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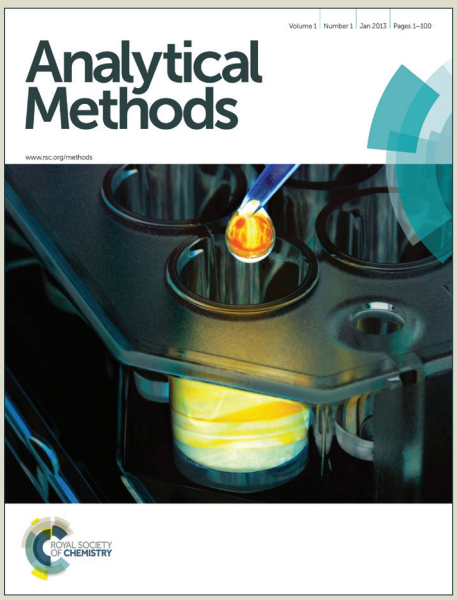

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Sensitive fluorescence "off-on" switch system for catechins detection based on water-soluble polythiophene derivatives

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Abstract

Due to catechins (CCs) play an important role in human health, we proposed a new CCs detection method relying on a novel fluorescence "off-on" switch system. Water-soluble cationic polythiophene derivatives, denoted as poly 3-{[1-(2-hydrazino-2-oxopropyl)piperidin-4-ylidene]methyl}thiophene hydrochloride (PMTH • HCl), was prepared as a novel fluorescent material. Initially, the fluorescence of PMTH • HCl can be quenched by Cu^{2+}, as the spontaneous formation of Cu^{2+} - PMTH • HCl assembling dyads, resulting in fluorescence turn off. Then, on addition of CCs in Cu^{2+} - PMTH • HCl, Cu^{2+} was separated from PMTH • HCl, producing free PMTH • HCl and stable complex Cu^{2+}-CCs, so as to turn on the fluorescence signal of PMTH • HCl. Meanwhile, the mechanisms of fluorescence quenching and recovery were discussed to provide new thoughts to design sensor based on water-soluble conjugated polymers. This fluorescence "off-on" signal was sensitive to the concentration of CCs. Under optimized condition, the fluorescence intensity was linear to CCs concentration varying from1.0×10^{-8} to 9.0×10^{-6} mol/L ($\gamma = 0.9968$), with a detection limit of 3.34×10^{-9} mol/L. Therefore, an efficient, sensitive, highly selective and practically applicable detection system for CCs has been designed.

Keywords: fluorescence; off-on switch; water-soluble polythiophene derivatives; copper ions; catechins

1. Introduction

Tea, including green and red tea, is becoming a more popular drink in the world, due to a number of bioactive chemicals, particularly rich in catechins (CCs). CCs, belonging to the family of flavonoids, have been shown to play an important role in human health with attention to their various biological and pharmacological effects [1-4], such as anti-carcinogenic activities, anti-oxidant activities, anti-inflammatory and reducing obesity. Therefore, a simple and reliable analytical method for the detection of CCs is a pressing need in developing high quality tea product. To date, many methods such as electrochemical detection [4,5], high-performance liquid chromatography [6-9] and chemiluminescence [10] have been established for assaying CCs. However, these protocols are insensitive, time-consuming and laborious. In recent years, researchers made significant progress in developing "off-on" switch system [11-15]. The "off-on" switch system could provide accurate results because of the specific adsorption and it is not restricted in the detection compared with "turn-off" [16, 17] or "turn-on" switch system [18-20].

Fluorescent probes play an important role in designing fluorescence sensors. Water-soluble conjugated polymers (CPs) [21-25] as optical transducers provided a useful platform for constructing high sensitive chemo-sensors and bio-sensors, owing to good water solubility, light-harvesting, electrical, and strong fluorescence properties. Meanwhile, CPs have the characteristics of amplification and superquenching properties [26-28], thanks to delocalized electronic structures, the rapid transfer of excitation throughout an isolated conjugated polymer chains [29] is supported. As a result, CPs offered many sensing advantages [30-34] for fluorescence sensors compared with quantum dots [35, 36] and small molecule dyes [37].

Based on the above facts, we used a novel water-soluble conjugated polymer denoted as poly

3-{[1-(2-hydrazino-2-oxopropyl)piperidin-4-ylidene]methyl}thiophene hydrochloride (PMTH • HCl) to develop a novel "off-on" switch system for the detection of CCs. In the system, the fluorescence of PMTH • HCl was efficiently quenched by the quencher Cu^{2+}, so as to turn off the fluorescence signal. Based on the stronger affinity between CCs and Cu^{2+}, addition of CCs can remove Cu^{2+} away from PMTH • HCl, resulting in fluorescent turn-on.

2. Experimental

2.1. Reagents and apparatus

All chemicals were of guaranteed analytical grade. Double distilled water was used in all experiments. CCs were purchased from Sigma-Aldrich.

Infrared spectra (IR) were recorded in potassium bromide pellets on a Perkin Elmer 1750. ^{1}H NMR and ^{13}C NMR was measured on a Bruker AV III400. Fluorescence measurements were carried out on a RF-5301 fluorescence spectrophotometer.

2.2. Synthesis of PMTH • HCl

PMTH • HCl was synthesized according to literature [38]. Firstly, 4-(thiophen-3-ylmethylidene)piperidine was used as starting material and then through nucleophilic addition reaction, hydrazinolysis reaction, and oxidative polymerization. PMTH was obtained after purification treatment by chromatography. PMTH • HCl was synthesized by acidification in chloroform. The structure is described as follows (Scheme 1).

(Scheme 1)

2.3. Fluorescence measurement

1.0 mL (1.0×10^{-5} mol/L) of Cu^{2+} was added to a solution of 1.0 mL (2.5×10^{-5} mol/L) of PMTH •HCl and 10 mL of Tris-HCl solution (pH 7.8) in10 mL colorimeter tube. Upon addition of

CCs in PMTH • HCl, fluorescence recovered. The fluorescence spectra were recorded with emission at 515 nm, excitation at 410 nm and slit width at 10 cm.

2.4 Preparation of samples

For sample analysis, 1.0 g green tea or 5.0 g sun cream was immersed in anhydrous ethanol (4:1 v/v) containing 0.10 mol/L HCl for 3 h. The mixture was filtrated and evaporated under vacuum. The residue was dissolved in double distilled water. Human serum was mixed with anhydrous ethanol (1:4 v/v), and then the mixture was allowed to stand at room temperature for 12 h. The supernatant was diluted 5 times after centrifugation.

3. Results and discussions

3.1. Fluorescence intensity changes of PMTH • HCl caused by Cu^{2+}

Fluorescence spectroscopy could be used to explain the interaction between PMTH • HCl and Cu^{2+} such as quenching mechanism. The fluorescence reduction efficiency increased quickly in the first few minutes of reaction and the quenching system could maintain at least 6 h. On addition of Cu^{2+}, the fluorescence intensity of PMTH • HCl decreased, which indicated the strong interaction of PMTH • HCl and Cu^{2+}. When the Cu^{2+} concentration was less than 1.6×10^{-7} mol/L, the fluorescence intensity was linear with the Cu^{2+} concentration (Fig. 1 (a)). Meanwhile, It could be deduced that the molar ratio between PMTH • HCl and Cu^{2+} was 1:1, approximately.

The quenching mechanisms are usually divided into static quenching and dynamic quenching, where static quenching results from the formation of non-luminous complex or intermolecular complex between fluorophor and quenching agent and dynamic quenching is due to the collision. In general, the values of quenching rate constant (K_q) were greater than the limiting diffusion rate constant of the biopolymer (2×10^{10} L/mol·s), which indicated that the quenching mechanism of

followed a dynamic quenching. In order to testify that it is dynamic quenching, Stern-Volmer equation was used:

$$I_0/I = 1 + K_{SV}[Cu^{2+}] = 1 + K_q\tau_0[Cu^{2+}] \quad (1)$$

Where I_0 and I are the fluorescence intensities in the absence and presence of quencher, respectively. K_{SV} and K_q are the Stern-Volmer dynamic quenching constant and the quenching rate constant. τ_0, the lifetime of PMTH • HCl, is 10^{-8} s, approximately. According to the linear regression plots of I_0/ I vs. [Cu^{2+}] (Fig. 1 (b)), the value of Kq is 8.98×10^{14} L/mol·s. Thus, the suitable quenching follows a static quenching.

(Fig. 1)

In order to evaluate the binding affinity or the binding constant (K), the following equation was used:

$$lg[(I_0-I)/I] = lgK + nlg[Cu^{2+}] \quad (2)$$

The linear regression plots of lg [(I_0-I)/I] vs. lg[Cu^{2+}] which were analyzed at three different temperatures (293K, 298K and 303K) yields lgK as the intercept and n (the number of bonding sites) as the slope (Fig. 2). The calculated results were shown in Table 1. The large binding constant indicated that the binding affinity is strong and Cu^{2+} was strong ion chelator to form stable complex with PMTH • HCl. Meanwhile, the value of n and the molar ratio between PMTH • HCl and Cu^{2+} testified the original description that the quenching mode was static.

(Fig. 2)

(Table 1)

There are four binding forces between macromolecule and small molecule [39]: H-bonding, Vander Waals, hydrophobic strength, and electrostatic. Vant't Hoff equation was used to study the

binding force between PMTH • HCl and Cu^{2+}:

$$ln\ (K_2 / K_1) = (\triangle H / R) \cdot (1 / T_1 - 1 / T_2) \quad (3)$$

$$\triangle S = -(\triangle G - \triangle H) / T \quad (4)$$

$$\triangle G = -RT\ lnK = \triangle H - T\triangle S \quad (5)$$

Where $\triangle H$, $\triangle S$ and $\triangle G$ are enthalpy change, entropy change and free enthalpy change, respectively. According to the views of Ross [40], The negative △H and △S values are associated with H-bonding and Van der Waals. The positive △H and △S values are characterized by hydrophobic strength. Very low positive or negative △H and positive △S values are associated with electrostatic interactions. The calculated results are revealed in Table 2. The negative $\triangle G$ value indicated that it is a spontaneous process. The negative entropy (△S) and enthalpy (△H) values indicated that the interaction forces were H-bonding and Van der Waals.

(Table 2)

3.2 Performance evalution of the fluorescence "off-on" switch system

In this type of system, PMTH • HCl was allowed to interact with the quencher (Cu^{2+}), which can be attributed to strong Cu-N binding. Upon addition of CCs to Cu^{2+} - PMTH • HCl, oxygen atom could bond with Cu^{2+} (Scheme 2). Therefore, CCs could remove Cu^{2+} away from PMTH •HCl, which lead to the recovery of PMTH •HCl fluorescence (Fig. 3). The mechanism was described in Scheme 3.

(Scheme 2)

(Fig. 3)

(Scheme 3)

The stability constants for complexes of CCs-Cu^{2+} and Cu^{2+}- PMTH • HCl have been

determined by a potentiometric titration technique [41-44]. The stability constants of CCs-Cu^{2+} and Cu^{2+}-PMTH • HCl were $10^{10.18}$ and $10^{7.50}$, respectively. Therefore, CCs were stronger chelators than PMTH • HCl to form more stable complexes with Cu^{2+}, resulting in the fluorescence signal of PMTH • HCl turn on.

When the molar ratio between CCs and Cu^{2+} was 1:1, the fluorescence recovered efficiency was maximum. The recovered fluorescence was stable. The fluorescence recovered linearly by the CCs concentration within the range of 1.0×10^{-8} to 9.0×10^{-6} mol/L (n = 5). The equation of the calibration curve was ΔI =79.615 + 11.496 c / (μmol/L), r = 0.9968, $\Delta I = I_0 - I$, where I_0 and I were the fluorescence intensity before and after the addition of CCs, respectively. Based on S/N = 3, a limit of detection (3.34×10^{-9} mol/L) was obtained.

3.3 Optimization of experimental condition

Due to hydroxyl existing in structure of CCs, pH has a great influence on sensitivity of the fluorescence "off-on" switch system. When pH was less than 4, the fluorescence of PMTH • HCl could not be quenched by Cu^{2+} or stable complexes could not be formed between Cu^{2+} and PMTH • HCl. When pH was more than 9, phenolic hydroxyl could react with base. Meanwhile, Cu^{2+} can catalyze the oxidation of CCs. The fluorescence intensity achieved maximum in the range of 7.2 to 8.6. The optimum pH 7.8 can be used in all subsequent experiments.

In order to achieve optimal fluorescence efficiency, the incubation time and temperature play important roles in designing the fluorescence "off-on" switch system. The fluorescence intensity achieved maximum value after incubating for 30 min at room temperature. Although the incubation time was shortened after rising temperature, the stable time of the system was shortened and the operation became complicated. Therefore, 30 min and room temperature were

used as the optimal experimental condition.

3.4 Specificity of the "off-on" switch system

The specificity of the system was investigated by testing the assay in response to common ions, different amino acid, starch and sucrose. The concentrations were at least 50 times greater than that of CCs (4.0×10^{-6} mol/L) under the same conditions. The results (Table 3) demonstrated that the proposed method could be applied in the detection of CCs with high specificity.

(Table 3)

3.5 Application to detection of samples

In order to demonstrate the feasibility of the new fluorescence "off-on" chemodosimeter for practical analysis, it was used to detect the recovery efficiency of different concentrations of CCs in real sample, such as human serum by standard addition methods. The recovery efficiency was in the range of 96.5% -105% and RSD was below 3.02% (Table 4).

(Table 4)

3.6 Comparison of methods

The analytical performance of the fluorescence "off-on" switch system was compared with the previously reported methods in Table 5. Compared with previous methods, the proposed method shows relatively higher sensitivity and lower detection limit.

(Table 5)

4. Conclusion

In summary, an excellent sensitive, specificity and fast detection method for CCs has been constructed by using PMTH • HCl as a fluorescence "off-on" probe. In the fluorescence "off-on" switch system, Cu^{2+} was modified with the ligand for binding with PMTH • HCl, resulting in turn off the fluorescence of PMTH •HCl. Addition of CCs leaded to the recovery of fluorescence based

on the stronger binding affinity between CCs and PMTH • HCl. The fluorescence "off-on" switch system with the limit of detection as low as 3.34×10^{-9} mol/L provides a sensitized recognition platform for CCs and possesses promising application in sample analysis.

Acknowledgments

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Figure captions

Scheme 1 Structure of PMTH • HCl.

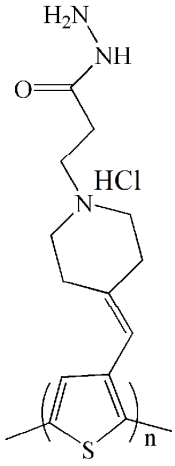
Fig. 1 (a) Plot of I_0/I vs. $[Cu^{2+}]$. [PMTH •HCl]= 2.5×10^{-6} mol/L; Tris-HCl buffer solution pH = 7.8; (b) Stern-Volmer plots of fluorescence quenching.

Fig. 2 Plot of $\lg[(I_0-I)/I]$ vs . $\lg[Cu^{2+}]$ at 303K(c), 298K(b) and 293K(a).

Fig. 3 Emission spectra of PMTH • HCl), Cu^{2+}+ PMTH • HCl and Cu^{2+}+ PMTH • HCl +CCs. a: PMTH •HCl; b: Cu^{2+}+ PMTH •HCl +CCs ; c: Cu^{2+}+ PMTH •HCl; [PMTH •HCl]=4.0×10^{-6} mol/L; $[Cu^{2+}]$ = 2.0×10^{-6} mol/L; [CCs]= 1.5×10^{-6} mol/L; Tris-HCl buffer solution pH=7.8.

Scheme 2 Binding mode of CCs toward Cu^{2+}.

Scheme 3 Schematic representations of the fluorescence “off-on” switch system for the detections of CCs.



Scheme 1

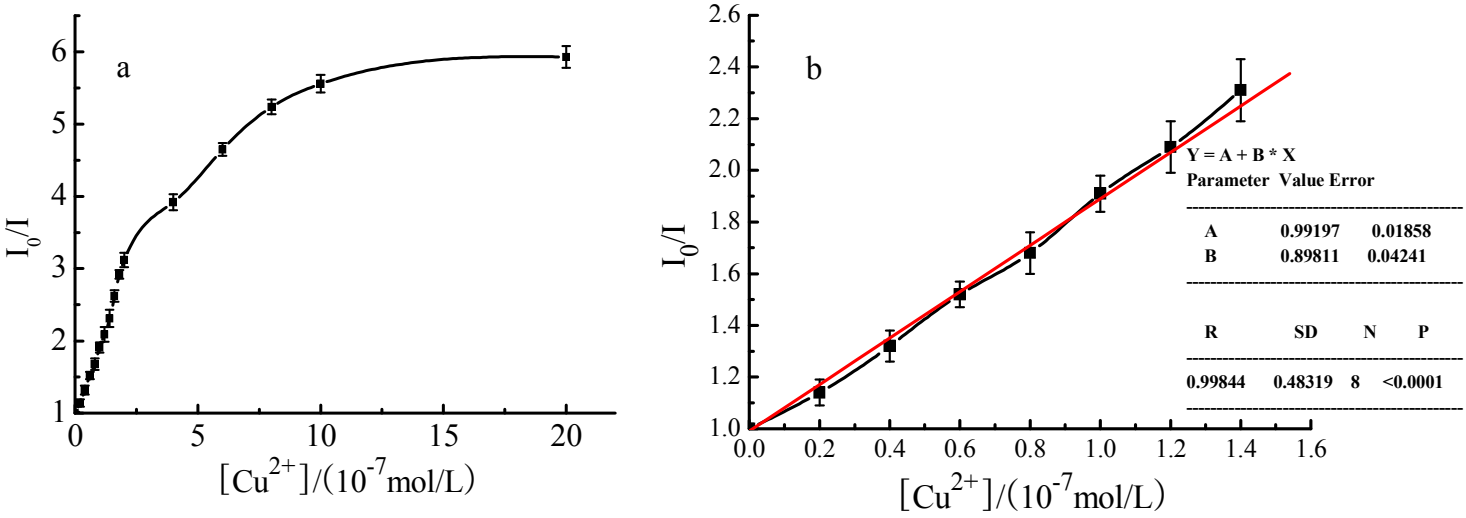


Fig. 1

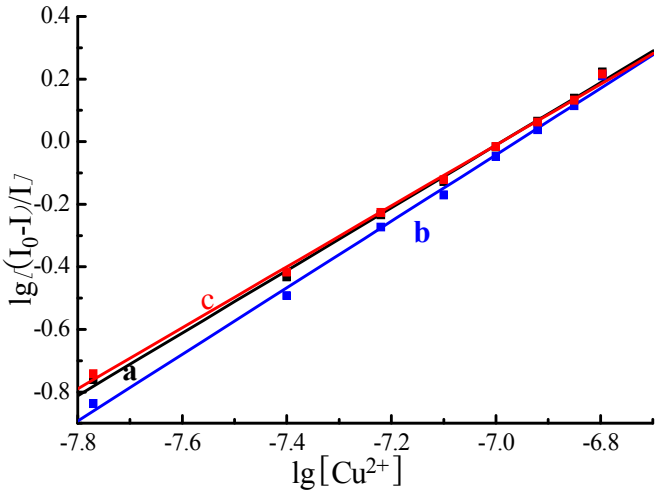


Fig. 2

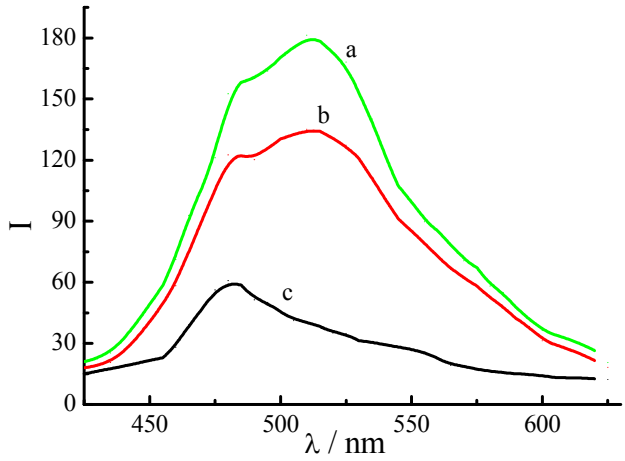
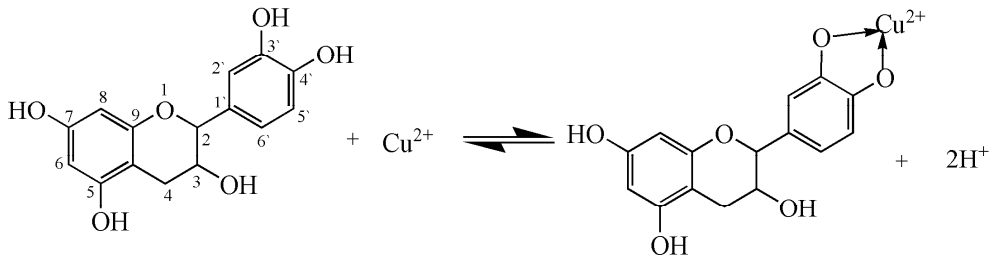
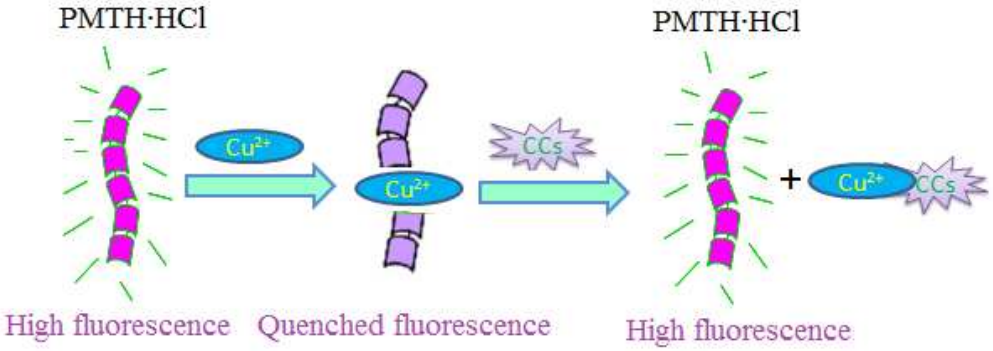


Fig. 3



Scheme 2



Scheme 3

Tables

Table 1 The quenching constant of the interaction between [Cu^{2+}] and PMTH • HCl at different temperatures.

T /(K)	K / (L/mol)	n	γ
293	3.162×10^{7}	1.048	0.9988
298	2.505×10^{7}	1.063	0.9983
303	1.950×10^{7}	1.078	0.9986

Table 2 Thermodynamic parameters of the Cu^{2+} - PMTH • HCl system.

T /(K)	ΔG/ (J/mol)	ΔS /(J/mol·K)	ΔH /(J/mol)
293	-4.207×10^{4}	-1.042×10^{7}	-3.569×10^{4}
298	-4.221×10^{4}	-1.067×10^{7}	-3.569×10^{4}
303	-4.229×10^{4}	-1.077×10^{7}	-3.569×10^{4}

Table 3 Effects of interferents

interferents	C / (mol/L)	Interference level / (%)
urea	8.0×10^{-4}	1.83
L-histidine	8.0×10^{-4}	1.45
L-serine	8.0×10^{-4}	0.90
Al^{3+}	8.0×10^{-4}	3.78
glucose	4.0×10^{-4}	2.87
sucrose	4.0×10^{-4}	1.63
L-proline	4.0×10^{-4}	0.83
Fe^{3+}	4.0×10^{-4}	1. 79
Mn^{2+}	4.0×10^{-4}	4.35
DL-alanine	2.0×10^{-4}	2.94
L-tryptophan	2.0×10^{-4}	4.12
L-arginine	2.0×10^{-4}	1.79
L-cysteine	2.0×10^{-4}	2.57

Table 4 Determination of CCs in real samples (n = 9)

Sample	Amount found (μmol/L ± RSD%)	Added (μmol/L	Recovered (μmol/L ± RSD%)	Recover (%)
Green tea extract 1	2.92 ± 1.56	2.0	5.02 ± 1. 06	105.0
		4.0	6.85 ± 1.98	98.25
Green tea extract 2	1.82 ± 1.22	2.0	3.75 ± 1.12	96.5
		4.0	5.93 ± 2.09	102.75
Human serum	0.28 ± 2.26	2.0	2.23 ± 3.02	97.5
		4.0	4.41 ± 1.62	103.25

Table 5 Comparison of analytical parameters for the determination of CCs

Methods	Reagents or condition	Linear range (mol/L)	Detection limit (mol/L)	Reference
fluorimetry	PMTH·HCl	1.0×10^{-8} to 9.0×10^{-6}	3.34×10^{-9}	This paper
electrochemical	multiwalled carbon nanotube paste electrode	1.0×10^{-7} to 2.69×10^{-6}	1.70×10^{-8}	4
electrochemical	ionic liquid, n-octylpyridinium hexafluorophosphate carbon paste electrode	5.0×10^{-7} to 1.25×10^{-5}	1.32×10^{-7}	5
HPLC	monolithic column	1.62×10^{-6} to 2.60×10^{-6}	3.57×10^{-7}	6
HPLC	solid-phase extraction	1.0×10^{-8} to 1.0×10^{-6}	1.0×10^{-7}	7
HPLC	sulfated-b-cyclodextrin	4.06×10^{-6} to 3.25×10^{-4}	3.57×10^{-7}	8
HPLC	liquid chromatography in tandem mass spectrometry	1.09×10^{-8} to 1.379×10^{-6}	2.60×10^{-9}	9