

Analytical Methods

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3 **1 Highly Sensitive ‘Naked-eye’ Colorimetric Detection of**
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5 **2 Thiourea using Gold Nanoparticles**
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3 **1 ABSTRACT**
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5 2 Thiourea (TU) has been widely used in agriculture, industry and other application
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7 3 fields. Due to its serious toxicity and hazard of the environment, it is necessary to
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9 4 found a sensitive and selective method for detecting thiourea. Herein, a facile, fast,
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11 5 and specific colorimetric method was established for the determination of thiourea
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13 6 with high sensitivity based on leaching of gold nanoparticles (Au NPs) by thiourea. In
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15 7 hydrochloric acid media, the absorbance of the Au NPs at 519 nm decreased with the
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17 8 increasing of the concentration of thiourea. The color change of the Au NPs with
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19 9 different concentration of thiourea could make it convenient to be observed by the
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21 10 naked eye. This method allowed the determination of thiourea in a range of 5-350 nM
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23 11 with a detection limit of 2.14 nM. Various foreign species do not interfere with the
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25 12 determination. The method was found to be sensitive, selective and was applied for
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27 13 the determination of thiourea in natural water, industrial waste water and fruit juices.
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33 14 Keywords: Gold nanopartilces, high selectivity, naked-eye colorimetric detection,
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35 15 thiourea.
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1. Introduction

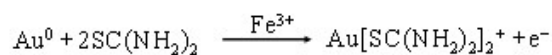
As an important material, thiourea (TU) and its derivatives have been widely applied in various fields, including industry, medicine, agriculture and chemistry.¹⁻¹¹ For instances, thiourea is usually used for electrodeposition of metals, rubber vulcanization, cleaning, detecting, and leaching of gold in industry.¹⁻⁶ In agriculture, thiourea and its derivatives are also employed as a fertilizer, fungicide for some fruits, and inhibitor for the dormancy of seeds and tubers.² As a result, thiourea might be appearance in industrial waste water, river water, the surface of fruits, even in fruit juices. Despite a significant role applied in many fields, thiourea is confirmed as a typical contaminator and prohibited in environment due to its serious toxicity on the environment and human healthy.¹² For examples, thiourea could cause allergy, hypothyroidism, and other glandular diseases. Besides the above hazards, thiourea and its derivatives could also disturb the metabolism of carbohydrate and result in carcinogenic for human.¹³ For these reasons, thiourea and its derivatives are considered as toxic and hazardous and forbidden to appearance in environment. Therefore, it is necessary to develop a determination method for thiourea and its derivatives in real samples, such as water, soil, or fruits.

To date, various methods have been found to detect thiourea in the last three decades. In 1958, D. Amin¹⁴ firstly reported a method to detect thiourea with utilize iodine. With the development of analysis science, other analytical technologies were reported for detecting thiourea gradually, but these methods have their own advantages and intrinsic disadvantages. For instances, flow injections fluorimetry methods is simple, rapid and cost effective, but it has a small detection range only from 3 to 8 μM and poor selectivity because the assay result can be severely interfered by some co-existing molecules and ions, such as Fe^{3+} , Pb^{2+} and EDTA with a concentration of

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3 1 only as the same as that of thiourea.¹⁵ Although Raman spectroscopy,¹⁶ kinetic
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5 2 methods,¹⁷ haloamines,¹⁸ voltammetry,¹⁹ spectrophotometry,² ion selective electrode
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7 3 potentiometry²⁰ and chemiluminescence²¹ have excellent selectivity and large
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9 4 detection range (six orders of magnitude), but the limit of detection (LOD) of these
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11 5 above methods is from 7.6×10^{-4} to 0.9 ppm, which indicated that their sensitivity is
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13 6 poor. Especially, the Fourier transform infrared spectroscopy (FTIR)²² has the largest
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15 7 linear rang (60-800 ppm) among these detection methods, it also has the lowest
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17 8 sensitivity which was investigated by a large LOD of 10 ppm. High performance
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19 9 liquid chromatography¹³ has large detection range (0-304.5 mg/L), but the
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21 10 disadvantage of this method is the complicated procedure for the pretreatment of the
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23 11 samples. In addition, tandem mass spectrometry²³ has both outstanding selectivity and
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25 12 sensitivity for detection of thiourea (LOD of 0.001 ppm), but it need expensive assay
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27 13 instrument, complicated procedure and time consuming for the samples pretreatment
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29 14 before the detection. For these reasons, it attracts great interest to establish a facile,
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31 15 rapid, high selectivity and sensitivity method for the detection of thiourea.
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36 16 Comparing with the other detective methods, colorimetry is a simple, fast, and
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38 17 economical analytical technique. Owing to the unique properties on chemistry,
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40 18 electricity and optics, noble-metal nanoparticles have attract more attention as
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42 19 significant colorimetric probes for detecting metal ions²⁴, biomolecules²⁵⁻²⁷ and
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44 20 organic molecules, especially thiourea. Wang *et al.*²⁸ proposed one of the earliest
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46 21 attempts to develop a colorimetric sensor for measuring thiourea without any
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48 22 complicated procedure.²⁸ The mechanism of this method is that thiourea displaced the
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50 23 stabilizer citrate ions and neutralized surface charges, which induced aggregation of
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52 24 Ag NPs.²⁸ Though this method is facile and rapid, there is still potentiality for
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54 25 improving the sensitivity and linearity range of this method.
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3 In this work, a rapid colorimetric method with high sensitivity and selectivity was
4 reported for detecting thiourea based on the leaching of thiourea to gold nanoparticles
5 (Au NPs) Firstly, Au NPs were synthesized by a common one-step route using
6 trisodium citrate to reduce chloroauric acid and characterized by high resolution
7 transmission electron microscopy (HRTEM) and Uv-vis spectroscopy. Then, the
8 absorbance of the Au NPs at 519 nm was notably decreased by the appearance of
9 thiourea in the mixture. It is linear relation between the decreasing of the absorbance
10 and the increasing of the thiourea concentration. In addition, the color of the Au NPs
11 solution could be changed gradually from dark red to light red when the concentration
12 of thiourea increased due to the leaching of thiourea to Au NPs. And the color change
13 of the solution can be observed conveniently by naked eye. Comparing with the
14 colorimetric probe for detecting thiourea based on Ag NPs, the color of Au NPs can
15 change more fast with ferric ions in acidic solution,²⁹ because the leaching of thiourea
16 to Au NPs has a better kinetics and can be sped by ferric ion as a catalyst.³³ The
17 dissolution of gold in acidic thiourea solutions may be represented by:



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19 As a result, ferric ions were added into acidic Au NPs solution to increase the rate
20 of the leaching reaction. Thus, a non-aggregation colorimetric system was developed
21 for detecting thiourea using unlabeled gold nanoparticles probe. The linearity range
22 and detection limit of this method is 5-350 nM and 2.14 nM, respectively. The present
23 method exhibits outstanding selectivity and reveals excellent capability for resisting
24 interference from coexisting ions on the detection of thiourea. In the percent of 2-3
25 orders of magnitude of K^+ , Na^+ , Zn^{2+} , NH_4^+ , Cl^- , F^- , $\text{S}_2\text{O}_3^{2-}$, EDTA, oxalic acid, xylose,
26 glucose, citric acid, ascorbic acid, urea, glycine, the co-existing species showed
27 negligible effects on detection of thiourea. Furthermore, it was also successfully
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1 applied for detecting thiourea in real samples including natural water, industrial waste
2 water and juice samples.

3 **2. Materials and methods**

4 **2.1. Materials and Chemicals**

5 Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from Aladdin Reagent (Shanghai,
6 China). Trisodium Citrate, KCl, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ were purchased from Tianjin
7 Northern Tianyi Chemical Reagent Co., Ltd (Tianjin, China). $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was
8 purchased from Tianjin Fuchen Chemical Reagent Co., Ltd (Tianjin, China). Thiourea
9 (TU), glucose, H_3PO_4 (85%), NaOH, ZnCl_2 , NH_4F , $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$,
10 $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, NaCl, $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$, ethylene diamine tetraacetic acid (EDTA) were
11 purchased from Tianjin Kewei Co., Ltd (Tianjin, China). D (+)-xylose was purchased
12 from Sinopharm Beijing Co., Ltd (Beijing, China). Urea was purchased from Tianjin
13 Fengchuan Co., Ltd. (Tianjin, China). Ascorbic acid (AA) was purchased from
14 Tianjin First Chemical Reagent Factory (Tianjin, China). H_3BO_3 was purchased from
15 Tianjin Third Chemical Reagent Factory. CH_3COOH (36%) was purchased from
16 Tianjin Yongda Co., Ltd. (Tianjin, China). Tris was purchased from Beijing Dingguo
17 Biological Technology Co., Ltd. (Beijing, China). Glycine was purchased from
18 Tianjin Zhujiang Medicines (Tianjin, China). All chemicals involved in this work
19 were analytical grade and used without further purification. Ultrapure water was
20 purchased from Wahaha Group Co. Ltd. (Hangzhou, China).

21 **2.2. Apparatus**

22 The absorption spectra of the as-prepared Au NPs were recorded on a DH-2000-BAL
23 Uv-vis spectrophotometer (Ocean Optics, USA) using quartz cuvettes with an optical
24 path length of 1 cm at 519 nm at room temperature. The size and morphology of Au
25 NPs were characterized by transmission electron microscopy (TEM) and

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3 1 high-resolution TEM (HRTEM) on a Tecnai G² F20 microscope (FEI, USA)
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5 2 operating at an acceleration voltage of 200 kV.
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7 **2.3. Synthesis of the gold nanoparticles**

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9 4 All glassware used in the experiment was soaked in aqua regia ($V_{\text{HNO}_3} / V_{\text{HCl}}=1:3$) for
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11 5 30 min and thoroughly cleaned with ultrapure water before to be used. Gold
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13 6 nanoparticles were synthesized by the reduction of HAuCl₄ using trisodium citrate
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15 7 following the previous method with slight modification.^{30,31} Typically, 50 mL HAuCl₄
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17 8 aqueous solution (1 mM, 37 °C) was heated to boil under vigorous stirring. Then, 5
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19 9 mL sodium citrate solution (38.8 mM) was added to the HAuCl₄ solution rapidly. Ten
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21 10 minutes later, the color of the solution changed from light yellow to wine red.
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23 11 Afterwards, the reaction mixture was kept on boiling for 30 min and cooled down to
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25 12 room temperature with stirring. The excessive trisodium citrate in this reaction
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27 13 mixture was removed by centrifugal filtration at 15000 rpm for 20 min. Then the Au
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29 14 NPs were resuspended in 4 mM trisodium citrate solution. The purified Au NPs were
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31 15 stored at 4 °C in dark.
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36 **2.4. Procedure for the detection of thiourea**

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38 17 1 mL 28 mM HCl was diluted with 0.5 mL ultrapure water and added with 500 μL of
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40 18 Au NPs solution. Then 20 μL thiourea solution was added to the system. About 30
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42 19 min later, the Uv-vis absorbance peak of the solution was stable, Fe³⁺ solution was
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44 20 added. After stirring for 15 min, Uv-vis absorbance of this solution was detected and
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46 21 signed as A. The absorbance of blank sample was determined using the same
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48 22 procedure without the addition of thiourea and signed as A₀. The variation of Uv-vis
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50 23 absorbance (A₀-A) of the Au NPs solution were recorded to quantify the
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52 24 concentration of thiourea.
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55 **2.5. Preparation of real samples**

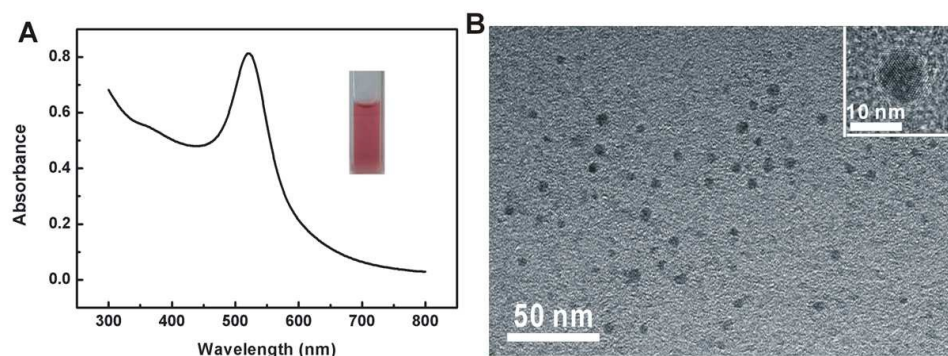
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3 1 According to the applications of thiourea in industry and agriculture mentioned before,
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5 2 one river water sample, one rain water sample, two industry waste water samples and
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7 3 three juice (orange juice, lemon juice and apple juice) samples were collected as the
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10 4 real samples. Both of the two waste water samples were obtained from two
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12 5 galvanization factories in Wuqing District, Tianjin, China. These samples were
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14 6 filtered with 0.22- μm micropore membranes and diluted to 10 folds. Juice samples
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16 7 were obtained from squeezing fruits. The three fruit (orange, lemon and apple)
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18 8 samples were purchased from a local market in Tianjin, China. The collected samples
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20 9 were rinsed with tap water, distilled water, and ultrapure water, three times
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22 10 respectively, and then dried and stored in clean vessels at room temperature for
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24 11 several hours. After drying, fruits were squeezed to get juice. The juice samples were
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26 12 centrifuged for 15 min with 5000 rpm. Supernatant liquid was filtered by 0.22- μm
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28 13 micropore membranes. To remove the excess of ascorbic acid, the juice samples were
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30 14 stirred for 10 min.³² Before analysis, the juice samples were diluted to 100 folds. All
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32 15 of samples were analyzed immediately after sampling.
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36 3. Result and discussion

37 3.1. Synthesis and characterization of the Au NPs probe

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39 18 In the present work, the Au NPs was synthesized by a typical route via reducing
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41 19 chloroauric acid with trisodium citrate.^{30, 31} Citrate was not only chosen as the
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43 20 reducing and stabilizing agent for preparing the Au NPs, but also increased the
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45 21 solubility of the Au NPs in aqueous solutions. The optical property of the Au NPs was
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47 22 investigated by Uv-vis absorption spectra. It illustrated that the maximum absorption
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49 23 peak located at 519 nm in the absorbance spectrum of the Au NPs (Fig. 1A). The inset
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51 24 of Fig. 1A exhibited that the color of the as-prepared Au NPs solution was is wine red
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53 25 under visible light. The size and morphology of the as-prepared Au NPs were
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1 characterized by HRTEM images. As shown in Fig. 1B, the as-prepared Au NPs
2 showed spherical shape and size uniformity with an average diameter of 8 nm.

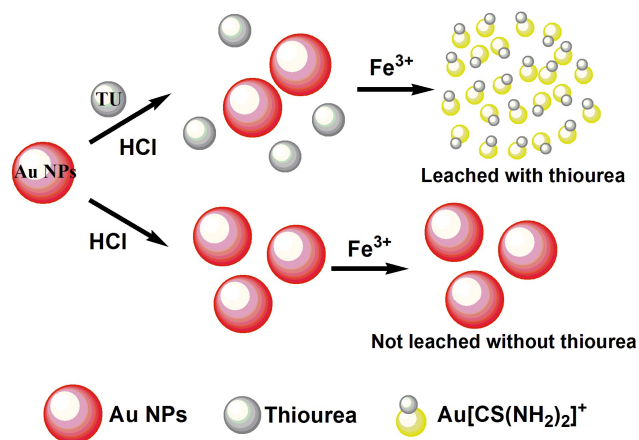


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4 **Fig. 1** (A) Uv-vis absorption spectrum of Au NPs aqueous solution. Inset image
5 shows the corresponding photograph of Au NPs under room light. (B) HRTEM image
6 of the as-prepared Au NPs.

7 **3.2. Optimization of the sensitivity of the Au NPs probe for the detection of** 8 **thiourea.**

9 Colorimetric detecting method based on Au NPs is a simple, rapid and sensitive
10 method for detecting thiourea. The quantity of thiourea can be measured via
11 investigating the varying of the Au NPs probe's absorbance due to the concentration
12 change of the Au NPs with the appearance of thiourea. As shown in Scheme 1, when
13 thiourea was added into an acidic mixture of Au NPs and Fe^{3+} ions, the Au NPs can
14 be leached by thiourea with the catalysis of Fe^{3+} ions. As a result, the concentration of
15 the Au NPs was decreased by the leaching process which can reduce the absorbance
16 of the Au NPs and shallow the color of the Au NPs. If thiourea was absent in the
17 mixture, the leaching did not appear. The decrease of absorbance was measured by a
18 common Uv-vis spectrometer and the change of color was observed by naked eye.
19 Therefore, this colorimetric approach is a facile and highly sensitive method for

1 detecting thiourea because it does not need expensive instruments and provides
 2 excellent sensitivity by the color change of the solution.



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 4 **Scheme 1.** Schematic illustration for selective detection of thiourea using the
 5 developed Au NPs probe.

6 Since the leaching process of thiourea to the Au NPs only occurred with the
 7 catalysis of Fe^{3+} ions in acidic media, the influence of reaction media and
 8 concentration of Fe^{3+} ions on the difference between the absorbance of the Au NPs
 9 prior to and after the addition of thiourea ($A_0 - A$, ΔA) was investigated respectively to
 10 obtain the most sensitive Au NPs probe. In this section, ΔA was used as the index of
 11 the sensitivity of the Au NPs probes. Firstly, the ΔA of the Au NPs mixture was
 12 measured in a series of media including hydrochloric acid (HCl), BR (10 mM H_3PO_4 ,
 13 10 mM CH_3COOH , 10 mM H_3BO_3) buffer solution, acetic acid-sodium acetate
 14 ($\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$) buffer solution, Tris-HCl buffer solution. It reveals that
 15 HCl could provide a stable condition for the optical property of the Au NPs than the
 16 other buffers owing to the little content of metal ions in HCl solution.^{15, 33} Therefore,
 17 HCl was chosen as the optimal reaction media, and the influence of HCl concentration
 18 on the ΔA of the Au NPs was systemic studied in the range of 1.0-50.0 mM. It
 19 demonstrated that the ΔA of the Au NPs was improved with the increasing of the

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3 concentration of HCl, but when the concentration of HCl was above 14 mM, the ΔA
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5 of the Au NPs decreased. It means that the Au NPs probe get the optimal sensitivity
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7 for determining thiourea when 14 mM HCl was added into the Au NPs solution (Fig.
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9 2A).
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11 Besides the reaction media, the influence of Fe^{3+} ions concentration on the ΔA of
12 the Au NPs was also studied. The performance of the leaching reaction of thiourea to
13 Au NPs was interrelated with the concentration of the ferric ions. As shown in Fig. 2B,
14 there is only a weak influence on the ΔA of Au NPs with the Fe^{3+} ions concentration
15 inferior to 1 μM . But it can be observed that the ΔA of the Au NPs increased
16 obviously when the concentration of Fe^{3+} ions was larger than 1 μM . As a catalyst,
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11 Fe^{3+} ions can accelerate the leaching progress of thiourea to Au NPs, but extra Fe^{3+}

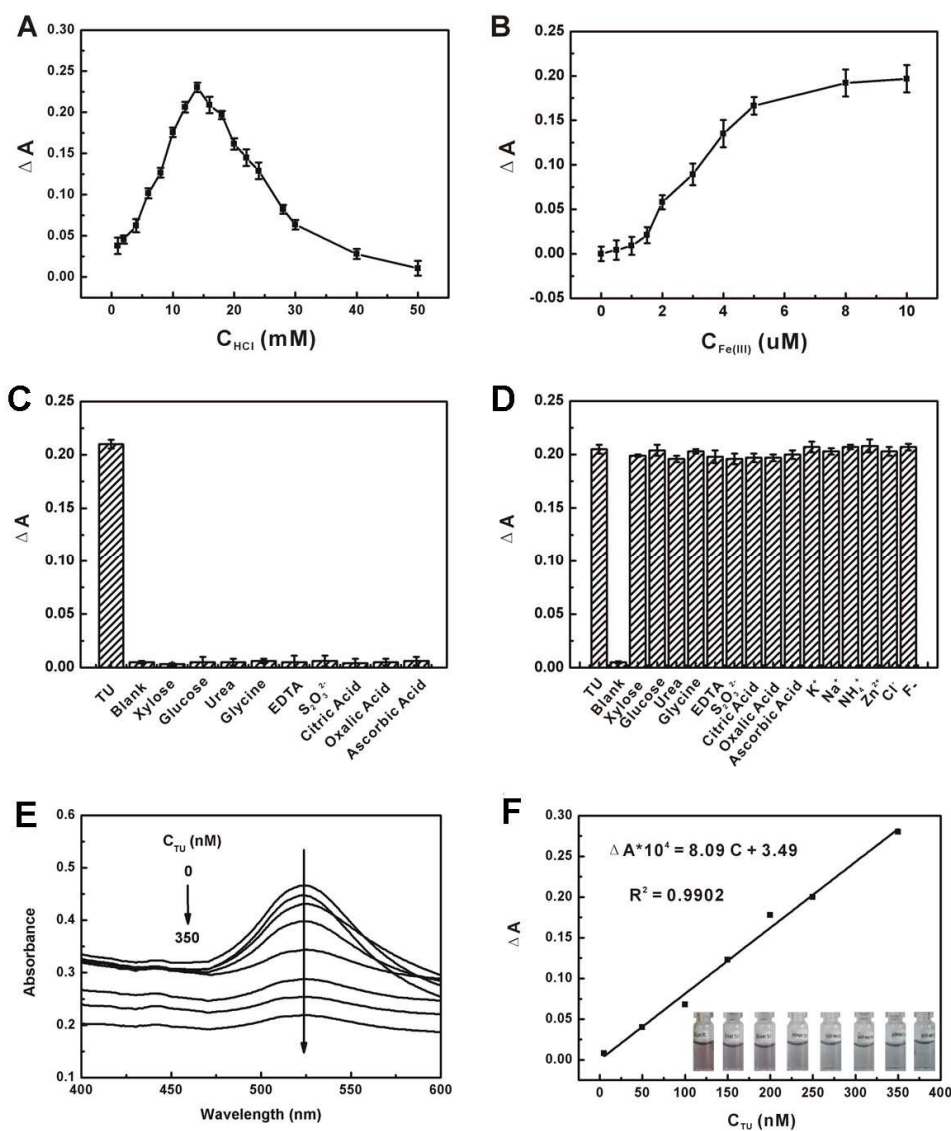


Fig. 2 Effect of gold nanoparticles test solution in the presence of (A) different concentration of hydrochloric acid, (B) ferric ions on ΔA value. Specificity test in this method, (C) is the specificity test and (D) is the co-existing test. (E) Uv-vis absorbance single of the label free Au NPs probe toward different concentrations of thiourea. (F) The corresponding calibration curve of detecting thiourea. The inset displays the color change for different concentrations of thiourea with naked-eyes.

1 ions might notably enhance the ΔA of the Au NPs because of strong catalysis even the
2 concentration of thiourea was at a lower level. The increasing of the ΔA contributed
3 to the extra Fe^{3+} ions can provide large error for the detection of thiourea. Therefore,
4 the concentration of Fe^{3+} ions should be controlled under an optimal level to increase
5 the stability of the detection system based on the as-prepared Au NPs. In this work, 1
6 μM was chosen as the optimum concentration of Fe^{3+} ions for detecting thiourea using
7 this Au NPs probe.

8 **3.3. Specificity of co-existing molecule and ions on the detection of thiourea**

9 To investigate the selectivity and specificity of the developed method for detecting
10 thiourea, interference study was employed. Under the same assay conditions, the ΔA
11 value of thiourea, some common molecules and inorganic ions was measured using
12 the as-prepared Au NPs probe, respectively. In this section, ΔA was defined as the
13 absorbance difference of the Au NPs between prior to and after the addition of each
14 kind of measurand into the Au NPs solution. The measurands were chosen as 0.1 μM
15 thiourea, 100 μM xylose, 100 μM glucose, 200 μM urea, 200 μM glycine, 10 μM
16 EDTA, $\text{S}_2\text{O}_3^{2-}$ 1 μM and 100 μM citric acid, 10 μM oxalic acid and 100 mM ascorbic
17 acid. As shown in Fig. 2C, the ΔA value of thiourea was much higher than that of the
18 other measurands, even their concentration was at least ten times higher than that of
19 thiourea. It was demonstrated that the other measurands produced negligible response
20 to the as-developed colorimetric detection method based on Au NPs. Thus, this
21 developed method exhibit outstanding selectivity for detecting thiourea.

22 To evaluate the anti-interference ability of the as-developed method for detecting
23 thiourea, an assay was carried out by measuring the ΔA of the Au NPs probe for
24 detecting 0.1 μM thiourea with a series of co-existing molecule and ions, including
25 100 μM xylose, 100 μM glucose, 200 μM urea, 200 μM glycine, 10 μM EDTA, 1 μM

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3 1 $S_2O_3^{2-}$, 100 μM citric acid, 10 μM oxalic acid, 100 mM ascorbic acid, 20 μM K^+ , 20
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5 2 μM Na^+ , 10 μM NH_4^+ , 1 μM Zn^{2+} , 10 μM Cl^- , 10 μM F^- , respectively. It revealed that
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7 3 the ΔA value of the Au NPs for detecting thiourea was negligibly reduced by the
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9 4 co-existing molecules and ions with much higher concentration than that of thiourea,
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11 5 although the concentration of urea and glycine was 2000 times higher than that of thiourea
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13 6 (Fig. 2D). This result indicated that the developed method shows an excellent
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15 7 performance for anti-interference in the detection of thiourea with the present of so
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17 8 much co-existing molecules and ions. Hence this method has a high specificity to
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19 9 detect thiourea and can be further applied for real samples.

10 3.4 Analytical performance of the AuNPs probe for detecting of thiourea

11 The figures of merit for the c under selected assay conditions are revealed in Table 1.
12 The decrease of Uv-vis absorbance is proportional to the increase of thiourea
13 concentration. As a result, thiourea can be detected over the linear range from 5 to
14 350 nM with a limit of detection (LOD) of 2.14 nM (3s). A calibration curve of
15 Uv-vis absorbance signal versus concentration of thiourea was obtained with a linear
16 coefficient $R^2 = 0.9902$ and a linear regression equation of $\Delta A \times 10^4 = 8.09C + 3.49$
17 (where C is the concentration of thiourea in nM) (Fig. 2E and F). Furthermore, the
18 relative standard deviation (RSD) was 1.5%, which was measured by detecting 0.1
19 μM thiourea for 11 replicates. It was revealed that this method exhibited excellent
20 precision for the detection of thiourea.

21 **Table 1.** Analytical figures of merit of the determination for thiourea

LOD / nM	2.14
Linear range / nM	5-350
Calibration function (R, $A_0 - A$; C, conc./ nM)	$\Delta A \times 10^4 = 8.09C + 3.49$
Correlation coefficient / R^2	0.9902
RSD (n=11) / %	1.5

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3 1 A comparison of the detection limits and the linear range for thiourea obtained by
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5 2 the present method and other techniques is made in Table 2. Clearly, the Au NPs
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7 3 probe for detecting thiourea fabricated in this work has a LOD higher than that of
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9 4 adsorptive stripping voltammetry,³⁴ but it is one or four orders of magnitude lower
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11 5 than those of the other methods.^{2, 17, 19, 21, 22, 23, 35} The linear rang of the Au NPs probe
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13 6 for detecting thiourea developed in this work is a little narrower than that of catalytic
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15 7 kinetic spectrophotometry¹⁷ and mass spectrometry²³, but it is one or two orders of
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17 8 magnitude wider than those by spectrophotometry,² flow injection amperometry,³⁵
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19 9 FTIR²² and adsorptive stripping voltammetry³⁴ and comparable with that obtained by
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21 10 flow injection fluorimetry,¹⁵ voltammetry¹⁹ and chemiluminescence²¹. It is
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23 11 demonstrated that the present method can provide the outstanding selectivity and
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25 12 sensitivity for the detection of thiourea in the real samples.
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Table 2. Some critical properties of this work with previous studies about determination of thiourea.

Method	Linear range		LOD		Remark	Reference
	ppm	nM	ppm	nM		
This work	3.8×10^{-4} – 2.7×10^{-2}	5.0 – 3.5×10^2	1.6×10^{-4}	2.14	Simple, rapid, high sensitivity and selectivity	This work
Voltammetry	0.3–76.1	3.9×10^3 – 1.0×10^6	Not given	Not given	Low sensitivity	19
Spectrophotometry	7.6–60.0	1.0×10^5 – 7.9×10^5	0.9	1.2×10^4	Low sensitivity	2
Mass spectrometry	0.01–5.0	1.3×10^2 – 6.6×10^4	0.001	13.1	Expensive instrument	23
FTIR	60.0–800.0	7.9×10^5 – 1.1×10^7	10.0	1.3×10^5	Low sensitivity	22
Flow injection fluorimetry	0.04–0.76	5.3×10^2 – 1.0×10^4	Not given	Not given	Low sensitivity	15
Flow injection amperometry	3.8–76.1	5.0×10^4 – 1.0×10^6	4.1	5.4×10^4	Low sensitivity	35
Adsorptive stripping voltammetry	Up to 0.30	Up to 3.9×10^3	2.5×10^{-6}	3.3×10^{-2}	High sensitivity	34
Catalytic kinetic spectrophotometry	0.03–10.0	3.9×10^2 – 1.3×10^5	0.02	2.6×10^2	Low sensitivity	17
Chemiluminescence	7.6×10^{-4} – 7.6×10^{-2}	10.0 – 1.0×10^3	7.6×10^{-4}	10.0	Low sensitivity	21

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3 1 For instances, flow injections fluorimetry methods is simple, rapid and cost
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5 2 effective, but it has a small detection range only from 3 to 8 μM and poor selectivity
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7 3 because the assay result can be severely interfered by some co-existing molecules and
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9 4 ions, such as Fe^{3+} , Pb^{2+} and EDTA with a concentration of only as the same as that of
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11 5 thiourea.¹⁵ Although Raman spectroscopy,¹⁶ kinetic methods,¹⁷ haloamines,¹⁸
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13 6 voltammetry,¹⁹ spectrophotometry,² ion selective electrode potentiometry²⁰ and
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15 7 chemiluminescence²¹ have excellent selectivity and large detection range (six orders
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17 8 of magnitude), but the limit of detection (LOD) of these above methods is from
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19 9 7.6×10^{-4} to 0.9 ppm, which indicated that their sensitivity is poor. Especially, the
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21 10 Fourier transform infrared spectroscopy (FTIR)²² has the largest linear rang (60-800
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23 11 ppm) among these detection methods, it also has the lowest sensitivity which was
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25 12 investigated by a large LOD of 10 ppm. High performance liquid chromatography¹³
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27 13 has large detection range (0-304.5 mg/L), but the disadvantage of this method is the
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29 14 complicated procedure for the pretreatment of the samples. In addition, tandem mass
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31 15 spectrometry²³ has both outstanding selectivity and sensitivity for detection of
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33 16 thiourea (LOD of 0.001 ppm), but it need expensive assay instrument, complicated
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35 17 procedure and time consuming for the samples pretreatment before the detection. For
36
37 18 these reasons, it attracts great interest to establish a facile, rapid, highly selective and
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39 19 sensitive method for the detection of thiourea.

20 **3.5 Analytical application for determination of thiourea in real samples**

21 With high specificity and selectivity in connection with thiourea leaching Au
22 nanoparticles, the probe exhibited a good performance to detection of thiourea, as
23 supported by the neglectable changes of the Uv-vis absorbance when interferences
24 present. Method in this work was applied for real samples such as water samples (two
25 industry waste water samples, one rain water sample and one river water sample) and

1 juice samples (one orange juice, one lemon juice and one apple juice). These samples
 2 were treated according to the sample preparation as reported before.² The
 3 concentrations of thiourea were determined in two industrial wastewater samples. No
 4 thiourea could be detected in the natural water samples and fruit juice samples. The
 5 recoveries of thiourea at 100 nM level and 300 nM level, respectively, spiked in the
 6 prepared water samples and fruit juice samples ranged from 92 to 107%, indicating no
 7 or little interference encountered from the complex matrices.

8 **Table 3.** Determination of thiourea in real samples

sample	Concentration found in solution / nM	Concentration of added TU / nM	Concentration found after addition thiourea (mean±s, n=3) / nM	Recovery (mean±s, n=3) / %
industrial wastewater 1	123.3±0.6	—	—	—
	123.3±0.6	100	231±1.0	104±2
industrial wastewater 2	145.0±0.8	—	—	—
	145.0±0.8	100	238±0.3	97±2
River water	nd	—	—	—
	nd	100	103.3±0.8	103±3
	nd	300	302.7±0.5	101±3
Rain water	nd	—	—	—
	nd	100	98.3±0.6	98±2
	nd	300	301.3±0.7	102±5
Orange juice	nd	—	—	—
	nd	100	105±0.4	105±2
	nd	300	303.1±0.9	103±2
Lemon juice	nd	—	—	—
	nd	100	103.3±0.9	103±3
	nd	300	301.5±1.0	102±4
Apple juice	nd	—	—	—
	nd	100	95±0.7	95±3
	nd	300	300.9±0.6	100±3

9 *nd: Not detectable

10

11 4. Conclusion

12 In summary, a non aggregation colorimetric system was developed to detect thiourea
 13 based on catalytic and oxydic effect on the unlabeled gold nanoparticles probe via the
 14 Fe³⁺ ions in acidity media. Compared with other techniques, this method has

1
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3 1 advantages such as simple and fast detecting process, high sensitivity and specificity,
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5 2 and powerful tolerance to complex coexisting matrices. This method has been applied
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7 3 for the thiourea determination in various water samples and fruit juice samples
8
9
10 4 available on the local market.

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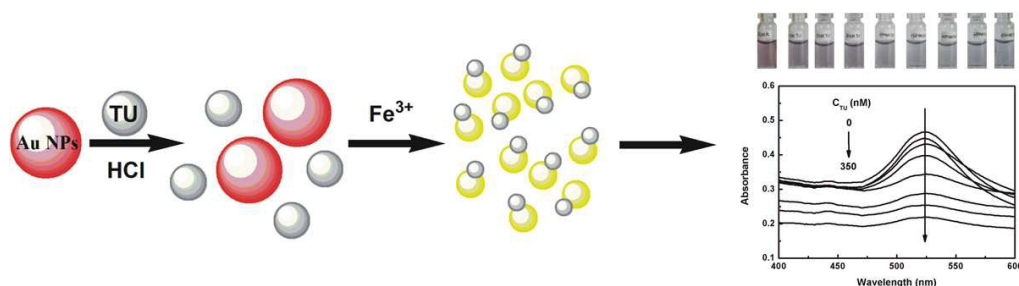
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1 Highly Sensitive ‘Naked-eye’ Colorimetric Detection of 2 Thiourea using Gold Nanoparticles

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11

12 A non aggregation colorimetric system was developed for the fast and sensitive
13 detection of thiourea in aqueous environments using unlabeled gold nanoparticles
14 probe.

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