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A Novel Fluorescence Probe Based on P-acid-Br and its Application in Thiourea Detection

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Xiu Wang[a], Chunlei Yang[a], Mei Yan*[, a], Shenguang Ge[b], Jinghua Yu[a]

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In this paper, a novel phenyleneethynylene derivative 4,4'-(2,5-dimethoxy-1,4-phenylene)bis(ethyne-2,1-diyl) dibenzoic acid (p-acid) and its derivative p-acid-Br were synthesized. Infrared spectroscopy (IR), fluorescence (FL) spectroscopy and ultraviolet visible (UV-vis) spectroscopy were applied to characterize p-acid and p-acid-Br. To research the practical applicability, a sample thiourea sensor was constructed using p-acid-Br label, in which the FL intensity response was proportion to the thiourea concentration in the range of 0.5~1000 nM, with a detection limit of 0.26 nM. Furthermore, the sensor showed high specificity, excellent stability, and good reproducibility. The p-acid-Br -based thiourea sensor can also provide potential application for detection of other organics. The method showed low detection limit, good specificity, high sensitivity and reproducibility. Satisfactory results were obtained for determination of thiourea in various water samples and fruit juice samples. This work is to open new avenues in the application of phenyleneethynylene derivative for sensitive thiourea assay. Hence, the proposed fluorescence sensor could become a promising method for local market.

Introduction

Thiourea, a sulfur-containing organic compound, has many industrial applications. Thiourea and its derivatives have been widely applied in various fields, including industry, medicine, agriculture and chemistry.[1-4] The reaction of thiourea with hydrogen peroxide under certain conditions produces a powerful reductive bleaching agent which is routinely used in the textile industry. Thiourea and its derivatives are known corrosion inhibitors. For instance, thiourea is usually used for electrodeposition of metals, rubber vulcanization, cleaning, detection, and leaching of gold in industry.[5-8] In agriculture, thiourea and its derivatives are also employed as a fertilizer, fungicide for some fruits, and inhibiter for the dormancy of seeds and tubers.[9,10] As a result, thiourea might be appearing in industrial waste water, river water, on the surface of fruits, and even in fruit juices. The boiler chemical cleaning wastes are then disposed of according to the guidelines established by environmental agencies.[11] Due to its significant role in many fields, thiourea is confirmed as a typical contaminator and prohibited in the environment due to its serious toxicity to the environment and human health.[12] It is also used as reagent for spectrophotometric determination of metals. Thiourea is less toxic than cyanide but it is still a toxic substance, dangerous for its effects on carbohydrate metabolism, carcinogenic, potentially allergenic, showing in addition inhibitory effects on nitrification in soils and water.[13-15] High concentrations of thiourea in industrial wastes may not be acceptable because of its high oxygen demand and organic nitrogen content. For example, thiourea could cause allergy, hypothyroidism, and other glandular diseases. Organic sulfur compounds are common in anoxic environments of wastewater and sediments and are environmentally significant due to their offensive odor. Besides, thiourea is toxic and a cancer support agent, from the above mentioned hazards, thiourea and its derivatives could also disturb the metabolism of carbohydrates and result in carcinogenic for humans.[16] For these reasons, thiourea and its derivatives are considered as toxic and hazardous are forbidden to appear in the environment. Therefore, it is necessary to develop a determination method for thiourea and its derivatives in real samples, such as water, soil, or fruits. Several methods have been employed in the determination of thiourea: raman spectroscopy,[17] mass spectrometry,[18] fourier transform infrared spectroscopy,[19] high performance liquid chromatography[20] and fluorescence.

Fluorescence based detection, homogeneous systems that allow direct measurement of binding in solution,[21] has played significant role at the forefront of the bio-analytical area, because it possesses ultrahigh sensitivity and selectivity.[22,23] One of the most exciting aspects of fluorescence technology is that it can decrease the size of a sample down to the single-molecule detection level, and it is this fact that provides an opportunity for miniaturization and high throughput screening.[24] In addition, fluorescence has enabled the elucidation of many biological processes, studying the structure and dynamics of biological macromolecules and determining the trace amounts of biological macromolecules.[25] Fluorescence sensors employ a fluorescent signal for the detection of analysis. In order to detect different organics at an earlier stage of a disease development, a low detection limit of the organics is desirable.

Among fluorescence-based detection, conjugated compounds are stars of fluorescent dyes for their well-known high absorption coefficients and high fluorescence efficiency, which lead to a wide range of applications in optoelectronic sensors.[26-31] Phenylene ethynylenes are an interesting kind of rigid rod molecular system which maintains p-electron conjugation at any degree of rotation of the phenyl rings and is proposed as a linker for electronic communication in molecular electronic devices.[32,33] Phenylene

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ethynylenes possess tunable optical properties both in solution and in the solid state. They are proposed as components in optoelectronic sensors. For sensor applications, it is extremely important to understand the self-organization of phenylene ethynylenes,[34,35] since the interchromophoric interactions significantly influence their sensor properties.[36] In an effort to prepare an efficient and uniform fluorescence probe, the phenylene ethynylene derivative possessing carboxylic acid groups at the para-position was called p-acid and its ramification p-acid-Br which make it reasonable to predict that they may have potential capability in biological and organic macromolecules analysis.

In this study, we report a novel fluorescence probe for targeted imaging FL overexpressed cancer cells based on the self-assembly of thiourea and p-acid-Br. The primary fluorescence of p-acid-Br turns on first upon the electrostatic adsorption of thiourea onto p-acid-Br based on cyclization reaction to produce a probe with negligible fluorescent background. The competition of thiourea expressed in the cancer cells enables thiourea desorption from p-acid-Br. Thus, the electron transfer between thiourea and p-acid-Br is inhibited. In this way, thiourea overexpressed cells are illuminated. We first examined the effect of the solvent polarity on the fluorescence of p-acid and then explored thiourea in water and selective detection of thiourea in cells with p-acid-Br. The identification and quantification of thiourea in living cells are significant issues in medical and clinical research, given that thiourea has severe toxic effects and have been implicated in hypothyroidism. For these reasons, establishing a facile, rapid, highly selective and sensitive method for the detection of thiourea is a great interest.

Experimental

Reagents

All reagents were of analytical-reagent grade or the highest purity available. Dimethylsulfoxide (DMSO), sodium hydroxide (NaOH), potassium hydroxide (KOH), para-dimethoxybenzene ($C_8H_{10}O_2$), N,N'-dimethylformamide (DMF), sodium sulfite (Na_2SO_3), tetrahydrofuran (THF), methanol (CH_3OH), ethanol (CH_3CH_2OH), aqueous ammonia (NH_4OH, 25%) and TEOS were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bis(triphenylphosphine)- palladiumchloride ($Pd(PPh_3)_2Cl_2$), iodine (I_2), cuprous iodide (CuI), triethylamine ($(C_2H_5)_3N$), potassium iodate (KIO_3) and trimethylsilylacetylene (TMSA) were purchased from Alfa Aesar China Ltd. Acetic acid (CH_3COOH), sulphuric acid (H_2SO_4, 98%), hydrochloric acid (HCl, 37.5%), sodium carbonate (Na_2CO_3), ammonium chloride (NH_4Cl), sodium chloride (NaCl), methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), ethoxyethane ($C_2H_5OC_2H_5$), magnesium sulfate ($MgSO_4$), sodium sulfate (Na_2SO_4) and petroleum ether (30-60°C) were obtained from Sigma-Aldrich. The 0.1 mol·L^{-1} phosphate buffer solution (PBS), which was made from Na_2HPO_4, NaH_2PO_4, and H_3PO_4, was employed as a supporting electrolyte.

Apparatus

Infrared (IR) spectra were recorded using a Nicolet 400 Fourier transform infrared spectrometer (Madison, WI). Absorption spectra were recorded using a UV3101 spectrophotometer. Fluorescence excitation and emission spectra were obtained with a Perkin-Elmer LS-55 luminescence spectrometer.

Synthesis of p-acid-Br

P-acid-Br was synthesized according to a previous protocol with major modifications[37], the procedure is shown in Scheme 1.

Synthesis of 2 : To a solution of 1,4-dimethoxy benzene (2.76 g, 20 mmol) in 20 mL of acetic acid, 2 mL of water and 1 mL of concentrated H_2SO_4 , KIO_4 (5.52 g, 24 mmol) and I_2 (6.09 g, 24 mmol) were added. The reaction mixture was stirred at 120 °C for 24 h and then cooled to room temperature. The excess iodine was removed by the addition of sodium thiosulphate solution until the brown color of iodine disappeared. The excess H_2SO_4 was neutralized with Na_2CO_3 solution until the brisk effervescence stopped. The residue was extracted with chloroform. The white solid product (7.79 g, 69%) was separated using a silica column with hexane as eluent.

Synthesis of 3: To the solvent of triethylamine and tetrahydrofuran (THF) (1:3), compound 2 (1.17 g, 3.90 mmol), CuI (0.023 g, 0.12 mmol) and $Pd(PPh_3)_2Cl_2$ (0.020 g, 0.03 mmol) in 37.72 mL of diisopropylamine, trimethylsilylacetylene (TMSA) (0.90 mL, 6.40 mmol) was slowly added. The mixture was stirred at reflux using ice-cold condensing circulation for about 3 h. The reaction mixture passed through a short celite column and further purified by column chromatography using silica column with petroleum ether/methylene chloride (4:1) as eluent to obtain white crystals of compound 3 (0.93 g, 72%).

Synthesis of 4: To a solution of 3 (0.58 g) in THF (25 mL), methanol (25 mL) and K_2CO_3 (0.974 g, 7.06 mmol) were added and the mixture stirred for 5 h. The solvent was evaporated and the residue was poured into water and extracted with chloroform. The organic layer was washed with salt-saturated solution and dried over anhydrous sodium sulphate. A yellow solid product (0.27 g) was obtained after the solvent was removed.

Synthesis of 5: To a solution of 4 (1.16 g, 6.24 mmol), CuI (0.118 g, 0.62 mmol), methyl 4-iodobenzoate (3.27 g, 12.48 mmol) and $Pd(PPh_3)_2Cl_2$ (0.2 g, 0.29 mmol) in triethylamine and THF (1:3) was added and refluxed for 12 h. The residue was concentrated and the yellow solid product (2.21 g) purified by column chromatography over silica using methylene chloride/petroleum ether (2:1) as eluent.

Synthesis of 6: To a solution of HPLC purified 5 (0.1 g) in THF (15 mL), KOH (1.0 g) and methanol (8 mL) were added and refluxed at a temperature of 70 ° C for about 5 h. The reaction mixture neutralized with dilute HCl to remove excess alkali and 50 mL water was added to precipitate the product. The mixture extracted with chloroform and the combined chloroform layer was evaporated under reduced pressure to yield the yellow solid product 6 (0.09 g).

Synthesis of 7: To a solution of 6 (0.58 g) in THF (25 mL), methanol (25 mL) and CuI (1.724 g) were added and the mixture stirred for 12 h. The solvent was evaporated and the residue was poured into water and extracted with chloroform. The organic layer was washed with salt-saturated solution and dried over anhydrous sodium sulphate. A light brown solid product p-acid-Br (0.27 g) was obtained after the solvent was removed. The product was used to detect the concentration of thiourea.

Fluorescence intensity detection

For the detection of fluorescence intensity, thiourea of various concentrations was added into the solution of p-acid-Br (dissolved in PBS containing 1 mol·L^{-1} DMF, pH7.4) and incubated for 60 min at

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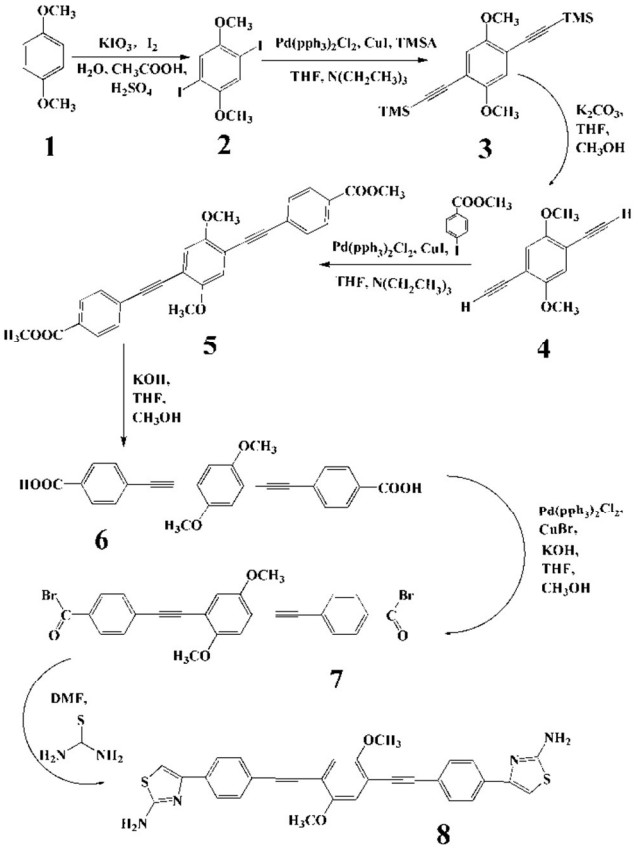
room temperature. Subsequently, the fluorescence intensity of thiourea was detected.



Scheme 1 Synthesis procedure of p-acid-Br and detect of thiourea.

Result and discussion

Absorption and emission

The prepared p-acid exhibited a blue color under ultraviolet radiation (λ = 365 nm). The fluorescence spectrum of p-acid is shown in Figure 1A: excitation into the long absorption band produces room-temperature photoluminescence and the maximum fluorescent emission was obtained at the wavelength of 481 nm, indicated that the p-acid molecule has favorable properties for fluorescence. Moreover, the small energy difference between excitation and emission spectra indicates the existence of a π-conjugated structure. Moreover, as Figure 1B showed the FL spectrum of p-acid (curve a), p-acid-Br (curve b) and p-acid-Br-thiourea (curve c) were also investigated. We observe that the p-acid have the excitation wavelength (Ex) at 376 nm and emission wavelength (Em) at 438 nm. The p-acid-Br do not have a FL intensity, because the C=O and Br- have quenching the FL intensity of p-acid. The Em positions between the p-acid and p-acid-Br-thiourea have a clear distinction, which shows that there have chemical bonds between the p-acid-Br and thiourea. Nevertheless, the FL intensity of p-acid-Br-thiourea is greatly increased compared to p-acid.

To further monitor the formation of p-acid, p-acid-Br and p-acid-Br-thiourea, the UV-vis absorption spectrum (Figure 1C) of p-acid (curve a), p-acid-Br (curve b) and p-acid-Br-thiourea (curve c) were also investigated. The p-acid (curve a on Figure 3) showed two absorption peaks at 382 and 456 nm, while there was one peak at 281 nm for the p-acid-Br (curve b on Figure 1C). When p-acid-Br-thiourea was prepared (curve c on Figure 1C), the peak at 443 nm were mainly ascribed to the molecule π -conjugated structure.

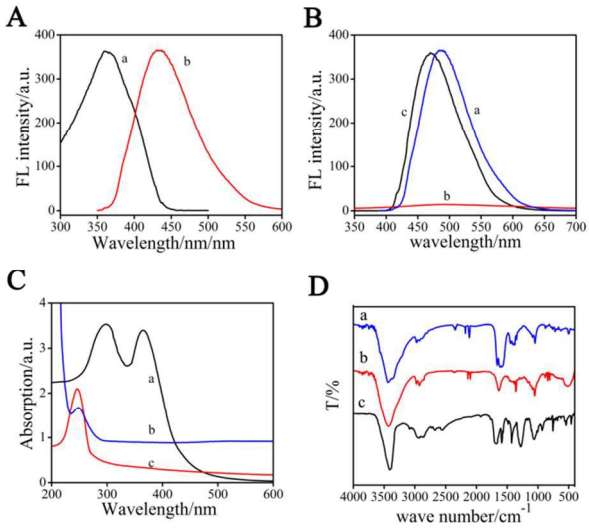


Figure 1 (A) The fluorogram of the 50 mmol·L^{-1} p-acid (curve a for Ex, curve b for Em). (B) Emission spectra recorded from (a) p-acid, (b) p-acid-Br and (c) p-acid-Br-thiourea, (the excitation wavelength is 396 nm, slit: 5 nm/5 nm) in 10 mmol·L^{-1} PBS at pH 7.4 containing 1 mol·L^{-1} DMF. (C) UV-vis spectra recorded from (a) p-acid, (b) p-acid-Br and (c) p-acid-Br-thiourea solution in 10 mmol·L^{-1} PBS at pH 7.4 containing 1 mol·L^{-1} DMF. (D) Infrared spectra of compounds: (a) p-acid, (b) p-acid-Br and (c) p-acid-Br-thiourea.

FT-IR analysis and NMR analysis

FT-IR analysis (Figure 1D) was performed to characterize p-acid (curve a), p-acid-Br (curve b) and p-acid-Br-thiourea (curve c). The strong peaks at 1644 cm^{-1}, 1498 cm^{-1} and 1484 cm^{-1} (curve a); 1657 cm^{-1} and 1546 cm^{-1} 1398 cm^{-1} (curve b); 1602 cm^{-1}, 1508 cm^{-1} and 1492 cm^{-1} (curve c) belong to C=C stretching vibration modes of benzene. The peaks at 2959 cm^{-1}, 2930 cm^{-1} and 2832 cm^{-1} (curve a); 2957 cm^{-1} and 2852 cm^{-1} (curve b); 2947 cm^{-1} (curve c) are assigned to C–H stretching vibration of CH_3–. The characteristic band at 1275 cm^{-1} (curve a) represented the C–O–C stretching vibration. The C=O stretching vibration and the C–O–C stretching vibration of p-acid are a means to prove the successful synthesis of p-acid. The characteristic band at 3415 cm^{-1} (curve c) corresponds to the stretching vibration of –OH group, together with existence of the C=O stretching vibration. The peaks at 2150 cm^{-1} (curve b), 2202 cm^{-1} (curve c) belong to C≡C stretching vibration. From the C≡C stretching vibration, we can clearly see the difference between p-acid-Br and p-acid. The peaks at 1719 cm^{-1} (curve b) belong to the C-Br stretching vibration 3336 cm^{-1} and 1638 cm^{-1} (curve c) belong to the C-S stretching vibration. Furthermore, compared with p-acid-Br, p-acid-Br-thiourea has red shifted on C≡C stretching vibration, we conclude that the p-acid-Br can compound with thiourea. All of these preliminary prove that the p-acid, p-acid-Br, p-acid-Br-

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thiourea were synthesized successfully. Data from ^{1}H NMR spectra of p-acid are shown in Figure SI ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 7.26 (2H), 8.00 (4H), 7.65–7.67 (4H), 3.85–3.87 (6H). Elemental analysis of $C_{26}O_6H_{18}$ gave a composition (%) of C 71.05, O 24.33, H 4.62, which was in good agreement with the theoretical composition of $C_{26}O_6H_{18}$: C 73.23, O 22.53, H 4.23.

Possible mechanism for FL production of p-acid and p-acid-Br-thiourea

FL is a means of converting optical energy to another optical energy. It involves the production of reactive intermediates from stable precursors. These intermediates then react under a variety of conditions to form excited states emitting light. The mechanism for the FL production of p-acid could be inferred according to the study of FL. The electrons within the p-acid are firstly excited to the excited state under photo-irradiation ($h\nu_1$), the p-acid could be oxidated to p-acid$^{\bullet+}$. The electron lost from one p-acid molecule can further react with another p-acid molecule forming the strong oxidant p-acid$^{\bullet-}$. p-acid$^{\bullet-}$ could react with p-acid$^{\bullet+}$ through electron transfer generating the excited state of polymer (p-acid*) which could emit light ($h\nu_2$). All possible FL mechanisms of p-acid are described as following equations (1)-(4):

$$h\nu_1 + \text{p-acid} \rightarrow \text{p-acid}^{\bullet+} + e^- \quad (1)$$

$$\text{p-acid} + e^- \rightarrow \text{p-acid}^{\bullet-} \quad (2)$$

$$\text{p-acid}^{\bullet+} + \text{p-acid}^{\bullet-} \rightarrow \text{p-acid}^{*} + \text{p-acid} \quad (3)$$

$$\text{p-acid}^{*} \rightarrow \text{p-acid} + h\nu_2 \quad (4)$$

The mechanism between p-acid-Br and thiourea (Tu), just as in the mechanism of p-acid, in the first step, p-acid-Br could react with Tu to form a new compound of p-acid-Br-Tu. Then, the electrons within the p-acid-Br-Tu are firstly excited to the excited state under photo-irradiation ($h\nu_1$), the p-acid-Br-Tu could be oxidated to p-acid-Br-Tu$^{\bullet+}$. The electron lost from one p-acid-Br-Tu molecule can further react with another p-acid-Br-Tu molecule forming the strong oxidant p-acid-Br-Tu$^{\bullet-}$. p-acid-Br-Tu$^{\bullet-}$ could react with p-acid-Br-Tu$^{\bullet+}$ through electron transfer generating the excited state of polymer (p-acid-Br-Tu*) which could emit light ($h\nu_2$). In summary, the possible FL mechanisms between p-acid-Br-Tu are described in equations (5)-(9):

$$\text{p-acid-Br} + \text{Tu} \rightarrow \text{p-acid-Br-Tu} \quad (5)$$

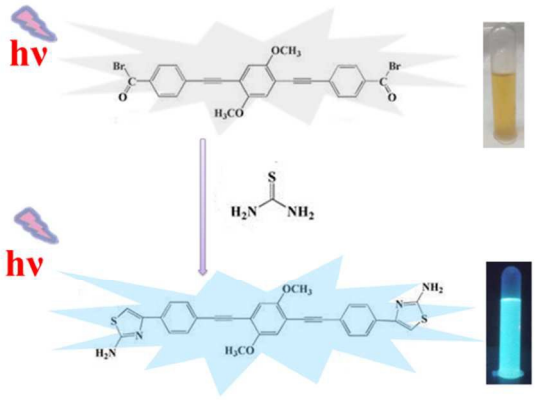
$$h\nu_1 + \text{p-acid-Br-Tu} \rightarrow \text{p-acid-Br-Tu}^{\bullet+} + e^- \quad (6)$$

$$\text{p-acid-Br-Tu} + e^- \rightarrow \text{p-acid-Br-Tu}^{\bullet-} \quad (7)$$

$$\text{p-acid-Br-Tu}^{\bullet+} + \text{p-acid-Br-Tu}^{\bullet-} \rightarrow \text{p-acid-Br-Tu}^{*} + \text{p-acid-Br-Tu} \quad (8)$$

$$\text{p-acid-Br-Tu}^{*} \rightarrow \text{p-acid-Br-Tu} + h\nu_2 \quad (9)$$

The kinetic behavior of p-acid-Br reacting with thiourea was studied by monitoring the fluorescence recovery as a function of time. As shown in Figure 2A and Scheme 2, the fluorescence intensity of p-acid-Br gradually increased with the elongation of time and reached equilibrium after 60 min, revealing a cyclization reaction between p-acid-Br with –Br, C=O and thiourea with C=S, C-NH_2 at room temperature. P-acid-Br has no FL because of the presence of –Br and C=O were made the FL disappeared, with the addition of thiourea which reacted with –Br and C=O, so that the FL appeared. Therefore, this fluorescence sensor could provide a promising platform for rapid screening and quantification of thiourea levels. An incubation time of 60 min was selected for sensitive determination of thiourea in the subsequent experiments.



Scheme 2 The FL mechanism of thiourea

Optimization of experimental conditions

The time and temperature of the p-acid-Br-thiourea reaction also greatly affected the analytical performance of the proposed detection. The effect of the incubation time of thiourea with p-acid-Br (Figure 2A) on the fluorescence intensity of the system is investigated, from which it can be seen that the optimal incubation time is 50 minutes. The fluorescence intensity increases with the increasing of the incubation time, and inclines to a constant value after 50 minutes, ascribing to the saturated binding between the thiourea and p-acid-Br.

Furthermore, with an increasing incubation temperature from 10 °C to 50 °C (Figure 2B), the FL detection after incubation for 50 min with 10 nM thiourea showed a maximum fluorescence response at 35 °C. To simplify the analytical process, all the experiments were carried out at room temperature. At this temperature, no further significant changes were observed in the fluorescence generated.

The influence of the pH value of the detection solution is an important parameter because the acidity of the solution greatly affects the activity of the p-acid-Br. The effect of the solution pH on the fluorescence responses of the analysis was investigated with 10 nM thiourea. Figure 2C shows the effect of pH value of the detection solution on the fluorescence change. The fluorescence change was increased with the increment of pH value from pH 5.5 to 7.4 and then when the pH value was above 7.4 the fluorescence change decreased. The optimal fluorescence response was therefore achieved at pH 7.4. The reason is that highly acidic or alkaline surroundings would damage the p-acid-Br, especially in an acidic environment.

Analytical Performance

Figure 3A showed the FL intensity of the solution in the absence (a) and presence (b~k) of different concentrations of thiourea. It can be seen that the FL intensity in the presence of

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thiourea (b) was higher than that in the absence of thiourea (a), and the FL intensity increased gradually with increasing concentrations of thiourea (b~k). The reason was that the specific binding of p-acid-Br and thiourea could produce the FL singal in the solution, and thus increased the FL intensity, which suggested that the thiourea concentration could be determined by the FL measurement of this reaction. The standard calibration curve for the thiourea detection is shown in Figure 3 B and C. The FL intensity increased linearly with the thiourea concentration from 0.5 nM to 1000 nM with a detection limit of 0.26 nM. The linear fit equation is FL = 194.68+ 212.86 $lgc_{thiourea}$ (unit of c is nM), with a correlation coefficient of 0.9889. According to the linear equation, we could detect trace thiourea concentration quantitatively. Higher plasma thiourea levels could be detected by an appropriate dilution with pH 7.4 PBS. To further clarify the performances of the developed analysis method toward the p-acid-Br and thiourea reaction, the analytical performance of the proposed fluorescence analysis has been compared with those of other thiourea detection. Characteristics such as the linear range and detection limit are summarized for all of them in Table 1. As can be observed, the detection limit of the developed fluorescence method exhibited a higher sensitivity and lower detection limit than those of other method. The reason might be the fact that the p-acid-Br-thiourea has a high quantum yield. Importantly, the high quantum yield could make it especially suitable for ultrasensitive bioanalysis without the need for additional reagents or signal amplification steps.

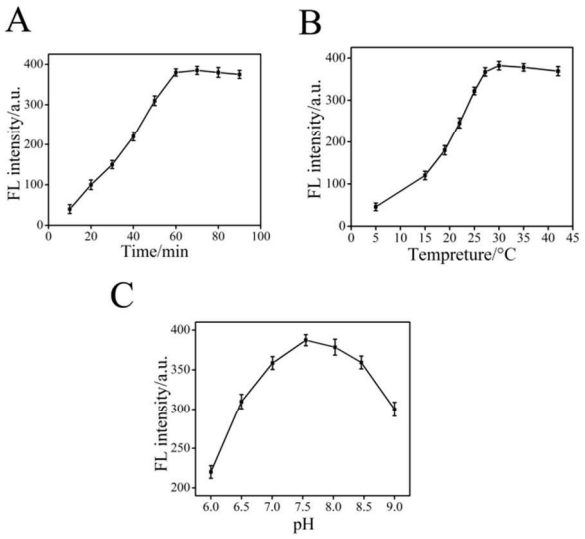

Figure 2 Effect of reaction time (A), reaction temperature (B), and pH (C) on the FL intensity.

Table 1 The results of comparing with other reported methods for the linear range and LOD of thiourea.

Method	Linear range /nM	LOD /nM	Ref.
Mass spectrometry	1.3×10^2~6.6×10^4	13.1	38
FTIR	7.9×10^5~1.1×10^7	1.3×10^5	39
Chemiluminescence	10.0~1.0×10^3	10.0	40
Catalytic kinetic spectrophotometry	3.9×10^2~1.3×10^5	2.6×10^2	41
Spectrophotometry	5.0~2.3×10^2	2.14	42
Fluorescence	0.5~1.0×10^3	0.26	This work

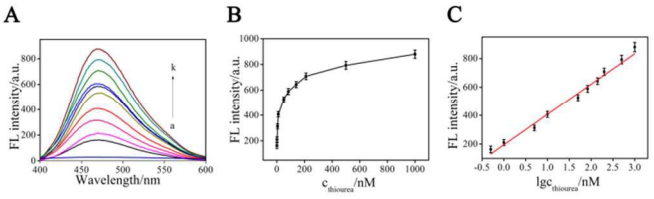

Figure 3 (A) FL profiles of the sensors in the presence of different concentrations of thiourea in PBS containing 1 mol·L^{-1} DMF, pH 7.4. (B) Relationship between FL and thiourea concentration each point is the average of three measurements. (C) Calibration curve for thiourea determination, thiourea determination (nM): (a) 0, (b) 0.5, (c) 1, (d) 5, (e) 10, (f) 50, (g) 85, (h) 140, (i) 200, (j) 500, (k) 1000.

Specificity of co-existing molecules and ions on the detection of thiourea

To investigate the selectivity and specificity of the developed method for detecting thiourea, interference study was employed. Under the same assay conditions, the FL value of thiourea, some common molecules and inorganic ions were measured using the as-prepared p-acid-Br probe, respectively. In this section, the measurands were chosen as 100 nM thiourea, 100 mM xylose, 100 mM glucose, 100 mM citric acid, 200 mM urea. As shown in Figure 4A, the FL value of thiourea was much higher than that of the other measurands, even when their concentration was at least ten times higher than that of thiourea. Thus, this developed method exhibits outstanding selectivity for detecting thiourea. In order to study the interfering effects or selectivity of the procedure, many of the coexisting species in real waters should be investigated. To evaluate the anti-interference ability of the as-developed method for detecting thiourea, an assay was carried out by measuring the FL of the p-acid-Br probe for detecting 100 nM thiourea with a series of co-existing ions, including 10 mM $S_2O_3^{2-}$, 10 mM K^+, 10 mM NH_4^+, 10 mM Cl^-, NO_3^-, SO_4^{2-}, CH_3COO^-, Ca^{2+} and Na^+, respectively. It revealed that the FL value of the p-acid-Br for detecting thiourea was negligibly reduced by the co-existing molecules and ions with much higher concentration than that of thiourea, although the concentration of urea was 2000 times higher that of thiourea (Figure 4B). This result indicated that the developed method shows an excellent performance for anti-interference in the detection of thiourea with the presence of so much co-existing molecules and ions. when the various coexisting species were added to the thourea solution, the FL intensity was almost not changed because they do not have the group of C=S, C-NH_2 in $S_2O_3^{2-}$, K^+, NH_4^+, Cl^-, NO_3^-, SO_4^{2-}, CH_3COO^-, Ca^{2+} and Na^+. So that they were just revealing a cyclization reaction between p-acid-Br with –Br, C=O and thiourea with C=S, C-NH_2 at room temperature, $S_2O_3^{2-}$, K^+, NH_4^+, Cl^-, NO_3^-, SO_4^{2-}, CH_3COO^-, Ca^{2+} and Na^+ were not revealing a cyclization reaction with p-acid-Br. So that p-acid-Br can specifically react with thiourea. Hence this method has a high specificity to detect thiourea and can be further applied for real samples.

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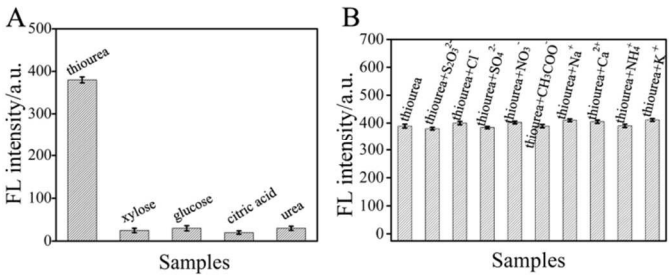

Figure 4 (A) is the specificity test and (B) is the co-existing test.

Table 2 Determination of thiourea in real samples.

Sample	Concentration found /nm	Concentration of added thiourea /nm	Concentration found after added thiourea /nm	Recovery /%
DI water	none	--	--	--
	131.4	--	--	--
Waste water 1	131.4	100	228.3	98.7
	131.4	300	305.2	101.7
	106.6	--	--	--
Waste water 2	106.6	100	210.4	101.8
	106.6	300	289.3	96.4
	none	--	--	--
River water	none	100	98.6	98.6
	none	300	302.4	100.8
	none	--	--	--
Orange juice	none	100	102.1	102.1
	none	300	301.8	100.6
	none	--	--	--
Apple juice	none	100	99.1	99.1
	none	300	302.2	100.7
	none	--	--	--
Lemon juice	none	100	100.9	100.9
	none	300	298.1	99.4

Analytical application for determination of thiourea in real samples

Preparation of real samples. According to the applications of thiourea in industry and agriculture mentioned before, water samples (DI water sample, two industrial waste water samples, one river water sample) and juice samples (one orange juice, one lemon juice and one apple juice) were collected as the real samples. Both of the two industrial waste water samples were filtered with 0.22 μm micropore membranes and diluted to 10 folds. Juice samples were obtained by squeezing fruits. The three fruit samples were purchased from a local market in Jinan. The collected samples were rinsed with tap water, distilled water, and ultrapure water, three times respectively, and then dried and stored in clean beakers at room temperature for several hours. After drying, fruits were squeezed to obtain juices. The juice samples were centrifuged for 15 min with 5000 rpm. The supernatant liquid was filtered by 0.22 μm micropore membranes. To remove the excess of ascorbic acid, the juice samples were stirred for 10 min. Before analysis, the juice samples were diluted to 100 folds.[42,43] All of samples were analyzed immediately after sampling.

Procedure for the detection of thiourea. With high specificity and selectivity in connection with thiourea leaching p-acid-Br, the probe exhibited a good performance in the detection of thiourea, as supported by the neglectable changes of the FL in the presence of interference. The method in this work was applied for real samples such as water samples (double-distilled water sample, two industrial waste water samples, one river water sample) and juice samples (one orange juice, one lemon juice and one apple juice), as shown in Figure 5A. 1 mL PBS (pH 7.4) and added with 500 μL of p-acid-Br solution (dissolved in PBS containing 1 mol·L^{-1} DMF, pH7.4). Then different concentrations thiourea solution was added to the system. About 60 min later, the FL absorbance peak of the solution was stable, we signed the FL_1 intensity. The absorbance of the blank sample was determined using the same procedure without the addition of thiourea and signed as FL_0. The different of FL intensities (FL_1-FL_0) of the solution was recorded to quantify the concentration of thiourea. The thiourea was determined in two industrial waste water samples. No thiourea could be detected in the natural water samples and fruit juice samples. The recoveries of thiourea at the 100 nM level and 300 nM level, respectively. The results were shown in Table 2. From Table 2 we can calculate the standard deviations (RDs), RDs of waste water 1 were -1.36 and 1.10, RDs of waste water 2 were 1.81 and 3.44, RDs of river water were -1.42 and 0.79, RDs of orange juice were 2.06 and 0.60, RDs of apple juice were -0.91 and 0.7, RDs of lemon juice were 0.9 and -1.67. These results indicated that the standard deviations are all less than 4.00%, which suggested that the specificity of the as-prepared FL sensor was acceptable. Therefore, this method could be reasonably applied in the environmental detection of thiourea.

In an attempt to test the applicability of our FL sensor for detection in biological sample matrixe, we employed diluted human serum samples (1:10). We added with 500 μL of p-acid-Br solution (dissolved in PBS containing 1 mol·L^{-1} DMF, pH7.4) into the diluted human serum. Then different concentrations of thiourea solutions were added to the system. About 60 min later, the FL absorbance peak of the solution was stable, founding there was no thiourea in human serum samples. The different of FL intensities of the solution was recorded with adding different concentrations of thiourea (as shown in Figure 5B). Therefore, this method could be reasonably applied in the biological sample matrixes detection of thiourea.

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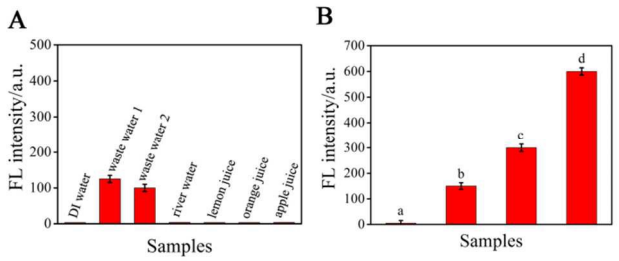

Figure 5 (A) Real samples detection, (B) Human serum samples without (a) and with different concentrations of thiourea: (b) 50 nM, (c) 100 nM, (d) 150 nM.

Conclusion

In summary, a novel organic fluorescence system was developed to detect thiourea based on p-acid-Br. Compared with other methods, this method has advantages such as a simple and fast detection process, high sensitivity and specificity, and powerful tolerance to complex coexisting matrices. This method has a potential in applied for thiourea determination in various water samples and fruit juice samples available in the local market, and thus provide a new promising platform for clinical analysis. Furthermore, it will be a suitable probe for efficient monitoring of intracellular thiourea levels within biological sample matrixes. This work is to open new avenues in the application of phenylene-ethynylene derivative for sensitive thiourea assay. Hence, the proposed fluorescence sensor could become a promising method for local market.

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Notes and references

[a] School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, P. R. China
E-mail: chm_yanm@126.com; Fax: +86-531-88959988;
Tel:+86-531-82767161
[b] Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan 250022, P. R. China

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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Graphical Abstract

A novel organic fluorescence system was developed to detect thiourea based on p-acid-Br, which in the range of 0.5~1000 nM and with a detection limit of 0.26 nM. And this method provides a new promising platform for clinical analysis.