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A sensitive method for the detection of catecholamine based on fluorescence quenching of CdSe nanocrystals

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Abstract

A sensitive method for the detection of catecholamine based on the fluorescence quenching of CdSe nanocrystals was developed. The sodium citrate-protected CdSe nanocrystals were synthesized in water solution. The fluorescence quenching of CdSe nanocrystals by dopamine, uric acid, ascorbic acid and catechol was studied; the results showed that all of these four kinds of compounds could quench the fluorescence of nanocrystals, and the quenching constant was 6.3×10^4, 2.57×10^3, 2.14×10^3 and 1.168×10^3, respectively. The order of sensitivity for the biosensor was: dopamine > lactic acid > ascorbic acid > catechol. This method shows good selectivity for dopamine, the detection limit reaches 5.8×10^{-8} M.

Keywords: CdSe nanocrystals; Fluorescence quenching; Catecholamine; Fluorescence detection

1. Introduction

Determination of catecholamine and their metabolites in body fluids such as urine, plasma and serum plays an important role in evaluating the activity of the sympathetic nervous system and supporting the diagnosis of many diseases. A lot of methods have been developed to detect catecholamine such as electrochemical [1–4], HPLC, capillary electrophoresis and fluorescence methods [5–9]. Fluorescence method attracted many attentions since it was a high sensitive method, such as 1,2-dipentylethylene diamine [10], ethylene diamine [11], *N*-hydroxysuccinimidyl-3-indolylacetate [12] and 1,2-bis (3-chlorophenyl) ethylenediamine [13] have been used as fluorescence derivation reagents for determining catecholamines. Terbium ion used as fluorescence probe for the detection of catecholamine has also been developed [14].

With the development of nanotechnology, many methods have been developed to detect catecholamine by using nanomaterials such as carbon nanotubes [15,16] and gold nanoparticles [17,18]. Semiconductor nanoparticles have attracted much attentions since they have unique optical and several other properties such as size-dependent, tunable adsorption and emission properties [19]. Fluorescence detection methods have led to major improvement in bioanalytical applications because of their extraordinary sensitivity and selectivity [20]. The traditional fluorescence probes used are organic dyes; semiconductor nanocrystals could probably be used to substitute the organic dyes based on their unique optical properties. A lot of methods have been developed for bioimaging [21–23], chemo [24,25] and biosensor [26,27] using the unique fluorescence properties of semiconductor nanocrystals.

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample, which has been widely studied both as a fundamental phenomenon and as a source of information about biochemical systems. Especially, one sensitive method for the detection of heme-containing proteins such as cytochrome *c*, hemoglobin and myoglobin based on the fluorescence quenching of conjugated polymer has been developed by Fan et al. [28], which opened a new area in fluorescence sensors. Based on their research, we have

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developed one sensor for the detection of electron transfer proteins using citrate-protected CdSe nanocrystals as sensitive fluorescence probe [29].

In this paper, we found that catecholamine could also quench the fluorescence of CdSe nanocrystals and the CdSe nanocrystals could also be used as sensitive fluorescence probe for the detection of catecholamine. The possible fluorescence quenching mechanism was explored. Four compounds could quench the fluorescence of CdSe nanocrystals, while good selectivity and sensitivity was acquired for dopamine by this method.

2. Experiments

2.1. Reagents and instrument

Selenium power, $CdCl_2$ and $NaBH_4$ were purchased from Acros. Dopamine and catechol, lactate acid and ascorbic acid were purchased from Fluka. All other reagents were reagent grade and used without further purification. Water used for preparation of aqueous solution was purified using Millipore Milli-Q water purification system.

UV–vis absorbance spectrometric was collected by a Cary 50 UV–vis–NIR Spectrophotometer (Varian, USA) and the fluorescence spectroscopy was measured by a Perkin-Elmer LS 50 B luminescence spectrometer.

2.2. Preparation of NaHSe

NaHSe solution was prepared according to the reported method [30]. Simply, 80 mg $NaBH_4$ was transferred into a small flask, then 1 ml deoxygenized water was added, after the solution was cooled with ice-water, 79.9 mg selenium power was added at continually stirring; the mixture was stirred for 1 h. The final solution was diluted with deoxygenized water and used for further experiment.

2.3. Preparation of citrate-protected CdSe nanocrystals

Citrate-protected CdSe nanocrystals were prepared using the reported method [31] with minor revision. Simply, 200 mg sodium citrate and 58.6 mg $CdCl_2$ were dissolved in 200 ml water; the pH of mixture was adjusted to 9.5 using 1 M NaOH, the mixture was bubbled with N_2 atmosphere for 20 min and then 800 μl 0.1 M NaHSe (diluted to 6 ml with water) was added drop by drop under continually stirring. The final solution was refluxed under N_2 atmosphere for 3 h.

2.4. The fluorescence quenching of CdSe nanocrystals by dopamine, catechol, lactic acid and ascorbic acid

Appropriated amounts of dopamine, catechol, lactic acid and ascorbic acid were added to each nanocrystals solution and the mixtures were vigorously stirred for 1 min before fluorescence measurement.

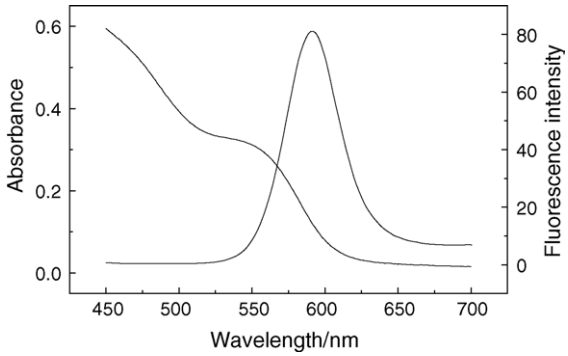

Fig. 1. UV–vis absorbance and fluorescence spectra (excited at 390 nm) of CdSe nanocrystals.

3. Results and discussions

3.1. UV–vis and fluorescence spectroscopy of CdSe nanocrystals

The growth of the CdSe nanocrystals can be identified from both the color change and the UV–vis spectra of the as-prepared products. The nanocrystals were orange-red after the reflux. The UV–vis absorbance and fluorescence spectra of nanocrystals were shown in Fig. 1; the nanocrystals show well-defined 1s–1s electronic transitions in the absorption spectroscopy, the absorbance edge of nanocrystals was 611 nm and the band gap was calculated to be 2.03 eV. The absorbance edge blue-shifts 101 nm compared with bulk CdSe (the band gap is 1.74 eV, and the absorbance edge is 712 nm). The fluorescence spectroscopy shows that the nanocrystals exhibit narrow near-band-edge or shallow trap emission, the emission spectroscopy is narrow and the stokes shift small suggests that the surface of these nanocrystals are relatively flat and regular, the fluorescence emission maximum is located at 591 nm. The nanocrystals are spherical in shape with the zinc blend structure.

3.2. The fluorescence quenching of CdSe nanocrsytals by catechol

If the CdSe nanocrystals were irradiated with photons of energy greater than its band gap, it would result in the promotion of electrons from the valence band (VB) to the conduction band (CB) of particles. The outcome of this process is a region of positive charge termed a hole (+) in the VB and a free electron in the CB. The whole process is shown in Eq. (1):

$$\mathrm{CdSe} + h\nu \rightarrow \mathrm{CdSe} + \mathrm{e}^{-1}(\mathrm{CB}) + h^{+}(\mathrm{VB}) \quad (1)$$

Without the presence of electron acceptor, the electron and hole would recombine and emit the fluorescence. Catechol molecular could adsorb on the surface of CdSe nanocrystals and be directly oxidized by the excited nanocrystals, the

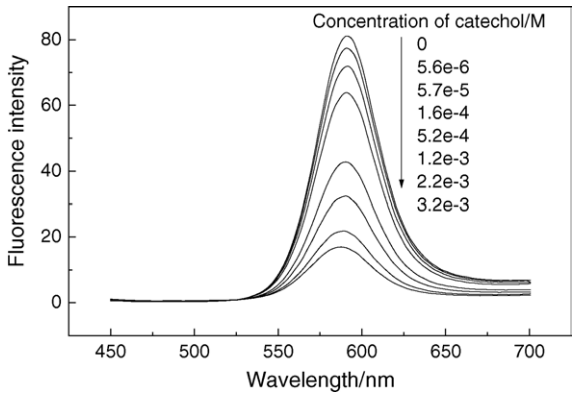

Fig. 2. Fluorescence spectra of CdSe nanocrystals in the absence and presence of different concentration of catechol.

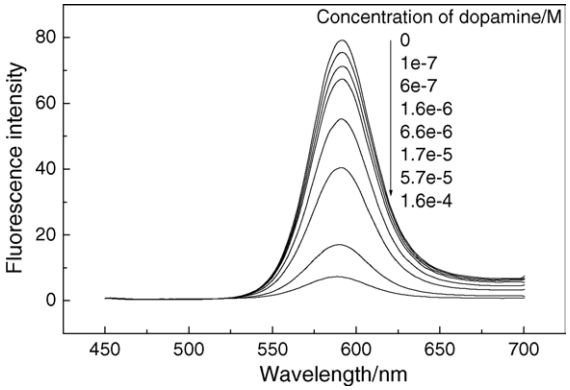

Fig. 4. Fluorescence spectra of CdSe nanocrystals in the absence and presence of different concentration of dopamine.

oxidized process is described as followings:

$$C_6H_6(OH)_2 + h^+ \rightarrow {}^{\bullet}C_6H_6 - (OH)_2{}^{\bullet+} \quad (2)$$

Eq. (2) shows that the hydroxyl groups of catechol are traps of the holes, which could block the recombination of electron-hole, and quench the fluorescence of nanocrystals.

The fluorescence quenching of CdSe nanocrystals by different concentration of catechol is shown in Fig. 2; the fluorescence intensity of nanocrystals decreases with the increase of catechol concentration. The fluorescence intensity decreases to 40% in the presence of 1.2 mM catechol.

The fluorescence quenching efficiency is described by the well-known Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K[Q] = 1 + k_q\tau_0[Q] \quad (3)$$

In this expression, F_0 and F are the fluorescence quantum efficiency in the absence and presence of quencher, K is the Stern–Volmer quenching constant, k_q is the biomolecule quenching constant, τ_0 is the unquenched lifetime and $[Q]$ is the quencher concentration. The fitted result using Eq. (3) is shown in Fig. 3; good linear relationship could be observed between 5.6×10^{-6} and 3.2×10^{-3} M, the k 1.168×10^3 and the correlated coefficient 0.998 could also be acquired.

3.3. The fluorescence quenching of CdSe nanocrystals by dopamine

The fluorescence spectra of CdSe nanocrystals in the presence of different concentration of dopamine are shown in Fig. 4; the fluorescence intensity decreases with the increase of dopamine concentration similar to catechol. However, the fluorescence intensity decreases to 21% in the presence of 5.7×10^{-5} M dopamine, compared with catechol, the fluorescence intensity only decreases to 89% in the presence of equal molar catechol, which shows that the dopamine is one more effective fluorescence quencher than catechol. Stern–Volmer equation was also used to fit the results (shown in Fig. 5); good linear relationship could be observed between 1×10^{-7} and 1.6×10^{-4} M. The K is 6.3×10^4 and the correlated coefficient is 0.9997, respectively. The K is almost 54 times larger than that of catechol. The detection limit reaches 5.6×10^{-8} M.

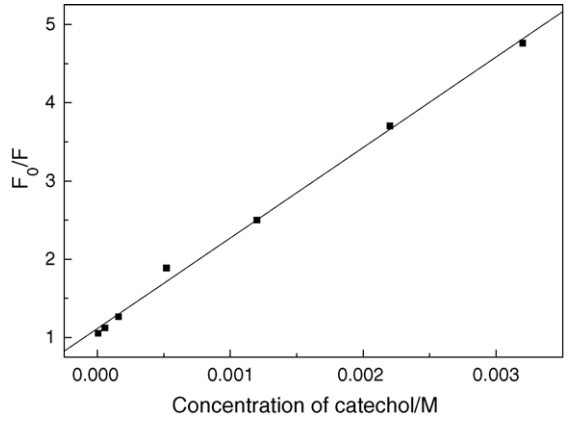

Fig. 3. The linear fitted result for catechol using Stern–Volmer equation.

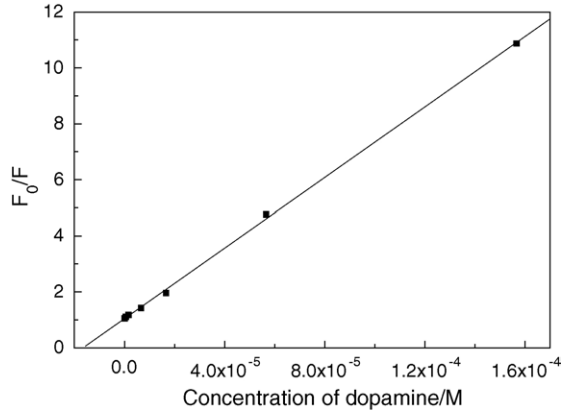

Fig. 5. Linear fitted result for dopamine using Stern–Volmer equation.

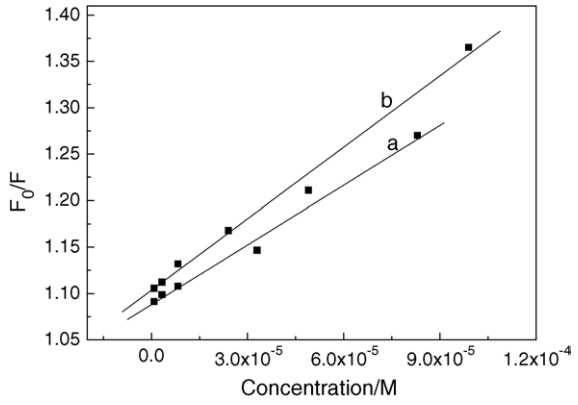

Fig. 6. The linear fitted results for ascorbic acid (a) and lactic acid (b).

It is well-known that dopamine is one kind of catecholamine, compared with catechol; only one site is substituted by ethylamine, but different fluorescence quenching efficiency could be observed for them. Two possible reasons are proposed to explain the fluorescence quenching of dopamine, the first reason is similar to catechol, the hydroxyl group could be oxidized and quench the fluorescence as hole traps. The second reason is that the amine group within the dopamine molecular could bind to the CdSe nanocrystals surface by serving as hole acceptor, thereby remove these sites from participation in radiative electron-hole recombination on the surface, the fluorescence could be quenched. This process is similar to butylamine, which has been studied [32]. Both of these two reasons cause the fluorescence quenching of CdSe nanocrystals, while only the hydroxyl group cause the fluorescence quenching for catechol, thus larger K and lower detection limit could be acquired for dopamine compared with catechol.

3.4. The fluorescence quenching of CdSe nanocrystals by lactic acid and ascorbic acid

We found that the fluorescence of CdSe nanocrstals could also be quenched by lactic acid and ascorbic acid. Fig. 6 shows the fitted results by Stern–Volmer equation, good linear relationship could also be observed and the K is 2.14×10^3 and 2.57×10^3 for ascorbic acid and lactic acid, respectively.

When all the variables are held constants, the higher the K, the lower the concentration of quencher required to achieve the fluorescence quenching. The use of a quenching with higher K will lead to higher sensitivity in biosensor application [28]. Since the K is 6.3×10^4, 2.57×10^3, 2.14×10^3 and 1.168×10^3 for dopamine, lactic acid, ascorbic acid and catechol, respectively, the order of sensitivity for the biosensor is dopamine > lactic acid > ascorbic acid > catechol. For dopamine, the K is almost 25, 29 and 53 times larger than that of lactic acid, ascorbic acid and catechol, respectively, which illustrates that this method has good selectivity for dopamine.

4. Conclusions

In this paper, we synthesized sodium citrate-protected CdSe nanocrystals, and the fluorescence quenching of CdSe nanocrystals by catechol, dopamine, ascorbic acid and lactate acid were investigated. The possible fluorescence quenching mechanism was proposed. The results show that all of these compounds could quench the fluorescence of CdSe nanocrystals while dopamine shows higher quenching efficiency than other compounds, illustrating that this method shows good selectivity for dopamine, the detection limit reaches 5.6×10^{-8} M.

Acknowledgements

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