Talanta 130 (2014) 536–541

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Nuclear fast red as highly sensitive "off/on" fluorescent probe for detecting guanine

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form
11 July 2014
Accepted 16 July 2014
Available online 24 July 2014

Keywords:
Guanine
Nuclear fast red
Cu^{2+}
Fluorescence

ABSTRACT

A novel fluorescence method for the determination of guanine was developed based on the fluorescence enhancement of Cu^{2+}–nuclear fast red complex in the presence of guanine in Tris–HCl buffer. The complex of Cu^{2+} with nuclear fast red resulted in a dramatic quenching of the fluorescence intensity. Nuclear fast red were dissociated from the complex with the addition of guanine due to the strong interaction between guanine and Cu^{2+}, which caused the fluorescence enhancement. The enhanced fluorescence intensity was well proportional to the concentration of guanine in the range of 4.96×10^{-8}–1.09×10^{-6} mol/L with the limit of detection 1.9×10^{-8} mol/L. The method has been applied successfully to the determination of guanine in serum and DNA samples, and the recoveries were from 96.0% to 104.8%.

1. Introduction

Guanine (G, Fig. 1A) is one of the building blocks of DNA that plays a crucial role in the storage of genetic information and protein biosynthesis [1]. The base is easily oxidized by various types of oxidants and free radicals [2,3]. Thus, its concentration level can be used to reflect the degree of oxidative damage to DNA [2]. Meanwhile, the abnormal changes of the bases in organism suggest the mutation and deficiency of the immunity system and may show the presence of various diseases [4]. In addition, guanine has widespread effects on cerebral circulation and coronary, control of blood flow, prevention of cardiac arrhythmias, modulation of adenylate cyclase activity and inhibition of neurotransmitter release [5]. Thus, the determination of guanine is a challenging and important task in the field of biological science, clinical medicine and analytical chemistry. So far, a variety of analytical methods are available for the determination of guanine in biological samples [4,6–13], such as DNA [4,6–10,13], human plasma [11,12] and urine samples [9], among which electrochemical [4,6–10,14], chromatography [11,15] and capillary electrophoresis [12,13] combined with amperometric detection have attracted extensive attention. In the above methods, chromatography generally requires complicated and expensive equipment. Electrochemical methods encounter the problem of labor-intensive electrode procedures and the poor stability of electrode.

Fluorescence method has the advantages of both sensitivity and simplicity. However, there are very little reports on the determination of guanine by using fluorescence method. Liu et al. [16] suggested a catalytic fluorimetry for guanine in urine samples based on guanine could be oxidized by hydrogen peroxide in the presence of Cu^{2+} as the catalyzer to produce 8-hydroyguanine in base medium, which possessed strong fluorescent characteristic at 397 nm. And the detection limit was 0.53×10^{-6} mol/L, but the substances such as adenine, thymine, cytosine examined have an obvious interference in determination. Kai et al. [17] described a fluorimetric method for determining guanine and its nucleosides and nucleotides based on the reaction of guanine with phenylglyoxal in a weakly acidic solution with a detection limit of 47 pmol/mL, yet the method needed to heat at 60 °C and consumed a long analysis time, and it was not used to assay any samples. At present, the optical detection and recognition have attracted wide attention in bioanalysis [18,19] and environmental monitoring [20–22] owing to the simplicity and sensitivity. Nevertheless, using fluorescent probe analysis method for determination of guanine was only presented in literature [2]. Based on the significant enhancement of fluorescence emission of CdTe fluorescence probes in the presence of adenine or guanine, a quantitative analysis method for these purines was proposed [2]. Whereas the method only can be applied to the detection in synthetic samples with the detection limit of 8.0×10^{-8} mol/L.

Among the fluorescence probe methods for various analytes, some were based on fluorescence recovery after quenching. For example, Wen et al. [23] designed a novel sensing system for the detection of sodium dodecyl sulfate (SDS) based on the

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http://dx.doi.org/10.1016/j.talanta.2014.07.044
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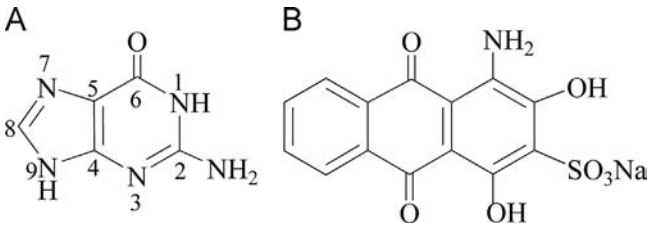

Fig. 1. . (a) Structure of guanine and (b) structure of nuclear fast red.

fluorescence recovery after the quenching of eosin Y–polyethyleneimine complexes. Patra et al. [24] reported the new optical sensors for uric acid or ascorbic acid based on the recovered fluorescence signal of curcumin and silver nanoparticle complex. Li's group [25] employed graphene quantum dots (GQDs) and a boronic acid-substituted bipyridinium salt (BBV) to build a fluorescence assay for glucose detection, which was realized via fluorescence quenching of GQDs by BBV and subsequent recovery by glucose. In this paper, we present a simple, convenient and sensitive fluorescence method for the quantitative determination of guanine based on the "off/on" fluorescent probe of Cu^{2+}–nuclear fast red (NFR, Fig. 1B). The complex of Cu^{2+} with nuclear fast red results in a dramatic quenching of the fluorescence intensity (turn-off), and it can be applied to determine guanine as a "turn-on" fluorescence probe. Nuclear fast red are dissociated from the complex with the addition of guanine due to the strong interaction between guanine and Cu^{2+}, which causes the fluorescence enhancement. The method shows the characteristics of simplicity and rapidity and the chemical reactions can be completed immediately at room temperature. Furthermore, it reveals a good selectivity owing to a higher interference level as thymine, cytosine, uracil and a certain ratio adenine can be tolerated in the system. It also displays a higher sensitivity than that of the literature [2,4–6,9,10,14,16] with the detection limit of 1.9×10^{-8} mol/L. Moreover, the method can be applied to the detection of the content of guanine in serum and DNA samples with excellent analytical performance.

2. Experimental

2.1. Apparatus

The fluorescence spectra were obtained on a FP-6500 spectrofluorophotometer (JASCO Corp.), with excitation and emission slit at 5 nm. A T6 double-beam ultraviolet–visible spectrophotometer (Beijing Pgeneral Instrument Co., Ltd., China) was used to record the absorption spectra. The pH values were measured by a pHS-3C pH meter (Hangzhou Dongxing Instrument & Equipment Plant, China).

2.2. Materials

The guanine (Sino-American Biotechnology) stock solution of 3.3×10^{-3} mol/L was prepared and kept in refrigerator at 4 °C. A 1.0×10^{-3} mol/L stock solution of nuclear fast red (Shanghai Specimen model Factory, China) was prepared by dissolving 0.0893 g of nuclear fast red and diluting to 250 mL with water. Copper sulfate was obtained from Shanghai Reagent Factory, and the stock solution (1.0×10^{-2} mol/L) was prepared by dissolving 0.6242 g copper sulfate in water. The corresponding stock solutions were diluted to acquire working solutions. Tris–HCl buffer solution was used to control acidity of the system. All of the used chemicals were of analytical grade and all solutions were prepared by doubly distilled water.

2.3. Procedures

To a 10 mL standard flask, add 0.5 mL of pH 5.8 Tris–HCl buffer solution, 0.5 mL of 1.0×10^{-3} mol/L nuclear fast red, 1.5 mL of 1.0×10^{-3} mol/L copper sulfate and a proper amount of 3.3×10^{-5} mol/L guanine working solution in turn. Then diluting it to the mark and mixing thoroughly. The fluorescence spectra of system were observed at emission wavelength of 576 nm and excitation wavelength of 555 nm with the excitation and emission slit of 5 nm. The enhanced intensity ($\Delta F=F-F_0$) was calculated (F and F_0 were fluorescence intensities of system and reagent blank, respectively).

3. Results and discussion

3.1. Fluorescence spectra

The fluorescence spectra are depicted in Fig. 2. NFR emits strong red fluorescence with the maximum emission wavelength at 576 nm owing to the large conjugated system and flat rigid structure. When Cu^{2+} was introduced to the NFR solution, the fluorescence emission intensity was greatly quenched. Subsequently, with the addition of guanine to the Cu^{2+}–NFR system, the significant fluorescence recovery was observed. And the enhanced intensity was proportional to the concentration of guanine in an appropriate rang. It suggested that the system can be used for the quantitative detection of guanine. Furthermore, it can be seen from Fig. 2 that the sensitivity of the Cu^{2+}–NFR–G system is higher than that of NFR–G system.

3.2. Binding selectivity of NFR to Cu^{2+} and the signal response to guanine

We investigated the effects of different metal ions such as Al^{3+}, Pb^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+}, Ag^{+} and Ca^{2+} on the fluorescence of NFR solution. As shown in Fig. 3A, a notable quenching effect was observed while interacting with Cu^{2+}. Furthermore, to investigate the fluorescence response of NFR-metal ion toward guanine, the metal ions mentioned above were tested (Fig. 3B). Cu^{2+} exhibits a high sensitivity and a good linear correlation because of the strong binding to guanine. Surprisedly, the reaction (Cu^{2+}–NFR–guanine) can be completed at room temperature and

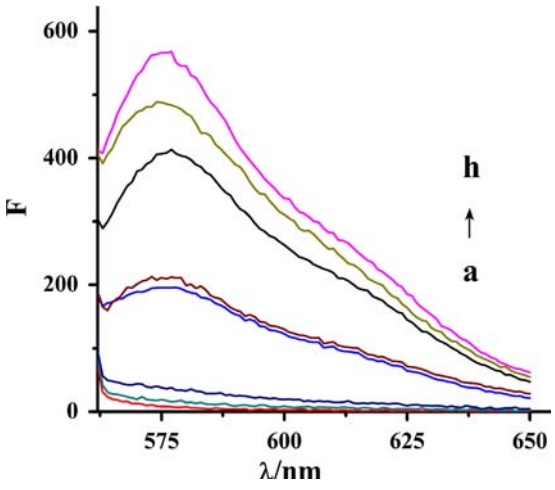

Fig. 2. Fluorescence spectra of system: (a) Cu^{2+}+Tris–HCl; (b) Tris–HCl+Cu^{2+}+G; (c) G+Tris–HCl; (d) Tris–HCl+NFR+Cu^{2+}; (e) Tris–HCl+NFR+Cu^{2+}+G (6.62×10^{-8} mol/L); (f) Tris–HCl+NFR+Cu^{2+}+G; (g) NFR+Tris–HCl; and (h) Tris–HCl+NFR+G. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8.

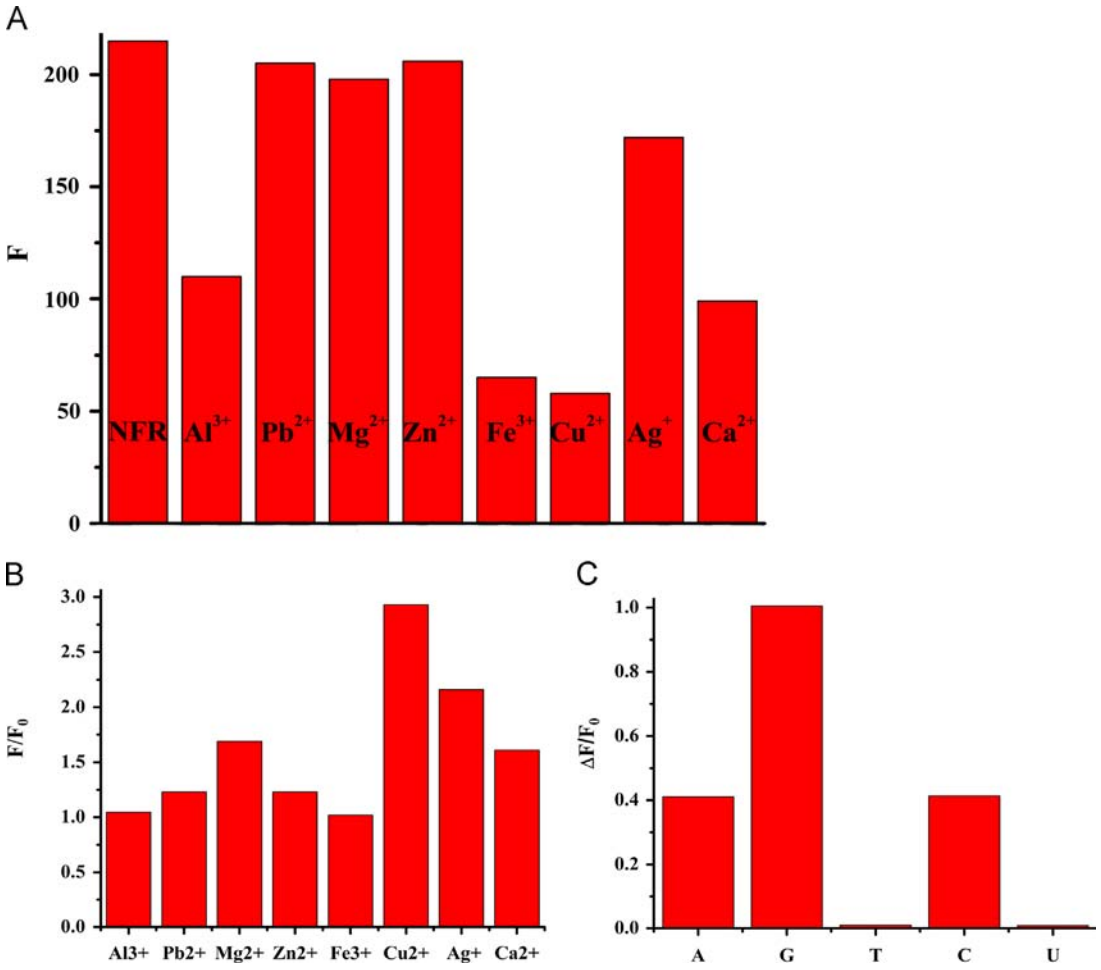

Fig. 3. (a) Fluorescence quenching of some metal ions to NFR. Conditions: M^{n+}, 1×10^{-4} mol/L; NFR,1×10^{-5} mol/L. (b) Sensitivity comparison of some metal ions–NFR–G system. Conditions: M^{n+}, 1×10^{-4} mol/L; NFR, 1×10^{-5} mol/L; G, 6.62×10^{-7} mol/mL. (c) Signal response of different bases–Cu^{2+}–NFR system. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; base(A; C; T; G; U), 6.62×10^{-7} mol/L.

the fluorescence intensity can remain stable at least for 1 h. Also, we investigated the signal response of Cu^{2+}–NFR toward other bases including adenine (A), thymine (T), cytosine (C), and uracil (U). It can be seen from Fig. 3C that a sensitive response was observed with guanine. It suggested that Cu^{2+}–NFR can be used as a selective fluorescence sensor for guanine.

3.3. Optimum experimental conditions

3.3.1. Effect of buffers and pH

The effects of some buffer solutions as HAc–NaAc, NaAc–HCl, succinic acid–sodium borate, Tris–HCl, potassium acid phthalate–NaOH and Britton–Robinson on the ΔF were studied. The results indicated that Tris–HCl buffer was the most appropriate, so it was chosen for the assay. The effect of pH on the ΔF was also investigated in the range of 5.4–6.6 and we used 5.8 as the optimum pH in this trial (Fig. 4A). Furthermore, the appropriate volume of Tris–HCl buffer solution was studied in Fig. 4B. The results indicated that the suitable amount was 0.5 mL. Hence, 0.5 mL pH 5.8 buffer was selected in this experiment.

3.3.2. Effect of adding sequence of the reagents

Several orders of adding reagents were investigated. The results showed that mixing Tris–HCl, NFR and Cu^{2+} first and then adding guanine had the higher sensitivity and the better linearity. Therefore, this order of addition was selected for this study. The results also showed that NFR reacted with Cu^{2+} to form chelate firstly.

3.3.3. Effect of NFR and Cu^{2+} concentration

The effect of the concentration of nuclear fast red is shown in Fig. 5A. It can be seen that when concentration of nuclear fast red is 5.0×10^{-5} mol/L, the ΔF value reached a maximum. So 5.0×10^{-5} mol/L nuclear fast red was used for further investigation. The influence of Cu^{2+} concentration upon ΔF was examined in the range of 1.3×10^{-4}–2.0×10^{-4} mol/L. ΔF reached the highest when the concentration of Cu^{2+} was 1.5×10^{-4} mol/L (Fig. 5B). So 1.5×10^{-4} mol/L Cu^{2+} was used for further research.

3.4. Effect of ionic strength

The effect of ionic strength on the fluorescence intensity was tested by addition of NaCl in the range of 0.0–4.0×10^{-2} mol/L (Fig. 6). The results showed that F_0, F and ΔF hardly had any variation when the NaCl concentration was lower than 2.5×10^{-2} mol/L. With further increase of NaCl concentration, F_0 presented a gradual decrease due to the heavy-atom effects, and F showed an obvious decrease result from that charge screening that weakens the combination between Cu^{2+} and guanine, which resulted in a decrease of ΔF.

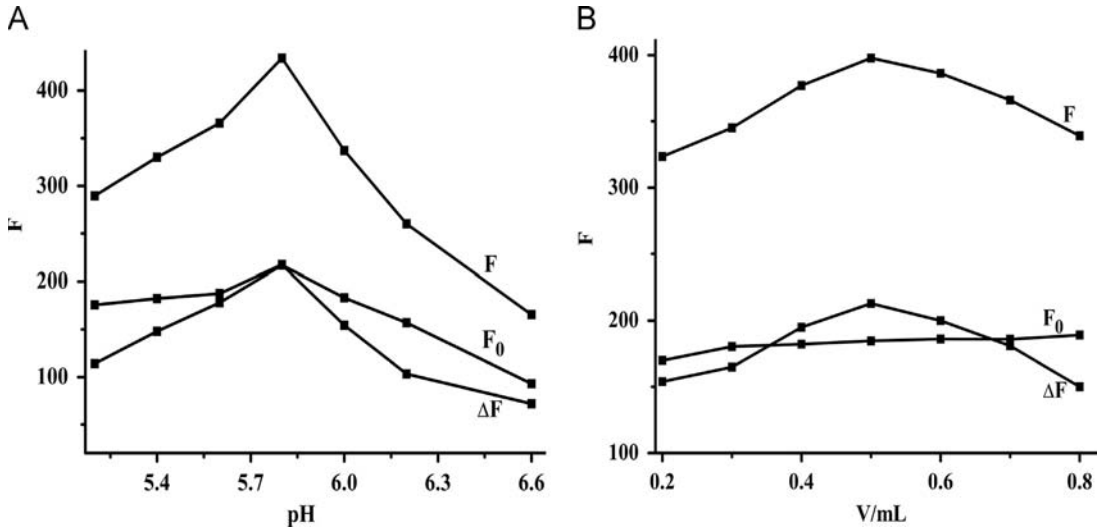

Fig. 4. (a) Effects of pH on fluorescence intensity. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/mL; λ_{ex}=555 nm; λ_{em}=576 nm. (b) Effect of Tris–HCl volumes on fluorescence intensity. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8; λ_{ex}=555 nm; λ_{em}=576 nm.

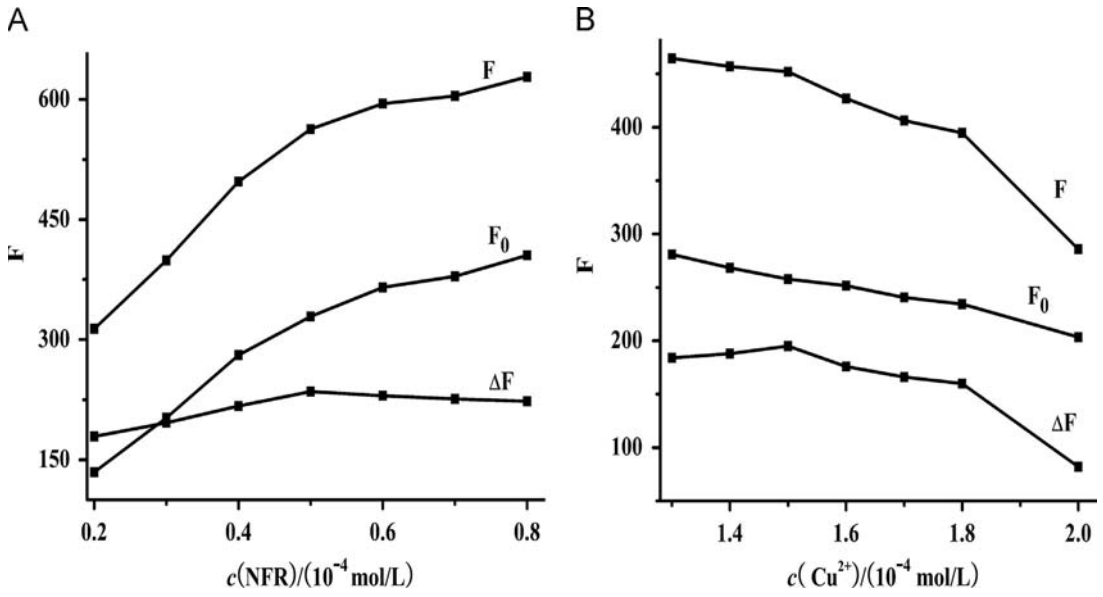

Fig. 5. (a) Effect of NFR concentration on fluorescence intensity. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8; λ_{ex}=555 nm; λ_{em}=576 nm. (b) Effect of Cu^{2+} concentration on fluorescence intensity. Conditions: NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8; λ_{ex}=555 nm; λ_{em}=576 nm.

3.4.1. The interaction of NFR, Cu^{2+} and guanine

The interaction of NFR, Cu^{2+} and guanine was investigated using the absorption spectra as shown in Fig. 7. As demonstrated in the absorption spectra of Cu^{2+}–guanine (Fig. 7A), the guanine showed a maximum absorption peak in the absence of Cu^{2+} at 260 nm. When Cu^{2+} was introduced to the guanine solution, the maximum absorption peak of guanine disappeared, indicating that Cu^{2+} have reacted with guanine. The reason is that Cu^{2+} is easy to combine with guanine N7 and O6 and form a pentacyclic compound with 1:1 coordination [26,27]. NFR solution presents two absorption bands corresponding to n–π conjugate and π–π conjugate in the ultraviolet (at 278 nm) and visible region (at 504 nm and 534 nm), respectively. With the addition of guanine to the NFR solution, the absorption intensity increased and the ultraviolet absorption profile changed. Because NFR belongs to anthraquinone compound showing the characteristics of affinity to electronics, and it is easy to interact with π electrons of the purine ring, which results in the large conjugation of electrons in NFR molecules. The location of the maximum absorption peak of NFR changed in the presence of Cu^{2+}. It indicated that Cu^{2+} and nuclear fast red formed a complex with 1:2 coordination [28]. Subsequently, a significant absorption intensity recovery in visible region was observed in the presence of guanine, indicating that the NFR molecules have been dissociated from Cu^{2+} due to the stronger interaction between guanine and Cu^{2+}, leading to the increase in dissociative NFR molecules in the system.

It can be seen from the fluorescence spectra (Fig. 2) that the fluorescence enhancement of nuclear fast red was observed in the presence of guanine. The reason for the phenomena is that the larger conjugation that existed in NFR molecules due to the electron-withdrawing property in anthraquinone can interact with π electrons of the purine ring. However, a prominent fluorescence quenching (turn-off) was surveyed owing to the non-radiative transitions in transition metal complexes as Cu^{2+} added to NFR solution [29]. When guanine existed in the Cu^{2+}–NFR system, the strong interaction between guanine and Cu^{2+} resulted in the NFR dissociated from the Cu^{2+}–NFR complex. Therefore, the fluorescence intensity increased (turn-on). The "off/on" fluorescence mechanism of Cu^{2+}–NFR–G system was inferred as Fig. 8.

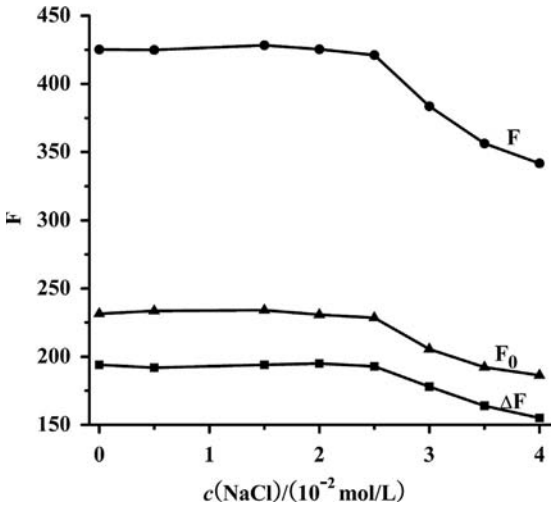

Fig. 6. Effect of ionic strength on fluorescence intensity. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8.

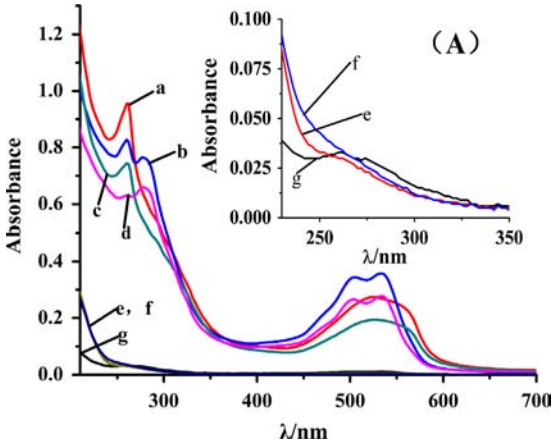

Fig. 7. Absorption spectra of system. (a) Tris–HCl+NFR+Cu^{2+}+G; (b) Tris–HCl+NFR+G; (c) Tris–HCl+NFR+Cu^{2+}; (d) NFR+Tris–HCl; (e) Tris–HCl+Cu^{2+}+G; (f) Cu^{2+}+Tris–HCl; (g) G+Tris–HCl. (A) Absorption spectra of Cu^{2+}–G. Conditions: Cu^{2+}, 1.5×10^{-4} mol/L; NFR, 5×10^{-5} mol/L; G, 6.62×10^{-7} mol/L; pH=5.8.

The quenching mechanism of nuclear fast red by Cu^{2+} could be described using the Stem–Volmer equation [30]:

$$F_0/F = 1 + K[Q] \quad (1)$$

where [Q] is the concentration of Cu^{2+}, F_0 and F are the emission intensity of NFR before and after the addition of Cu^{2+}, K is the Stem–Volmer quenching constant. The regression equation is $F_0/F=0.984+0.989\times10^4$ [Q] with correlation coefficient of 0.993 ($n=7$) and $F_0/F=0.908+0.881\times10^4$ [Q] with 0.992 ($n=7$) for 298 K and 318 K, respectively (Fig. 9). It was concluded from the curves slop that the binding constant for the complex of Cu^{2+} with NFR is 0.989×10^4 L/mol at 298 K and 0.881×10^4 L/mol at 318 K. It can be seen that the binding constant decreases with the increasing of temperature, which demonstrated that the quenching mechanism is static quenching resulting from the complex formation between NFR and Cu^{2+}.

3.4.2. Calibration graph

Under the optimum conditions, the calibration curve was constructed according to the procedure. The enhanced fluorescence intensity was well proportional to the concentration of guanine in the range of $4.96\times10^{-8}-1.09\times10^{-6}$ mol/L, and the

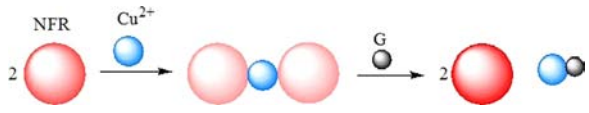

Fig. 8. Fluorescence mechanism of Cu^{2+}–NFR–G system.

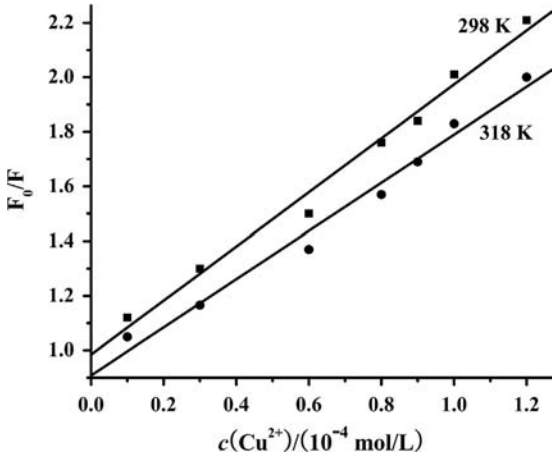

Fig. 9. Fluorescence quenching curves at 298 K and 318 K. Conditions: NFR, 5×10^{-5} mol/L; Cu^{2+}, 0.2, 0.4, 0.6, 0.8, 0.9, 1.0, 1.2 ($\times10^{-4}$ mol/L).

regression equation was $\Delta F=210.7C-2.08$ with the correlation coefficient (r) of 0.9991 ($n=8$). According to the 3σ, the detection limit was calculated to be 1.9×10^{-8} mol/L guanine. It can be seen from Table 1 that the method showed a higher sensitivity than those of the literature methods [2,4–6,9,10,14,16]. The relative standard deviation (RSD) was obtained to be 2.9% for 11 parallel determinations of 6.62×10^{-7} mol/L guanine. The lower RSD values provided a guarantee for the accurate determination of guanine.

3.4.3. Influence of foreign substances

The selectivity of the developed probe for sensitive determination of 6.62×10^{-7} mol/L guanine was analyzed and several compounds such as important biological substances and some metal ions were checked as potential interfering substances. When the relative error was less than 5%, the results are summarized in Table 2. It can be seen that most foreign substances can be tolerated at high concentrations. Besides, an amount of other nucleobases (uracil, adenine, cytosine and thymine) has almost no influence on the determination. It shows that this test presents good selectivity.

3.5. Analytical applications

3.5.1. Determination of guanine in serum and DNA samples

In order to illustrate the applicability of the proposed method to the analysis of biological samples, serum and DNA samples were assayed. (1) 2.0 mL fresh serum sample (from healthy humans) and 0.5 mL trichloroacetic acid were mixed thoroughly and centrifuged at 4000 r.p.m. for 30 min, then appropriate amount of supernatant fluid was diluted, and checked by the standard addition method. (2) The appropriate amount of DNA samples was dissolved in redistilled deionizer water and heated in a boiling water bath (100 °C) for 30 min. Finally, the sample was cooled rapidly in an ice-water bath. Standard addition method was used for assaying the prepared samples. The results for serum and DNA samples are shown in Table 3. It is thus clear that, this method

Table 1
Comparison of the proposed method with others.

Detection method	Linear range (mol/L × 10^{-6})	LOD (mol/L × 10^{-6})	r	References
Electrochemistry	2.5–150	0.75	0.9993	[4]
Electrochemical luminescence	5.0–100	1.4	0.998	[5]
Electrochemistry	1–132[a]	0.70[b]	–	[6]
Electrochemistry	2–200	0.58	0.9956	[9]
Electrochemistry	25–200	0.76	0.998	[10]
Electrochemistry	1.0–46	0.33	0.9889	[14]
Fluorometry	0.6–90	0.08	0.9993	[2]
Fluorometry	1.3–66[c]	0.53[d]	0.9926	[16]
Fluorometry	4.96×10^{-2}–1.09	1.9×10^{-2}	0.9991	This method

[a,b] Calculated according to the reported values of 0.2–20 μg/mL and 0.1 μg/mL in the literature [6].
[c,d] Calculated according to the reported values of 2.0×10^{-7}–1.0×10^{-5} g/mL and 8.0×10^{-8} g/mL in the literature [16].

Table 2
Tolerance of foreign substances.

Foreign interfering molecules	Tolerance level	Relative error (%)	Foreign interfering molecules	Tolerance level	Relative error (%)
Na^+	1000	−4.4	CO_3^{2-}	10	−3.2
K^+	700	−2.5	Glucose	300	3.3
Zn^{2+}	300	4.2	Vc	150	−2.2
Mg^{2+}	150	−2.5	Uric acid	50	3.9
Ba^{2+}	120	−3.3	Tyrosine	200	3.7
Mn^{2+}	100	−4.4	Threonine	180	2.1
Ca^{2+}	100	−2.7	Aspartic acid	150	−3.6
Ni^{2+}	80	−3.2	Tryptophan	150	−4.8
Pb^{2+}	80	−3.9	Phenylalanine	100	3.6
Fe^{3+}	8	−4.3	Serine	100	2.3
NH_4^+	150	1.7	Glutamic acid	50	−3.8
SO_4^{2-}	1000	−4.4	Glycine	40	−2.8
NO_3^-	700	−2.5	Lysine	20	3.6
Br^-	500	−3.5	Arginine	5	4.7
F^-	500	4.7	Uracil	100	−3.8
I^-	150	3.6	Thymine	70	4.1
$H_2PO_3^{2-}$	90	2.9	Cytosine	15	3.1
SO_3^{2-}	30	−4.6	Adenine	5	4.8

Table 3
Results for the determination of G in serum and DNA samples.

Samples	Founded (mol/L × 10^{-6})	Added (mol/L × 10^{-6})	Total founded (mol/L × 10^{-6})	Recovery % (n=8)	RSD % (n=8)
Serum 1	–	0.066	0.065	98.5	3.2
Serum 2	–	0.331	0.337	101.8	2.5
Serum 3	–	0.662	0.636	96.1	1.9
DNA 1	0.166	0.066	0.229	96.0	3.5
DNA 2	0.166	0. 331	0.512	104.8	3.1
DNA 3	0.166	0. 662	0.801	96.0	2.2

could be applied satisfactorily to the detection of guanine in serum and DNA samples.

4. Conclusions

Herein, we designed an efficient fluorescent probe of Cu^{2+}–nuclear fast red complex with high sensing ability for the selective detection of guanine. The complex of Cu^{2+} with nuclear fast red results in a dramatic quenching of the fluorescence intensity. Subsequently, nuclear fast red are dissociated from the complex with the addition of guanine due to the strong interaction between guanine and Cu^{2+}, which causes the fluorescence enhancement. We present the "turn-on" fluorescent probe for detecting guanine with excellent analytical performance such as wide linear response range, low detection limit, high selectivity, considerable time saving and low cost. The method can be used successfully to determine guanine in serum and DNA samples. Furthermore, this system uses the complex of metal ion binding to organic dye as fluorescent probe to measure guanine, which has a promote role in the improvement and development for the determination of purine compounds.

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