigated.

Medium is an important factor to influence the sensor. It was found that ARS did not emit fluorescence in Tris-HCl, HAc–NaAc, NaH_2PO_4–$NaHPO_4$, and HNO_3–hexamethylenetetramine buffer solution. However, when BR buffer solution was used into the system, ARS would emit intense fluorescence. It is attributed to boron that participated in action with ARS [31]. Thus, BR buffer solution was used as a medium. And the effect of pH in a range between 4.7 and 9.0 was detected. According to the experiments, ARS would emit more intense fluorescence in acidic solution than that in alkaline solution. In addition, the results showed the pH of the solution had little effect on the fluorescence quenching efficiency of the ARS by Cu^{2+} in the neutral or weak acid environment. However, the fluorescence quenching efficiency would decrease in the alkaline solutions due to the hydrolysis of Cu^{2+}. Moreover, shown in Fig. S3 (Supporting Information), the fluorescence restoration efficiency of the ARS in the presence of tiopronin was higher in the neutral environment (pH 6.0–7.0) than that in alkaline (pH 7.3–9.0) or acid (pH 4.7–5.8) solutions. It may be attributed to the higher affinity of tiopronin in neutral environment. Considering the results above, BR buffer solution of pH 6.5 was selected for further experiment.

The concentration of ARS affected not only the fluorescence intensity but also the sensitivity of the assay. When the concentration of ARS increased, a large amount of Cu^{2+} was needed to quench its fluorescence. As a result, the sensitivity of the assay decreased significantly. However, if the concentration of ARS was too low, the fluorescence intensity was also very low, which may sacrifice the linear range. Considering these factors, 6.0×10^{-7} M ARS was applied for further study.

Under the room temperature, fluorescence restoration of the system could be reached within 3 min, and no apparent emission change was observed within 30 min after adding tiopronin. Therefore, this method is a promising candidate for rapid tiopronin testing.

3.4. ARS–Cu^{2+} based sensor for tiopronin

As indicated in Fig. 2a, the intensity of fluorescence emission of ARS–Cu^{2+} was sensitive to tiopronin and increased as the concentration of tiopronin increased. The correlation between the

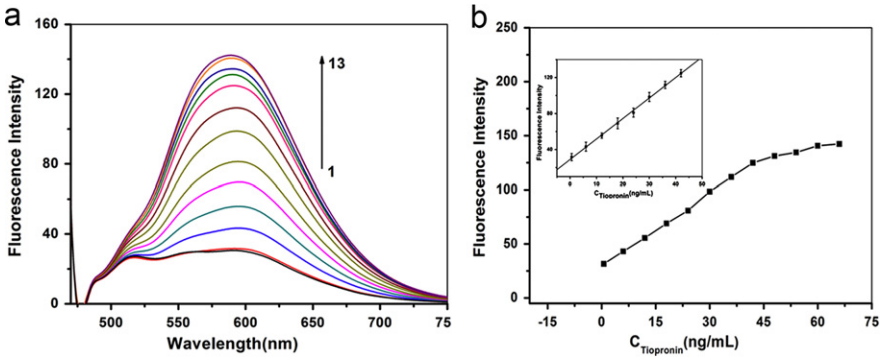

Fig. 2. (a) The fluorescence emission spectra of ARS (6.0×10^{-7} M) containing Cu^{2+} (300 nM) with increasing amounts of tiopronin (1 – 13) (ng/mL): 0.0, 0.6, 6.0, 12.0, 18.0, 24.0, 30.0, 36.0, 42.0, 48.0, 54.0, 60.0, 64.0. The inset shows photographs of the corresponding solutions of ARS+Cu^{2+} without (A) tiopronin and with (B) tiopronin (64.0 ng/mL) after incubation for 5 min. (b) The fluorescence emission intensity of ARS containing Cu^{2+}([ARS]=6.0×10^{-7} M, [Cu^{2+}]=300 nM) versus tiopronin concentration in pH 6.5 BR buffer solution. The inset displays the linear plots. The standard deviations of samples were calculated by a sample size of 5. Each point represents the mean ± standard deviation.

emission intensity and the concentration of tiopronin was shown in Fig. 2b. A linear correlation existed between the emission intensity and the concentration of tiopronin over the range 0.6–55 ng/mL. The standard regression equation is $F=28.95+2.28C_{tiopronin}$ (shown in the inset of Fig. 2b) and the corresponding regression coefficient is 0.9985. A series of 11 repetitive measurements of the 30 ng/mL tiopronin were used for estimating the precision, and the relative standard deviation was 1.40%, showing that the strategy had good reproducibility. According to the definition that the detection limit is the lowest analyte concentration required to produce a signal 3 times greater than that of the blank level, the detection limit for this system was 0.8 ng/mL($S/N=3$), a value which is about 100 times lower than that of the previous reports for tiopronin detection [8].

3.5. Dynamic detection of tiopronin

A tunable dynamic range is important for practical applications, as the desirable concentration for the same target analyte can be different from various applications. For example, besides the application in drug formulations, it also could be used to detect tiopronin in biological samples. We further investigated if the concentration of copper ions can be used as a tunable parameter to tune the dynamic range and fit the sensor for different detection requirements. The calibration curve can be obtained at different concentrations of copper ions, and accurate quantification of tiopronin over different concentration ranges also can be achieved. As shown in Fig. 3, the dynamic range varied from 0.6 to 55 ng/mL, in the presence of 3.0×10^{-7} M Cu^{2+}. When 6.0×10^{-7} M Cu^{2+} was used in the assay, the linear range shifted to 5.0–70 ng/mL. With the increase of Cu^{2+} concentration to 1.2×10^{-6} M, the dynamic range varied from 77 to 130 ng/mL. Thus, the dynamic range of the sensor can be tuned simply by adjusting the concentration of Cu^{2+} while other assay procedures are the same. Moreover, our system could be extended to a multimicrowell plate-based assay to achieve different sample detection at one time without the need to develop new sensors. And serial dilutions are less necessary to achieve an accurate measurement. For unknown samples, this effectiveness simplifies the range finding and eliminates errors introduced by pre-treatment [35].

3.6. Effect of interferences

For an excellent chemosensor, high selectivity is a matter of necessity. To assess the possibility of practical application in determination of pharmaceutical preparation, some metal ions and excipients often contained in tablets, such as lactose,

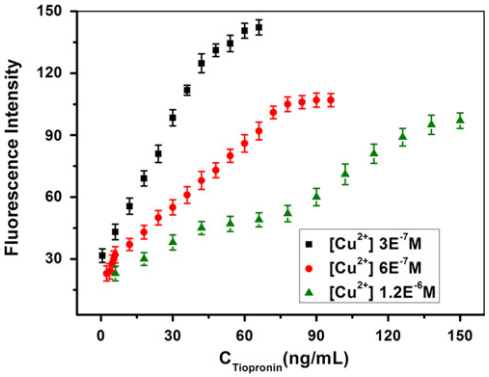

Fig. 3. The calibration curves of the label-free sensor. The complex ARS (6.0×10^{-7} M) was treated with different Cu^{2+} concentrations and then treated with increasing amounts of tiopronin in pH 6.5 BR buffer solution. Each point represents the mean ± standard deviation from five determinations.

microcrystalline cellulose (MCC), low-substituted hydroxypropyl cellulose (L-HPC), starch, magnesium stearate (MS), sodium dodecyl sulphate (SDS), D-glucose, β-cyclodextrin (β-CD) and mannitol, were tested under the optimal conditions. Due to the bare solublility of MCC, L-HPC, starch and magnesium stearate in water, we selected their saturated solutions to study the effects [22]. The results in Table S1 (Supporting Information) revealed that the tolerant concentrations of these excipients were much larger than those in the as-prepared tablet sample solution. Accordingly, we can conclude that these excipients did not restore the fluorescence of ARS–Cu^{2+} which in turn would give rise to erroneous tiopronin levels being determined. In addition, according to our experiment, the majority of metal ions such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Ba^{2+}, Ag^{+}, $Fe^{3+}$and Fe^{2+} can be tolerated at high concentrations due to the specificity of tiopronin to Cu^{2+}. Thus, the proposed assay has good selectivity for tiopronin.

There are the two factors responsible for the high selectivity of this method. First, the quenching of fluorescence of ARS in the presence of Cu^{2+} was attributed to the complexation between Cu^{2+} and ARS. It allows one to monitor the efficient recovery by addition of tiopronin, and this change provides the basis for the "turn-on" assay. Second, compared with ARS and other excipients, the tighter binding of tiopronin for Cu^{2+} led to an assay with high specificity.

3.7. Application

In order to test the applicability of the proposed assay, the analysis of different kinds of real samples was performed.

3.7.1. Detection of tiopronin in tablets and injections

The method was applied to determine tiopronin in commercial tablets and injections. In order to evaluate the validity of the proposed method, oxidation–reduction titration was also used for the determination according to the procedure described in literature [36]. The test samples were obtained from different allotments. The results were shown in Table 1. Each measurement was repeated for five times. The results showed that the content of tiopronin in tablets and injections assayed by the proposed method agreed with the titration result and the labeled value. The recovery of the method was also acquired using the standard addition method by adding a known amount of standard to the pre-analyzed tablet sample in three different levels. Results were shown in Table 2. The recoveries were 98.9% and 102.2%, indicating that the method is reliable for the determination of tiopronin in pharmaceutical preparations.

3.7.2. Detection of tiopronin in human serum

It is crucial to develop a selective and sensitive sensor for tiopronin in the physiological condition. However, many methods could not be performed at optimal conditions, since the determination process was carried out simultaneously with separation. For example, Kusmierek and Bald proposed a method for tiopronin, which is based on derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate followed by ion-pairing reversed-phase liquid chromatography separation and ultraviolet-absorbance [37]. Due to its time-consuming and complex process, it is far away from being a useful analytical method. Without the separation and time-consuming, our ARS–Cu^{2+} system imparted this sensor to be used conveniently and fast. As in the case for monitoring tiopronin in biological samples, it is important to consider the possible interference from physiological species. We subsequently investigated the fluorescence assay of tiopronin in the presence of serum. In the experiment, the similar detection procedure was used as described in aqueous solution, except that diluted human serum was applied as reaction matrix. The diluted human serum was prepared by adding fresh 1 ml human serum into the 99 mL BR buffer samples, and mixed well. The tiopronin spiked diluted human serum samples were prepared by adding different amounts of tiopronin into the as-prepared diluted human serum, and mixed well. Then each sample was measured for five times. The method had good recoveries, as given in Table 3. The recoveries of these measurements are in the range of 98.5–102.6% under the optimal conditions, indicating that this method is reliable and practical.

Table 1
Determination results of tiopronin in commercial tablets and injections.

Samples[a]	Labeled value (μg/mL)	Found ± S.D. (n=5) (μg/mL)		R.S.D. (%)
Tablets	10	10.2 ± 2.5[b]	10.5 ± 2.3[c]	1.6[b]
Injection	10	9.9 ± 0.9[b]	10.2 ± 1.5[c]	1.0[b]

[a] Samples were diluted 1000-fold with doubly distilled water. The data are given as the average value ± S.D. obtained from five independent experiments (n=5).
[b] Proposed method.
[c] Oxidation–reduction titration method according to literature [31].

Table 2
Determination results of tiopronin in commercial tablets by standard addition.

Background (ng/mL)	Added (ng/mL)	Found[a] (ng/mL)	Recovery (%)	R.S.D.(%) (n=5)
10.00	4.00	13.79	98.9	2.2
	8.00	18.32	101.8	1.3
	10.00	20.43	102.2	0.8

[a] The data are given as the average value obtained from five independent experiments (n=5).

Table 3
Determination of tiopronin in several samples containing human serum.

Sample number	Tiopronin (added) (ng/mL)	Tiopronin[a] (found) (ng/mL)	Recovery (%)	R.S.D. (%, n=5)
1	10	9.98	99.8	2.23
2	15	15.15	101.0	1.78
3	20	20.51	102.6	2.03
4	25	24.62	98.5	1.98
5	30	30.10	100.3	1.15

[a] The data are given as the average value obtained from five independent experiments (n=5).

3.8. Validation test

In order to illustrate the principle of the assay, the presumption was verified by UV–vis absorption spectra under the identical experimental condition. The results were shown in Figure S4a (in Supporting Information). The maximum absorption wavelength of ARS in pH 6.5 BR buffer aqueous solution was centered at 473 nm. Whereas, when Cu^{2+} was added into the solution above, the peak shifted to 506 nm along with color changing from yellow to red. The changes were owing to 1:2 (Cu^{2+}: ARS) complex formation [38]. However, the addition of tiopronin in ARS–Cu^{2+} solution resulted in opposite spectral change which is shown in Fig. S4b (Supporting Information). Moreover, the color of the solution changed from red to yellow as well. From the results above, it can be concluded that Cu^{2+}–ARS complex was decompounded and ARS was released. The results of the UV–vis spectra support the sensing mechanism of our method; it provides an exceptional simple approach for detecting tiopronin.

4. Conclusion

In summary, a new type of rapid, sensitive, and selective label-free fluorescence turn-on assay for detection of tiopronin using ARS/Cu^{2+} ensemble is developed. The assay is based on the highly specific interaction between the tiopronin and copper ions and the intense fluorescence ARS probe in a competition assay format. The system is simple in design and fast in operation and is more convenient and promising than other methods. The novel strategy eliminated the need of derivate reaction, separation process, and sophisticated instrumentations. More importantly, the detection process can be seen with the naked eye and can be easily adapted to automated high throughput screening. The detection limit of the method is 0.8 ng/mL, which is lower than previous fluorescence-based methods. Significantly, the sensor can achieve dynamic linear range simply by adjusting the concentration of copper ions. In addition, the protocol offers high selectivity for the determination of tiopronin in commercial tablet, injection, and human serum. Thus, the assay shows great potential applications in the fields of pharmaceuticals and clinical analysis.

Acknowledgments

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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.2012.07.021.

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