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A new fluorescence "off–on" chemodosimeter for L-cysteine based on water-soluble polythiophene

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ABSTRACT

A novel water-soluble cationic conjugated polymer, denoted as poly 2,5-[3-(1,1-dimethyl-4-piperidine methylene)thiophene] chloride (PDPMT-Cl) was a novel fluorescent material. Fluorescence can be quenched by $[AuCl_4]^-$ effectively. On addition of L-cysteine (L-Cys) in $[AuCl_4]^-$-PDPMT-Cl, fluorescence recovered. A new method to detect L-Cys was established successfully by designing a fluorescent "off-on" probe. The method showed good sensitivity and selectivity. Under optimized condition, the fluorescence intensity was linear to L-Cys concentration varying from 1.0×10^{-8} M to 6.0×10^{-5} M (γ=0.9982). The detection limit (3σ) was 1.39×10^{-10} M. The method was successfully used for the determination of L-Cys in human serum and compound amino acid injection.

1. Introduction

L-Cys, a kind of sulfhydryl amino acid has many physical functions such as involve in protein synthesis, detoxification and metabolic. It can also protect the liver cells and promote the action of liver function. The variation of L-Cys concentration in organisms can cause some disease, such as renal failure, Alzheimer's disease, neural tube defects and coronary heart disease [1–4]. Therefore, there is a pressing need to develop a device that can rapidly measure the L-Cys concentration.

Currently, many analytical techniques were described in the literature for the determination of L-Cys, such as electrochemical techniques [5,6], flow injection analysis (FIA) [7–10], high performance liquid chromatography (HPLC) [11–15], spectrophotometry [16–18], and spectrofluorimetry [19–22], etc. Spectrofluorimetry has attracted great interests of researchers because of the advantage of high sensitivity, low cost and easy operation. Spectrofluorimetry is used widely in biochemistry analysis. Recently, more attention on fuorescent sensor [23–25] has been received. Whereas, most of the sensors were constructed by designing a fluorescence turn-on probe [26–28] or they exhibited signal output of fluorescence emission quenching [29–31]. The construction of fluorescent chemosensors with emission "off-on" response based on water-soluble polythiophene derivatives is very novel.

Many materials, small molecule [32], and quantum dot [33,34] have been used as fluorescent sensor. Polymer [35–37] began to be using as sensing materials both in chemistry and biology in recent years. Polymer appear promising in determining biological macromolecule and small molecule [38–40] as a new type of fluorescent sensor due to their high molar absorption coefficient, high fluorescence quantum yield, good chemical stability and thermal stability. Among these polymers, water-soluble conjugated polymers [41] with ionic charges can be used to amplify fluorescence signals in aqueous solution and have drawn a great deal of attention as fluorescent biomolecular sensory materials.

In our present study, we used a novel water-soluble cationic conjugated polymer denoted as poly 2,5-[3-(1,1-dimethyl-4-piperidine methylene) thiophene]chloride (PDPMT-Cl) with excellent photoelectric properties [42,43]. We developed a new type of fluorescence "off-on" probe for L-Cys. Fluorescence can be quenched by $[AuCl_4]^-$ effectively. Upon addition of L-Cys to the $[AuCl_4]^-$-PDPMT-Cl system, the fluorescence emission restored, due to the stronger binding ability between L-Cys and $[AuCl_4]^-$.

2. Experimental

2.1. Reagent and apparatus

L-Cys was obtained from nopharm Chemical Reagent. Other chemicals and solvents were of guaranteed analytical grade. Double distilled water was used in all experiments. Buffer solution was prepared for pH 7.8–8.6 with NaH_2PO_4–NaOH.

Fluorescence measurements were performed on a RF-5301 fluorophotometer (Shimazu, Japan). UV spectra were recorded using TU-1901 ultraviolent and visible spectrophotometer (Shanghai, China).

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2.2. Synthesis of PDPMT-Cl

PDPMT-Cl was synthesized according to literature [43]. Firstly, 3-methylthiophene was used as starting material and then through bromination, Witting–Horner reaction, and methylation. The monomer was obtained. The polymer was synthesized by oxidative polymerization in chloroform using $FeCl_3$ as oxidant. The structure is described as follows (Scheme 1).

2.3. Analytical procedure

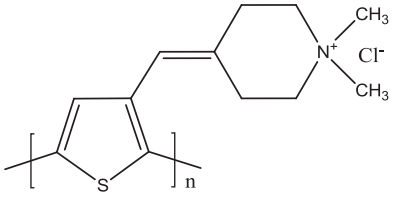
To 10 mL colorimeter tube, PDPMT-Cl (1.00 mL, 2.0×10^{-4} M) and $[AuCl_4]^-$ (2.00 mL, 2.0×10^{-5} M) were added and diluted with 0.5 mL buffer solution (pH 8.2). The fluorescence was quenched. Then L-Cys was added, the solution was allowed to stand at room temperature for 10 min. The fluorescence spectra were recorded at $\lambda_{ex}/\lambda_{em}$=455/600 nm. The width of slit was 10 cm for the absorption spectra.

For sample analysis, compound amino acid injection was diluted 1000 times by double distilled water. In order to avoid the interference of protein in human serum, human serum was mixed with anhydrous ethanol (1:4 v/v). After well mixed, the solution was allowed to stand at room temperature for 12 h. The supernatant was diluted 5 times after centrifugation.

3. Result and discussions

3.1. Fluorescence response of PDPMT-Cl to metal ions

Fluorescent experiments were carried out with different ions (Ru^{3+}, Cd^{2+}, Cu^{2+}, Rh^{3+}, $[AuCl_4]^-$, $[PtCl_4]^{2-}$). Fig. 1 showed the fluorescence response toward different ions. As can be seen from Fig. 1, fluorescence was quenched effectively by $[AuCl_4]^-$ and $[PtCl_4]^{2-}$. The system was more stable with the existence of $[AuCl_4]^-$. Therefore, $[AuCl_4]^-$ was used as quenching ion.



Scheme 1. Structure of PDPMT-Cl.

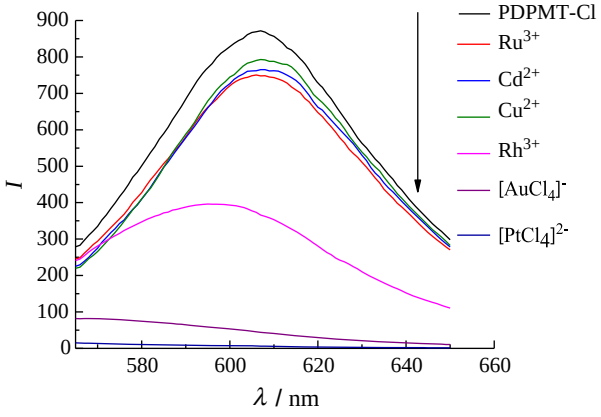


Fig. 1. Fluorescence emission spectra of PDPMT-Cl upon addition of different metal ions. [PDPMT-Cl]=4.0×10^{-5} M; NaH_2PO_4-NaOH buffer solution pH=8.2.

3.2. The interaction between $[AuCl_4]^-$ and polymer

Fluorescence response of PDPMT-Cl in the presence of $[AuCl_4]^-$ in various concentrations was investigated. The fluorescence intensity decreased upon the addition of $[AuCl_4]^-$. The relative fluorescence intensity was linear to the concentration of $[AuCl_4]^-$ when the concentration was below 4.0×10^{-6} M. Fig. 2 showed the calibration curve. It could be deduced that the molar ratio between PDPMT-Cl and $[AuCl_4]^-$ was 1:1.

The process of fluorescence quenching is usually divided into static quenching and dynamic quenching. Static quenching is due to the formation of non-luminous complex or intermolecular complex between quenching agent and fluorophor, which result in the decrease of the fluorescent intensity. Dynamic quenching results from collision between quenching agent and fluorophor. In order to prove dynamic quenching, Stern–Volmer equation was used

$$I_0/I = 1 + K_{SV}[[AuCl_4]^-] = 1 + K_q\tau_0[[AuCl_4]^-] \quad (1)$$

where I_0 and I are the fluorescence intensities in the absence and presence of quenching agent, respectively. K_{SV} is the Stern–Volmer dynamic quenching constant. K_q and τ_0 are the quenching rate constant and the lifetime of polymer. For PDPMT-Cl, τ_0 is known to be approximately 10^{-9} s [44]. The results were showed in Fig. 2 that the values of K_{SV} and K_q are 4.26×10^5 L mol^{-1} and 4.26×10^{14} L mol^{-1} s^{-1}, respectively (γ=0.9939). The values of K_q were much greater than the limiting diffusion rate constant (2×10^{10} L mol^{-1} s^{-1}) between macromolecules and small molecules. Therefore, the suitable way of quenching of PDPMT-Cl caused by $[AuCl_4]^-$ follows a static quenching.

For static quenching, we used Scatchard equation

$$\lg[(I_0-I)/I] = \lg K + n\lg[[AuCl_4]^-] \quad (2)$$

where K is the binding constant. Fig. 3 showed the linear regression plots of lg [$(I_0-I)/I$] vs. lg [$[AuCl_4]^-$] at two different temperatures (293 K and 303 K). The calculated results were revealed in Table 1. The binding constant is very large, which indicated that the binding affinity is strong. Therefore, in order to recover fluorescence, the material should have the stronger binding affinity with $[AuCl_4]^-$ than $[AuCl_4]^-$-PDPMT-Cl.

There are four interaction forces between macromolecule and small molecule [45]: H-bonding, hydrophobic strength, Van der Waals and electrostatic. According to the views of Ross [46], the positive ΔS (entropy change) and very low ΔH (enthalpy) values are associated with electrostatic interactions. The positive ΔS and ΔH values are characterized by hydrogen bonding. The negative ΔS and ΔH values are characterized by H-bonding and Van der Waals.

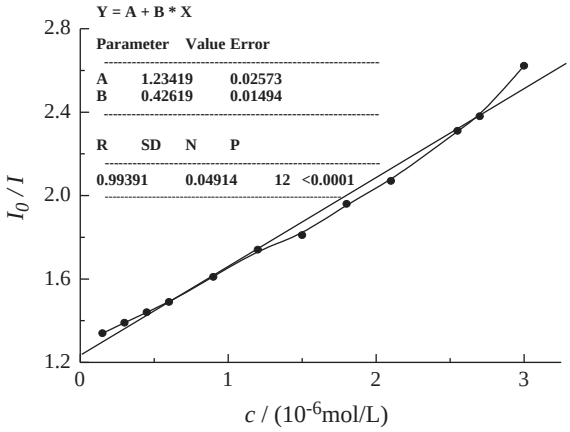


Fig. 2. Stern–Volmer plots of fluorescence quenching.

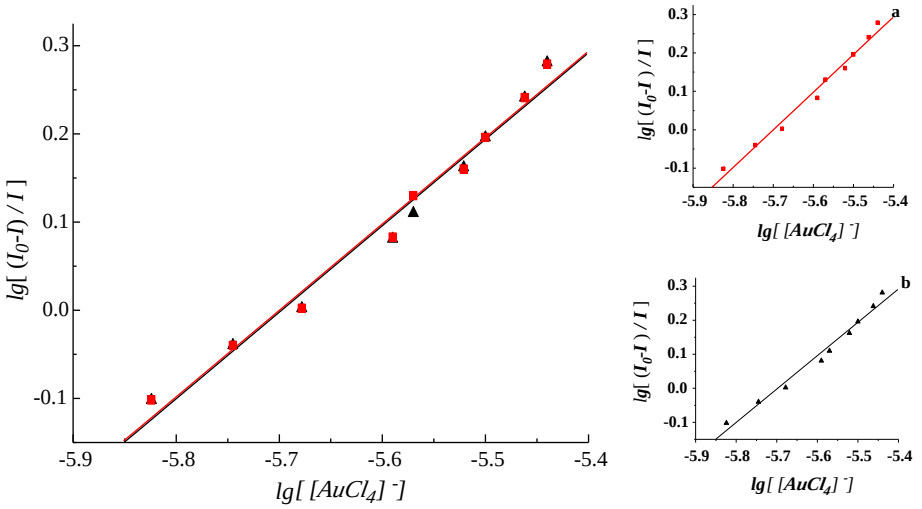


Fig. 3. Plot of lg [(I_0–I)/I] vs. lg [[$AuCl_4$]$^-$] at 293 K (a) and 303 K (b).

Table 1
The quenching constant of the interaction between [$AuCl_4$]$^-$ and PDPMT-Cl at different temperatures.

T/K	K/(L mol^{-1})	n	γ
293	3.865×10^5	0.9802	0.9915
303	3.866×10^5	0.9806	0.9901

In order to study the interaction force between PDPMT-Cl and [$AuCl_4$]$^-$, Vant't Hoff equation were used

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

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

The thermodynamic parameters, ΔS, ΔH and ΔG (free enthalpy change), calculated are revealed in Table 2. The values of ΔS and ΔH indicated that the interaction forces were electrostatic interactions.

3.3. Fluorescence response of [$AuCl_4$]$^-$-PDPMT-Cl to L-Cys

The fluorescence responses could be restored clearly upon addition of L-Cys to [$AuCl_4$]$^-$-PDPMT-Cl (Fig. 4). Upon addition of L-Cys, the ability of complexation between L-Cys with –SH and gold ion were powerful. Therefore, [$AuCl_4$]$^-$ and L-Cys can form more stable compound. The mechanism was depicted in Scheme 2.

3.4. Optimization of pH, the time of incubation and temperature

The pH effect on the fluorescence response was investigated by measuring the fluorescence intensity. The fluorescence intensity increased gradually from 7.8 to 8.2 and after achieving the maximum current at pH 8.2, the fluorescence intensity decreased, indicating that the optimum pH 8.2 can be used in all subsequent experiments.

After adding L-Cys to [$AuCl_4$]$^-$-PDPMT-Cl system, the fluorescence intensity gradually recovered. The fluorescence intensity achieved maximum value after incubating for 10 min at room temperature. And the fluorescence intensity remained stable for 4 h. The sensitivity could not be improved after rising the temperature, and the operation of experiment became complicated. Therefore, the optimum time of incubation and temperature were 10 min and room temperature.

Table 2
Thermodynamic parameters of [$AuCl_4$]$^-$-PDPMT-Cl.

T (K)	ΔG (J mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔH (J mol^{-1})
293	-3.13×10^4	106.9	18.7
303	-3.24×10^4	107.0	

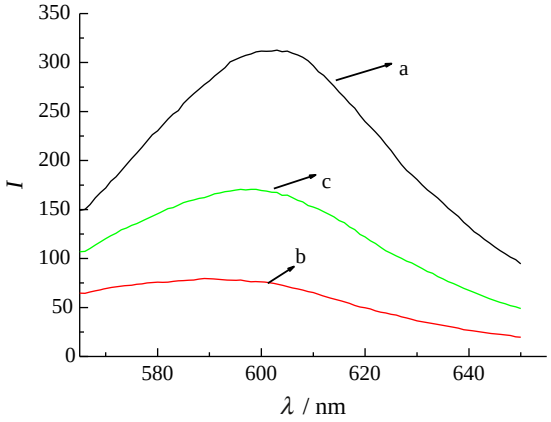


Fig. 4. Emission spectra of PDPMT-Cl, PDPMT-Cl+[$AuCl_4$]$^-$, PDPMT-Cl+[$AuCl_4$]$^-$+L-Cys; (a): PDPMT-Cl; (b): PDPMT-Cl+[$AuCl_4$]$^-$; (c): PDPMT-Cl+[$AuCl_4$]$^-$+L-Cys; [PDPMT-Cl]$=4.0 \times 10^{-5}$ M; [[$AuCl_4$]$^-$]$=2.0 \times 10^{-5}$ M; [L-Cys]$=1.0 \times 10^{-5}$ M; NaH_2PO_4-NaOH buffer solution pH$=8.2$.

3.5. Linear range and the limit of detection

To investigate the efficiency of the recovery of fluorescence, different concentration of L-Cys were added to [$AuCl_4$]$^-$-PDPMT-Cl system. Under the optimum condition, the calibration curve was constructed by plotting ΔI ($\Delta I = I_0 - I$) against L-Cys concentration, where I_0 and I were the fluorescence intensity before and after the addition of L-Cys, respectively. The fluorescence intensity increased as the concentration of L-Cys increased. When the molar ratio between L-Cys and [$AuCl_4$]$^-$ was 2:1, ΔI was maximum and stable. The linear range was found to be from 1.0×10^{-8} to 6.0×10^{-5} M with a correlation coefficient of 0.9982. The linear regression equation: $\Delta I = 18.21 + 60.89$ c/(μM). The limit of detection was 1.39×10^{-10} M.

3.6. Interference effects

The effect of interferents was studied on the fluorescent responses of the sensor in the presence of 1.0×10^{-5} M L-Cys. The interference effects of amino acid, starch, and sucrose, etc, were investigated. The effects were depicted in Table 3. The results indicated that the system exhibited good selectivity.

3.7. Real sample analysis

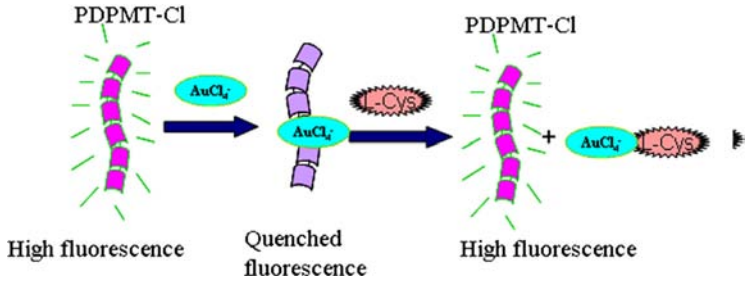
To evaluate the applicability of the proposed method, it was used for L-Cys assay in real sample, such as human serum. The analytical results were shown in Table 4. Recovery experiments were carried out at the same time. The recovery efficiency was in the range of 95.6%–104.1%. The calculated RSD were below 3.26%. The results obtained were satisfactory.

3.8. Comparison of methods

The proposed method for the determination of L-Cys 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.

4. Conclusion

In this study, a new method for the detection of L-Cys was successfully constructed by using PDPMT-Cl as a fluorescence "off-on" probe. The fluorescence can be quenched effectively by



Scheme 2. Schematic representations of the detections for L-Cys.

Table 3
Effects of interferents.

Interferents	C/(M)	Interference level/(%)
Starch	1.0×10^{-4}	0.89
ATP, L-proline	1.0×10^{-4}	2.02
L-histidine	1.0×10^{-4}	1.00
Glucose	1.0×10^{-4}	4.51
DL-alanine, L-serine	1.0×10^{-4}	3.97
L-phenylalanine, L-tryptophan	1.0×10^{-4}	4.26
DL-cysteine	1.0×10^{-4}	0.74
L-arginine	1.0×10^{-4}	3.67
Na^+, K^+	1.0×10^{-3}	0.62
Mg^{2+}, Fe^{3+}	1.0×10^{-3}	1.90
Ca^{2+}	1.0×10^{-3}	3.03
Al^{3+}	1.0×10^{-3}	1.26
BSA, threonine	1.0×10^{-4}	2.48
Citric acid, ascorbic acid	1.0×10^{-4}	2.16
Lactic acid, vitamin B6	1.0×10^{-4}	1.05
Cu^{2+}, Ni^{2+}, Co^{2+}	1.0×10^{-3}	0.92
F^-, Cl^-, CO_3^{2-}, NO_3^-	1.0×10^{-3}	0.32
Sucrose	1.0×10^{-3}	0.68

Table 4
Determination results of real samples ($n=9$).

Sample	Amount found (μM ± RSD%)	Labeled (μM)	Added (μM)	Recovered (μM ± RSD%)/(μg L^{-1})	Recover (%)
Amino acid injection 1	2.16 h ± 0.98	2.06	5.00	5.12 ± 1.28	102.4
(Diluted 1000 times)			10.0	10.41 ± 2.16	104.1
Amino acid injection 2	0.831 ± 1. 86	0.825	5.0	5.19 ± 1.62	103.8
(Diluted 1000 times)			10.0	9.78 ± 2.16	97.8
Human serum 1	13.28 ± 2.26	12.6	5.0	4.78 ± 3.26	95.6
			10.0	10.15 ± 1.22	101.5
Beer	88.22 ± 2.89		5.0	4.86 ± 2.19	97.2
			10.0	9.78 ± 1.82	97.8
Honey	212.36 ± 1. 62		5.0	5.16 ± 1.89	103.2
			10.0	9.85 ± 2.89	98.5

Table 5
Comparison of analytical parameters for the determination of L-Cys.

Methods	Reagents or condition	Linear range (M)	Detection limit (M)	Reference
Fluorimetry	PDPMT-Cl	1.0×10^{-8}–6.0×10^{-5}	1.39×10^{-10}	This paper
Electrochemical	Boron-doped carbon nanotube/glassy carbon electrode	7.8×10^{-7}–2.0×10^{-4}	2.6×10^{-6}	[5]
Electrochemical	Chemisorptions reactions	1.0×10^{-6}–6.0×10^{-6}	5.0×10^{-7}	[6]
FIA	Autocatalytic sodium sulfite/hydrogen peroxide reaction	5.0×10^{-8}–2.5×10^{-6}	1.8×10^{-8}	[9]
FIA	Electrocatalytic oxidation at pretreated platinum electrode	4.0×10^{-7}–4.0×10^{-5}	1×10^{-7}	[8]
HPLC	Postcolumn reagents; gold nanoparticles	5.0×10^{-6}–5.0×10^{-5}	5.9×10^{-9}	[13]
Spectrophotometry	Fourier transform infrared spectrometry	5.0×10^{-5}–2.5×10^{-3}	1.65×10^{-5}	[18]
Spectrofluorimetry	Fluorescence intensity enhancement	1.0×10^{-8}–8.0×10^{-7}	3.8×10^{-9}	[22]

$[AuCl_4]^-$. $[AuCl_4]^-$ could form complex with L-Cys. Therefore, the fluorescence could be restored after the addition of L-Cys. And the intensity of fluorescence was linear to the concentration of L-Cys. The proposed method showed high sensitivity, selectivity and speed, and was appropriated for L-Cys detection, including in real sample analysis.

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