Chiral recognition of phenylglycinol enantiomers based on N-acetyl-L-cysteine capped CdTe quantum dots in the presence of Ag⁺

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Abstract: In this study, a novel method for chiral recognition of phenylglycinol (PG) enantiomers was proposed. Firstly, water-soluble N-acetyl-L-cysteine (NALC)-capped CdTe quantum dots (QDs) were synthesized and experiment showed that the fluorescence intensity of the reaction system slightly enhancement when added PG enantiomers to NALC-capped CdTe quantum dots (QDs), but the R-PG and S-PG could not be distinguished. Secondly, when there was Ag⁺ presence in the reaction system, the experiment result was extremely interesting, the PG enantiomers could make NALC- capped CdTe QDs produce different fluorescence signal, in which the fluorescence of S-PG + Ag⁺ + NALC-CdTe system was significantly enhanced, and the fluorescence of R-PG + Ag⁺ + NALC-CdTe system was markedly decreased. Thirdly, all the enhanced and decreased of the fluorescence intensity were directly proportional to the concentration of R-PG and S-PG in the linearly range 10⁻⁵-10⁻⁷ mol·L⁻¹, respectively. So, the new method for simultaneous determination of the PG enantiomers was built too. The experiment result of the method was satisfactory with the detection limit of PG can reached 10⁻⁷ mol·L⁻¹ and the related coefficient of S-PG and R-PG are 0.995 and 0.980, respectively. The method was highly sensitive, selective and had wider detection range compared with other methods.

Keyword: Phenylglycinol enantiomers; quantum dots; N-acetyl-L-cysteine; Ag⁺

Introduction

In asymmetric synthesis field, chiral amino alcohols are the important chiral source for synthesis of chiral reagents, at the same time, it is widely used in drug synthesis and resolution of enantiomers. Phenylglycinol (PG) has wide application value in the utility of chiral amino alcohols, which was known as 2-amino-2-phenylethanol[1,2]. It is the intermediate of many medicines that has been used successfully in the prevention of nausea and vomiting associated with cancer chemotherapy [3]. It not only can be used as the starting material, but also can be used as chiral auxiliaries, chiral protective reagents, and chiral ligands[4].The chiral phenylglycinol as intermediates can be prepared to morpholine compound for treating asthma and helping ease the pain; the preparation of the drugs with isoquinoline structure, which have obvious effect on prevention and treatment of brain tissue pathological changes; and it also can be applied to prepare antitumor drug used for inhibiting tumor cell growth. As we all know, PG include R-PG and S-PG, which are both enantiomers. The recognition of the enantiomers is quite important because not all of the enantiomers are good for human's body.

Semiconductor quantum dots (QDs) is a kind of typical semiconductor nanomaterials, it will play an important role in the field of information technology. During the past decades, it have gained increasing attention owing to their size-dependent properties and novel optical properties[5-9]. Firstly, the emission spectrum of the quantum dots can be controlled through the size of quantum dots, therefore the fluorescence emission spectrum of QDs could cover the whole visible light region with different size or chemical composition[10-12]. CdTe quantum, for example, with its size range from 2 nm to 15 nm, their emission wavelength can be redshift from 510 to 660 nm[13-15]. Secondly, QDs have good light stability. It can be used to observe the long time of the labeled objects, which provides a powerful tool for studying long-term interactions between molecules in cells[16-19]. Thirdly, QDs with wide excitation spectrum and narrow emission spectrum[20-22], quantum dots with different particle sizes could be detected with the same excitation wavelength, so it can be applied to multi-color marking, which can greatly promote the application of

fluorescence labeling[23,24]. Fourth, the optical property of quantum dots[25,26], have is the large Stokes shift. This property can avoid the overlap of the emission spectrum and the excitation spectrum, which is beneficial to the detection of fluorescence spectrum signal.[27], which is beneficial to the application of fluorescence spectrum[28]. Fifth, the quantum dots have good biocompatibility, and can be specifically connected with different chemical modifications, which has low cytotoxicity, little harm to the living organisms, and can be used for living in vivo labeling and detection[29-32]. Sixth, The fluorescence lifetime of quantum dots is longer than organic dyes and can sustainable dozens of nanoseconds(20ns-50ns)[33]. After the excitation of light, most spontaneous fluorescence have decay and the fluorescence of quantum dots still exists, so the fluorescence signal without background interference[34]. All in all, the quantum dots have wide excitation spectrum and continuous distribution, meanwhile the emission spectrum is narrow and symmetrical. At the same time, it had the characteristics of adjusted color, high stable photochemical long fluorescence lifetime and et al, it is an ideal fluorescent probe.

In this work, a new fluorescence method was developed for the recognition of R-PG and S-PG based on N-acetyl-L-cysteine (NALC) capped CdTe quantum dots. As we all know, fluorescence method are used more and more popular by the virtue of simple, fast and high effective, compared with other methods. Hence, we used fluorescence method to recognize R-PG and S-PG. S-PG in the low concentration can make the fluorescence spectrum of NALC-capped CdTe QDs slight enhancement with the presence of Ag^+ , while R-PG with the opposite result, therefore PG enantiomers could be recognised indirectly. Compared with the enantioselective recognition methods reported before, the developed method had many advantages, including low detection limit, fast and have effective enantioselectivity. The method was highly sensitive, selective and had wider detection range compared with other methods and the results showed in table 1.

Table 1 The results of this method compared with others

Method	Linear range	Ref.
Fluorescence	$1\text{-}3\times 10^{-4}\text{mol/L}$	[42]
Capillary electrophoresis	$0\text{-}0.375\times 10^{-3}\text{mol/L}$	[43]
Molecular simulation	—	[44]
Fluorescence	$10^{-3}\text{-}10^{-5}\text{mol/L}$	[46]
RP-HPLC	$0.5\text{-}5\times 10^{-3}\text{mol/L}$	[45]
Fluorescence	$10^{-5}\text{-}10^{-7}\text{mol/L}$	This work

Experiment

Reagents

All chemical reagents were of analytical grade and were purchased from Aladdin Reagent Co., Shanghai, China with no additional purification. These reagents were: Cadmium chloride with 2.5 water ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$), Te powder (Te), sodium borohydride (NaBH_4) silver nitrate (AgNO_3), N-acetyl-L-cysteine(NALC), Sodium hydroxide (NaOH), R-phenylglycinol (R-PG) and S-phenylglycinol (S-PG). High-purity water with the resistivity of greater than $18\text{ M}\Omega\text{ cm}^{-1}$ was used in the experiments.

Apparatus

A Varian Cary Eclipse spectrofluorophotometer (Varian Company, California, America) was used to record the PL spectra of the NALC-capped CdTe QDs at ambient conditions operating with **Xe lamp as excited source**; the excitation and emission slits were 5 nm. A UV-2500 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was applied to record the absorption spectra. A Nicolet-6700 spectrometer was devoted to Fourier-transform infrared spectra. Low resolution Transmission Electron Microscopy (TEM) images were recorded on a JEOL JEM 1011 microscope operating at 100kV. a Bruker AXS D8-ADVANCE X-ray diffractometer with Cu/K α radiation over a 2θ range of $10\text{-}80^\circ$.

Synthesis of NALC-CdTe quantum dots

The method that synthesis of NALC-CdTe quantum dots was prepared according to the previously described method with small changes[7]. Tellurium powder(0.0638g) with excessive sodium borohydride (0.1136g) dissolved in DI-water, subsequently placement for a certain time to ensure product sodium hydrogen telluride(NaHTe). CdCl₂·2.5H₂O (0.2248 g) and NALC (0.2448g) were dissolved in 40 mL deionized water. Under magnetic stirring, the pH of the mixture was adjusted to 12 by using the dropwise addition of NaOH solution (1 mol·L⁻¹). The solution was deaerated by N₂ bubbling for about 30 min. The resulting solution mixture was then heated to 369 K and injected sodium hydrogen telluride solution with a syringe along with refluxed under nitrogen for regular time which heated different time can get different solution with different fluorescence. The salmon pink CdTe solution was obtained. The concentration of NALC-capped CdTe QDs was 2.5×10⁻⁵ mol·L⁻¹(determined by the Cd²⁺ concentration)

Reaction of Phenylglycinol with AgNO₃

This method is for the first time to used modify phenylglycinol with silver nitrate. Typically, 20μL of 0.2 mol·L⁻¹ silver nitrate was added into 4.0 mL of 5 mmol·L⁻¹ phenylglycinol solution dropwise, before adjusted the value of pH to 12 posput 15min to ensure react completely. Finally, put this solution into water path with 60°C for 20 min, when it cooled to room temperature and centrifuged to get purified solution with nonfluorescence. The concentration of phenylglycinol in the presence of Ag⁺ was about 5×10⁻³ mol·L⁻¹, and compared with phenylglycinol solution in the absence of Ag⁺ the color is a litter darker. In addition, the naked eye can not see other differences. The S-PG in the presence of Ag⁺ solution shown in Figure 1a in the ambient conditions and in the UV lamp with 365nm and that of R-PG in the presence of Ag⁺ shown in 1b.

Fig 1 (a) The solution of S-PG in the presence of Ag⁺ in the ambient conditions and in the 365nm UV lamp. (b) The solution of R-PG in the presence of Ag⁺.

Results and discussion

Characteristics of CdTe QDs

Fourier transform infrared (FT-IR) spectrum

The synthesised NALC-capped CdTe QDs with the above method has orange fluorescence under UV light. In order to ensure its structure, some tests have been done. Figure 2 demonstrates FT-IR spectrum of NALC-capped CdTe QDs. The obvious peak around 3410cm^{-1} is attributed to the stretching vibrations of the -OH group, 2968cm^{-1} peak can be assigned to the -CH₂ asymmetric stretching vibrations, 2840cm^{-1} peak can be perceived as -CH₂ symmetric stretching vibrations, 1640cm^{-1} refers to the stretching vibrations of N-H, C=O. The **bending vibrations adsorption** at 1597cm^{-1} peak belongs to N-H, 1380cm^{-1} can be regard as C-C stretching vibrations, and 1110cm^{-1} peak originates from the C-O stretching vibrations. And 1045cm^{-1} peak can be identified as C-N stretching vibrations.

Fig. 2 FT-IR spectrum of NALC-capped CdTe QDs

Transmission electron microscopy

The structures of the NALC-capped CdTe QDs were also investigated by the transmission electron microscopy (TEM), as shown in Fig. 3a, and the results displays that NALC-capped CdTe QDs were homogeneous distributed in the aqueous mean

grain size are about 3.0 nm, which is accordance with the Fig. 3b.

Fig.3 (a)TEM image of the NALC-capped CdTe QDs. (b) the corresponding size histogram of the NALC-capped CdTe QDs

Fluorescence spectra

To further investigate the optical properties and ligand chemical structure of the as prepared NALC-capped CdTe QDs. The UV-vis absorption, fluorescence excitation and emission spectra of as-prepared NALC-capped CdTe QDs were also recorded, shown in Fig. 4b, and a is the normalized PL spectra of NALC-capped CdTe QDs at different excitation wavelength (in 10 nm increment starting from 350 nm to 400 nm). In addition, inset in (b) is the NALC-capped CdTe QDs solution in the ambient conditions in the right and under the UV lamp with 365nm in the left. We also test the fluorescence stability of the NALC-capped CdTe QDs by measuring the fluorescence decay curve, and know the fluorescence lifetime form it. The fluorescence lifetime of NALC-capped CdTe QDs is 2.00ns in average. as presented in Fig.4c

Fig.4. (a) PL emission spectra at the different excitation (inset pHotograph of the NALC-capped CdTe QDs in the ambient conditions and under UV light with 365nm.). (b) PL excitation and

emission and UV-vis absorption spectra of the NALC-capped CdTe QDs in the aqueous solution

(c) fluorescence decay curve of the NALC-capped CdTe QDs

Powder X-ray diffraction and Energy Dispersive Spectrometer (EDS)

The X-ray diffraction can measured the crystallinity of the NALC-capped CdTe QDs. The X-ray diffraction analysis further verifies the cubic structure of NALC-capped CdTe QDs compared with standard spectrum, the three obvious diffraction peaks can be indexed to the (111), (220), and (311) diffraction of face-centered cubic (fcc) NALC-capped CdTe QDs(PDF,15-0770),so the cubic structure of the NALC-capped CdTe QDs can be sure, which presented in Fig.5a. As shown in Fig.5b, EDS was applied to study the elementary composition and molecular structure of the NALC-capped CdTe QDs. The EDS spectrum obvious peaks can be find with respect to Cd and Te.

Fig.5 (a) The X-ray diffractogram of the NALC-capped CdTe QDs. (b) The EDS spectrum of the NALC-capped CdTe QDs.

Recognition of phenylglycinol enantiomers

Recognition of phenylglycinol enantiomers with NALC-capped CdTe QDs

The fluorescence emission spectra of NALC-capped CdTe QDs in the absence and presence of phenylglycinol under the room temperature were shown Fig.6. The result indicated that addition of PG either R-PG or S-PG leads to the fluorescence intensity of NALC-capped CdTe QDs increase, and the degree of fluorescence enhancement basically coincide.

Fig.6. PL spectra of NALC-capped CdTe QDs with phenylglycinol

Chiral recognition of phenylglycinol enantiomers in the presence of Ag^+

In order to further study chiral recognition of phenylglycinol enantiomers with NALC-capped CdTe QDs. Some modifications have been made in phenylglycinol. The interesting finding is that R-PG and S-PG with Ag^+ have different signal response, just as Fig.7 exhibits. This method can be applied to recognize phenylglycinol enantiomers, which was not been reported before.

Fig.7. PL spectra of NALC-capped CdTe QDs phenylglycinol in the presence of Ag^+

Under the optimum conditions, a set of experiments were subjected to detect the PG with various concentration in the presence of Ag^+ , as shown in Fig.8a and Fig.8c. It pointed out that when the concentration of the S-PG in the presence of Ag^+ with $10^{-7} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence intensity of NALC-capped CdTe QDs was slight enhanced, but it clearly quenched with increasing the concentration of S-PG with Ag^+ in the range of 2×10^{-6} - $10^{-5} \text{ mol}\cdot\text{L}^{-1}$. In addition, the linear regression was $\Delta I_{\text{PL}}=452.05-19.39C$, (where C is the concentration of S-PG with Ag^+ in $\text{mol}\cdot\text{L}^{-1}$) and the correlation of 0.995(as shown in Fig.8b).

While R-PG in the presence Ag^+ , the fluorescence intensity always decreased with different concentration of R-PG with Ag^+ in the range of 0 - $10^{-5} \text{ mol}\cdot\text{L}^{-1}$, which can obviously observed this phenomenon in Fig.8c. Moreover, the linear regression equation of $\Delta I_{\text{PL}}=428.24-54.68C$, (where C is the concentration of R-PG with Ag^+ in $\text{mol}\cdot\text{L}^{-1}$) and the correlation of 0.980(as shown in Fig.8d). It is worthwhile mentioning that S-PG exhibited better linear relation on fluorescence intensity than that of R-PG in the presence Ag^+ , which indicated that NALC-capped CdTe QDs can achieve high enantioselective fluorescence recognition of PG enantiomers.

Fig.8 PL spectra of NALC-capped CdTe QDs with PG in the presence Ag⁺ different concentration. (a) S-PG in the presence Ag⁺. (b) the linear regression of the S-PG in the presence Ag⁺. (c) R-PG in the presence Ag⁺. (d) the linear regression of the S-PG in the presence Ag⁺.

Principle of the reaction between PG and NALC-capped CdTe QDs in the presence of Ag⁺

There are many factors could influence fluorescence signal, such as intermolecular electrostatic, hydrogen bonding interaction, hydrophobic and hydrophilic interaction, and aggregation interaction of biological molecules[35]. Moreover, the spectral properties and fluorescence intensity are strongly influenced by molecular size, shape, conformation, and interfacial properties[36], which is conducive to us for molecular recognition and detection. In this work, in order to recognize the enantiomers of PG, and NALC is a kind of chiral reagent, that is why NALC was chose as ligand[37], in addition, CdTe QDs can easily bind with NALC via -SH. Adding Ag⁺ to PG solution dropwise to form a kind of coordination complex. As chelating ligands, amino alcohols are highly associable with many metal ions[47], so they can become a complex through coordination-chelation

interaction with hydroxyl and amino of PG. When added NALC-capped CdTe QDs solution, it would compete with part of the PG and coordinate with Ag^+ . Comparing the sizes of PG and NALC-capped CdTe QDs, the size of the PG molecule is quite small, what is more, the capability of the hydroxyl carboxyl with Ag^+ is stronger than that of amino of PG[38-40]. The negative charge distribution on oxygen atoms of the carboxylic acid group and ring structure indicate the possibility of a strong electrostatic interaction with the positive metal ion[41]. That is to say, the coordination complex would change with adding NALC-capped CdTe QDs. As we all know, enantiomers are two kinds of substances produced by the arrangement of atom in molecule in space, then they can produce different space steric hindrance, when interacted with Ag^+ . On one hand, the chiral ligand of the CdTe QDs is N-acetyl-L-cysteine, which has similar spatial arrangement with S-PG and the ball-and-stick model of PG and N-acetyl-L-cysteine, as shown in Fig.9. Compared with R-PG, S-PG has more advantages in the PG+ Ag^+ +NALC-CdTe QDs system. Hence, the space steric hindrance between R-PG+ Ag^+ and NALC-CdTe QDs is stronger. On the other hand, in this system, when became the complex of PG+ Ag^+ +NALC-CdTe QDs, the π electron conjugation and the molecular flatness were changed. When added S-PG in the system made the π electron conjugation and the molecular flatness increase because it has similar spatial arrangement with N-acetyl-L-cysteine and benzene ring, which caused the enhancement of fluorescence. While R-PG has opposite spatial arrangement with N-acetyl-L-cysteine and it caused the molecular flatness decreased, which led to the quenching of fluorescence. And the scheme of the analytical principle was shown in Scheme 1

Scheme 1. Schematic illustration for the signal transduction mechanism for developed PL probe PG

Fig.9 The ball-and-stick model of PG and N-acetyl-L-cysteine

Optimization of method

In order to ensure the accuracy and validity, a set of work have been done to reduce the chance of false positives. There are many other ions in the system solution, such as Na^+ , Cl^- , which have brought in when fabrication the NALC-capped CdTe QDs and Ag^+ functionalized PG.

Effect of sample solution pH

In the course of the experiment, pH value plays a significant index, which can strongly influence the fluorescence intensity of the NALC-capped CdTe QDs, hence, the effect of the pH value was investigated for experiment system. According to the effects of pH, BR was selected as buffer solution, that is because NALC-capped CdTe QDs solution is alkaline and the range of BR buffer solution is from 1.8 to 11.9, which both contains acidic and alkaline. The results showed that with the pH range of acidity was 7.45-10.88 shown in Fig.10 and when pH value was 9.91, the condition of the experiment reach to the optimal.

Fig.10 Effect of pH fluorescence intensity of NALC-capped CdTe QDs in the BR buffer solution.

Effect of NaCl

The free ionic in the solution may influence the detection of PG. To further investigate the stability of this sensing system, the fluorescence intensity of NALC-capped CdTe QDs with various concentration of NALCl in the range of 10^{-3} - 10^{-7} mol·L⁻¹ was studied. As shown in Fig.11, whether Na⁺ or Cl⁻ has no effect on the fluorescence intensity of NALC-capped CdTe QDs.

Fig.11 Fluorescence intensity change of NALC-capped CdTe QDs with the concentration of NALCl in the range of 10^{-3} - 10^{-7} .

Effect of various ions

In order to ensure the effective of the experiment, the fluorescence spectra of NALC-capped CdTe QDs on various metal ions in the presence of PG (including Fe(III), Al(III), Pb(II), Mg(II), Ba(II), Co(II), Cd(II), Ni(II), Ca(II), Zn(II), Cr(III)) were investigated, as shown in Fig.12. The fluorescence intensity of NALC-capped CdTe QDs with PG could quench, when added Fe(III), Pb(II) and Mg(II), but cannot recognize the enantiomers of PG. The result is satisfactory.

Fig.12 The fluorescence spectra of various metal ions (Fe(III), Al(III), Pb(II), Mg(II), Ba(II), Co(II), Cd(II), Ni(II), Ca(II), Zn(II), Cr(III)), the concentration of metal ions is 2×10^{-4} mol · L⁻¹.

Conclusions

In summary, PG is an important drug intermediate with wide application prospect. The development of a sensitive, rapid and accurate method for measuring PG is essential. In this work an effective means has been developed to recognize the enantiomers of PG. At first, the NALC-capped CdTe QDs cannot direct recognize PG, hence, Ag^+ was added in PG solution. Interestingly, the enantiomers of the PG in the presence of Ag^+ can be recognized commendably and the concentration range reached $10^{-7} \text{ mol} \cdot \text{L}^{-1}$. The fluorescence intensity of NALC-capped CdTe QDs with S-PG and R-PG in presence of Ag^+ have different fluorescence signal response and the linear related coefficient is 0.995 and 0.980, respectively. In addition, the mechanism of the interaction between NALC-capped CdTe QDs and PG when Ag^+ is present in the solution, thus a novel and effective method to recognize the enantiomers of PG was found.

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In this study, Chiral recognition of phenylglycinol enantiomers based on N-acetyl-L-cysteine capped CdTe quantum dots in the presence of Ag^+ . The fluorescence intensity of NACL-capped CdTe QDs with pure phenylglycinol enantiomers both had slight enhancement, while the fluorescence signal made a difference with phenylglycinol enantiomers in the presence of Ag^+ , as shown in figure.