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ORIGINAL PAPER

An immediate luminescence enhancement method for determination of vitamin B_1 using long-wavelength emitting water-soluble CdTe nanorods

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Abstract A method for determination of vitamin B_1 has been developed that is based on the enhancement effect of vitamin B_1 on the luminescence of water-soluble CdTe nanorods modified with thioglycolic acid and cysteine. The effect of variables including the size of the nanorods on the enhancement of luminescence have been investigated. A preliminary mechanistic study showed that the passivating action of vitamin B_1 on the surface of the CdTe nanorods is likely to be responsible for the enhancement. Interferences by shortwave fluorescence are effectively eliminated because measurements are performed in the near-infrared. Due to the near-infrared measurement character, the fluorescence interference of vitamin B_2 can be effectively eliminated. Under the optimum conditions, the extent of luminescence enhancement is proportional to the concentration of vitamin B_1 in the range from 0.1 to 3.0 μmol L^{-1} and the detection limit is 0.03 μmol L^{-1}. The relative standard deviation for 2.0 μmol L^{-1} vitamin B_1 is 1.3% (n=6). The method is highly sensitive and selective, avoids the sample treatment needed in other procedures, and can be applied to the determination of vitamin B_1 in real samples with satisfactory results.

Keywords CdTe nanorods · Vitamin B_1 (Thiamine) determination · Luminescence enhancement

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Introduction

Compared with zero-dimensional nanostructures, one-dimensional nanostructures with the quite different quantum confinement energies [1] present some novel optical properties, and their applications in chemo and biosensors have received considerable attention at recent years [2–7]. Up to now, J. Li et al. employed zero- and one-dimensional CdTe nanomaterials to study the effect of divalent metal cations on their photoluminescence (PL) [8]. It was found that the response of CdTe nanorods to these metal cations was more sensitive comparing to CdTe QDs. Meanwhile, Tang and co-workers investigated water-soluble CdTe nanowires which displays high selectivity and sensitivity toward copper (II) in the presence of other physiologically relevant cations [9]. Nevertheless, the exploration of 1D semiconductor materials as luminescent probes for biological samples at present is still at the primary stage.

Vitamin B_1 (Thiamine) is a natural nutrient present in many foods. It is a biologically and pharmaceutically important compound, essential for carbohydrate metabolism, maintenance of neural activity and prevention of beriberi disease [10]. There have been numerous reports on the determination of vitamin B_1, e.g. spectrophotometry [11, 12], spectrofluorimetry [10, 13], chemiluminescence [14], selective membrane electrodes [15, 16], potentiometry [17, 18], high performance liquid chromatography [19] and capillary electrophoresis [20], resonance Rayleigh scattering (RRS) [21]. Among these methods, spectrofluorimetry are most often used due to the advantage of high sensitivity. But vitamin B_1 is a non-fluorescent compound which needs to be converted into an intensely fluorescent thiochrome derivative by appropriate oxidants before fluorescence detection. So it is desirable to develop an immediate,

simple and sensitive approach for determination of vitamin B_1.

Recently, Sun and co-workers first reported an immediate luminescent method for the vitamin B_1 assay based on the quenching of vitamin B_1 on the PL of CdSe QDs [22]. However, it was noted that the intense fluorescence spectral interference of vitamin B_2 (riboflavin) that often coexisted in real samples was not investigated and avoided, which would greatly limit the applications of these QDs.

In this work, we synthesized the CdTe nanorods according to previous work [23], and investigated the interaction between vitamin B_1 and CdTe nanorods. An obvious luminescence enhancement effect of vitamin B_1 on CdTe nanorods has been observed. Further study shows that the above luminescence enhancement effect is greatly determined by CdTe nanorods' size and state surface. This could be ascribed to the passivation action of vitamin B_1 on the surface of the CdTe nanorods. Compared with short CdTe nanorods, long CdTe nanorods obtained in our experiment conditions are a more suitable candidate for the vitamin B_1 assay according our observation. On the one hand, long CdTe nanorods with near-infrared emission feature can effectively eliminated the short-wavelength emission spectral interferes from coexisting substances such as vitamin B_2. On the other hand, the long CdTe nanorods, which may have larger trapping effect than that of short one, is readily passivated by trace vitamin B_1, facilitating the higher analytical sensitivity. In virtue of the resulting long CdTe nanorod, a sensitive and selective vitamin B_1 assay has been developed. To the best of our knowledge, the immediate luminescence enhancement method for the determination of vitamin B_1 in the presence of vitamin B_2 has not been reported up to now. This method has been applied to the determination of vitamin B_1 in the commercial vitamin B_1 tablets and vitamin B complex tablets, and satisfactory results have been obtained.

Experimental

Reagents

Tellurium powder (60 mesh, 99.999%), Thiamine hydrochloride, Riboflavine, Nicotinic acid and other biochemical reagent were purchased from Alfa Aesar (http://www.alfa.com/webapps/ec165w.pgm). Thioglycolic acid (TGA), L-cysteine (Cys) hydrochloric hydrate, $CdCl_2{\cdot}2.5H_2O$, $NaBH_4$, and other routine chemicals were purchased from Shanghai Reagent Company (Shanghai, China http://www.reagent.com.cn) and used as received without further purification. Stock standard solution of thiamine hydrochloride (1.0×10^{-3} mol L^{-1}) was prepared in water and stable for at least 1 month when kept refrigerated. A buffer solution of pH 10.83 was prepared by mixing Na_2CO_3 (0.1 mol L^{-1}) and $NaHCO_3$ (0.1 mol L^{-1}) solutions in a volume ratio of 9:1. The vitamin B_1 tablets and vitamin B complex tablets were purchased from Second People's Hospital of Wuhu (Wuhu, China http://www.whsph.com/). 21 Super VITA tablet were purchased from Hangzhou Minsheng Pharmaceutical Group Co., Ltd. (http://www.mspharm.com/). All chemicals used were of analytical grade or of the highest purity available. All solutions were prepared with doubly deionized water (DDW).

Apparatus

A Hitachi (http://www.hitachi-hitec.com/global) F-4500 fluorescence spectrophotometer (Tokyo, Japan) was adopted to record the luminescence spectra. UV-Vis absorption spectra were recorded with a Hitachi (http://www.hitachi-hitec.com/global) U-3010 spectrophotometer (Tokyo, Japan). Luminescence lifetimes were measured with the time-correlated single-photon counting technique on the Combined Steady State and Lifetime Spectrometer (Edinburgh Analytical Instruments F900, http://www.edinst.com). A Hitachi (http://www.hitachi-hitec.com/global) H-600 transmission electron microscope (TEM) was used to observe the appearance and size of nanocolloids. All pH values were measured with a Model pHs-3c meter (http://cn.dgbatglsse.cnele.com).

Synthesis of CdTe nanorods

CdTe nanorods was prepared according to the method [23] described previously with some slight modifications. When the molar ratio of Cd^{2+}:TGA:Cys:HTe^- was set at 1:1.8:0.6:0.5, the typical procedure is as follows: first, 10 mg Te powder and 6.25 mg $NaBH_4$ were added into 0.3 mL water, and reacted at 0°C for 8 h to obtain NaHTe. Second, fresh NaHTe solution was injected into 100 mL oxygen-free aqueous solution containing 20 μl TGA, 15.2 mg cysteine and 37.5 mg $CdCl_2{\cdot}2.5H_2O$ in pH 11. Then the mixture solution was heated and further refluxed in an oil-bath, until the color of solution changed from pale yellow to deep red. Last, the solution was allowed to cool to the room temperature naturally. Other CdTe nanorods were also prepared according to the similar procedure with different molar ratio of Cd^{2+}:TGA:Cys:HTe^- from 1:1.8:0.8:0.5 to 1:1.8:0.4:0.5 at same pH.

Sample preparation

First, grind five vitamin B_1 tablets, vitamin B complex tablets, or fourteen 21 Super VITA tablets into powder, and dissolve the 0.01 g powder (10.0 g for 21 Super VITA) in hydrochloric acid solution (0.01 mol L^{-1}), then transfer into

a 100 mL calibrated flask and dilute to the mark with water, last, take 0.60 ml above solution to determine the content of vitamin B_1, following the analytical procedure. The recovery test was carried out in each instance by adding 1 mL vitamin B_1 (10^{-5} mol L^{-1}) standard solution to 0.60 ml above treated sample solution, and the other procedures is the same.

Analytical procedure

0–3 mL 1.0×10^{-5} mol L^{-1} of vitamin B_1 solution was added into each of a series of 10 mL colorimetric tubes. And 1.0 mL 0.1 mol L^{-1} Na_2CO_3-$NaHCO_3$ buffer solution of pH 10.83 and 1.0 mL 1.55×10^{-4} mol L^{-1} CdTe nanorods solution, according to the concentration of Cd^{2+} [24], were added, then dilute to the mark with water. The mixture was shaken and equilibrated waiting for 10 min to uniform.

The luminescence intensity was measured at an excitation wavelength of 530 nm and an emission wavelength of 665 nm in a 1 cm quartz cell against a solvent blank. Both excitation and emission were performed with a slit width of 10 nm. All experiments were performed at room temperature.

Results and discussion

Luminescence behavior of CdTe nanorods and their interactions with vitamin B_1

No luminescence was observed for all the crude reaction solution, however, the band edge emission of the CdTe nanorods gradually appeared under reflux, and the emission color could also be tuned by prolonging the reflux time. Transmission electron microscopy (TEM) images (Fig. 1) show that the long axis of the samples with the molar ratio of Cd^{2+}:TGA:Cys:HTe^- at 1:1.8:0.6:0.5 changed from 200 nm to 3 μm with the refluxing time increased from 1.5 to 5.5 h, 12 h, 24 h. Fig. S-1A (Electronic Supplementary Material) shows the luminescence spectra of CdTe nanorods extracted at different reflux times. The red shift of band edge emission peaks from 550, 630, 665 nm and then to 720 nm, corresponding to the elongation of the long axis of the as-prepared CdTe nanorods, can be ascribed to the quantum confinement energies decrease. From Fig. S-1A, it also can be seen that the luminescence intensity of the nanorods gradually decreased with prolonging the reflux time, may be due to the larger surface trap of long CdTe nanorods than that of short one obtained under the same experimental conditions. Thus, the long CdTe nanorods seem to be more easily passivated by trace vitamin B_1, resulting in higher enhancement effect. It should be pointed out that, however, the stability of the CdTe nanorods with emission wavelength beyond of 665 nm is relatively low according to our observation. Therefore, the emission wavelength of 665 nm was chosen for further experiments taking higher analytical sensitivity and stability into account.

Fig. S-1B shows the size-dependent luminescence enhancement phenomenon by vitamin B_1. Short CdTe nanorods show hardly luminescence enhancement effect. When the length of CdTe nanorods further increased along the long axis, however, the characteristic luminescence intensities were significantly enhanced at the presence of vitamin B_1. Furthermore, the extent of luminescence enhancement was proportional to the concentration of vitamin B_1, which forms the basis of the immediate vitamin B_1 assay.

Vitamin B_1 molecule has an aminothiazole heterocyclic structure, on which the sulfur atom with isolated electron can readily coordinate with Cd^{2+}, resulting in luminescence enhancement effect by passivating CdTe nanorods surface. Interestingly, similar luminescence enhancement behaviors

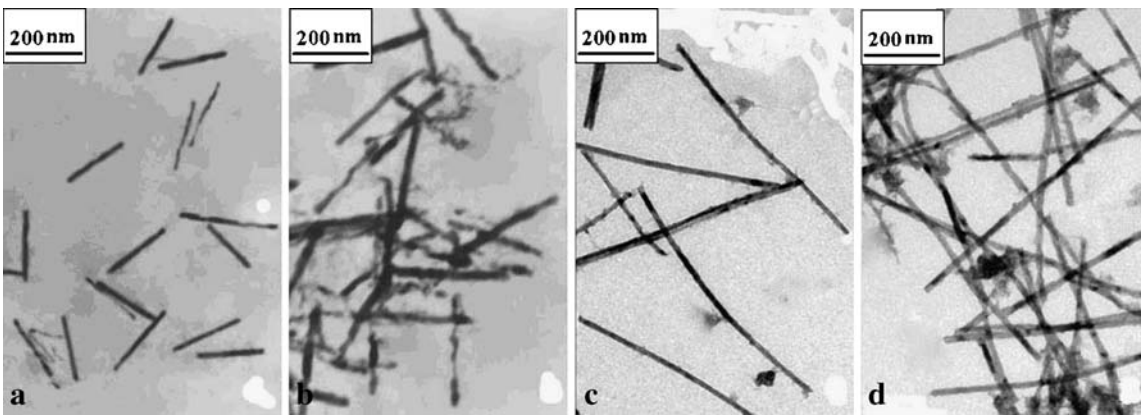

Fig. 1 TEM image of CdTe nanorods after refluxing for 1.5, 5.5, 12 and 24 h (from **a** to **d**). The molar ratio of Cd^{2+}:TGA:Cys:HTe^- of the nanorods used is set at 1:1.8:0.6:0.5

on the CdTe nanorods have also been observed for other samples with aminothiazole heterocyclic structure, such as aminothiazole hydrochloride and 4-methyl-5-thiazoleethanol (results not shown), suggesting that the passivation of vitamin B_1 on the surface of CdTe nanorods may be responsible for the luminescence enhancement observed.

The intensity decay curves of the CdTe nanorods with the emission wavelength of 665 nm in the absence and presence of vitamin B_1 are shown in Fig. S-2. The measurements were performed at room temperature with the excitation and emission wavelength at 530 nm and 665 nm, respectively. The decay times is increased from 17.34/99.60 to 19.81/117.08 ns after the addition of 2.0 μmol L^{-1} vitamin B_1. The enhanced decay time observed indicates that the some trap states of CdTe nanorods can be passivated by trace of vitamin B_1 [25].

Different molar ratio of Cd^{2+}: TGA: Cys: HTe^- from 1:1.8:0.8:0.5 to 1:1.8:0.4:0.5 show also dramatic influence on the luminescence intensity and stability of the CdTe nanorods prepared. With the decrease of L-cysteine addition, the luminescence intensity and stability of the CdTe nanorods prepared decreased while the luminescence enhancement effect by trace vitamin B_1 increased (the results not given). When the molar ratio was set at 1:1.8:0.8:0.5, no luminescence enhancement effect was observed, suggesting that a sufficient passivation on CdTe nanorods surface occurred in the presence of too much excessive L-cysteine. Thus, vitamin B_1 failed to significantly enhance the luminescence of CdTe nanorods through further passivating their surface. In the following work, accordingly, molar ratio of Cd^{2+}: TGA: Cys: HTe^- of 1:1.8:0.6:0.5 was taken for the further experiment.

Optimization of the analytical procedure

The effect of the concentration of CdTe nanorods on luminescence enhancement in the presence of vitamin B_1 was tested. Properly decreasing the concentration of the CdTe nanorods can induce a high luminescence enhancement effect, facilitating analytical sensitivity. However, when the concentration of the probe is too low, the linear range is very narrow according to our observation. In this work, therefore, 1.5×10^{-5} mol L^{-1} CdTe nanorods solution was used taking the analytical sensitivity and linear range into account.

The effect of pH was also investigated. The results were shown in Fig. S-3A. The luminescence enhancement increases with increasing pH, and reaches a maximum at pH=10.83. When the pH increases further, however, the luminescence enhancement significantly decreases. Obviously, the differences of surface passivation of the CdTe nanorods at different pH should account for the luminescence enhancement change. At low pH, the surface passivation effect of the CdTe nanorods is incomplete, due to the weaken coordination action of both capping reagents (thioglycolic acid and cysteine) and vitamin B_1 resulting from the competition of H^+. At higher pH, however, vitamin B_1 also failed to further passivating their surface, since that the uncoordinated surface sites have already saturated by capping molecules themselves due to the strong coordination effect.

In addition, the concentration of buffer solution was also studied. From Fig. S-3B it can be seen that the maximum change of luminescence appeared in the concentration range of 1.0×10^{-2}—2.0×10^{-2} mol L^{-1} buffer solution. Thus, 1.0 mL1.0×10^{-2} mol L^{-1} Na_2CO_3–$NaHCO_3$ solution of pH 10.83 was recommended.

Interference study

Following the described procedure, interference test was performed in the presence of some coexisted potentially interfering substances using a standard solution of vitamin B_1 (2.0 μmol L^{-1}). The results are summarized in Table S-1, where a relative error of less than ±5% is required. It was found that some inorganic ions, amino acids and vitamin scarcely interfere with the determination of vitamin B_1. Low concentration of Cu^{2+}, Mn^{2+} and Hg^{2+} cause obvious

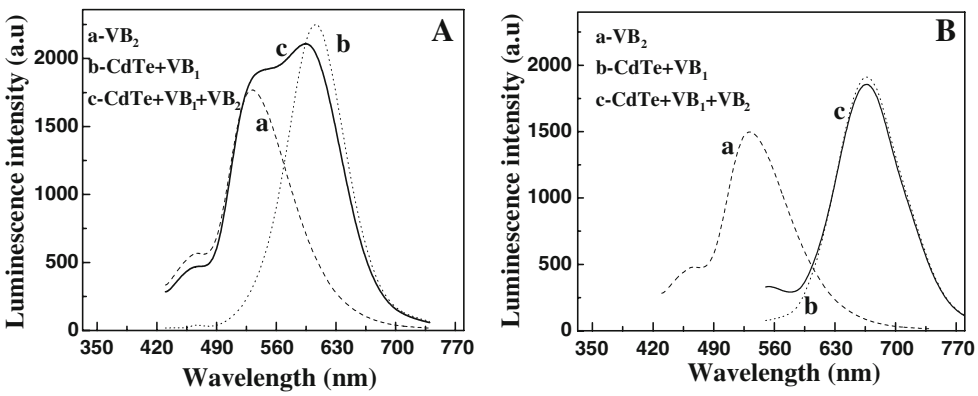

Fig. 2 Luminescence spectra of vitamin B_2 (**a**), CdTe nanorods in the presence of vitamin B_1 (**b**), and CdTe nanorods in the presence of vitamin B_1 and B_2 (**c**). A: excited at 400 nm for (**a**), (**b**) and (**c**); B: excited at 400 nm for (**a**) but excited at 530 nm for (**b**) and (**c**), respectively. Both the concentration of vitamin B_1 and B_2 are 2.0 μmol L^{-1}

Table 1 Comparison of analytical parameters between present method and some other methods reported for the determination of vitamin B_1

Method	Reagent/system	Linear range ($molL^{-1}$)	Detection limit ($ngmL^{-1}$)	Reference
Optosensor for UV spectrophotometry	Sephadex CMC-25 cation exchanger	$5.0–98\times10^{-6}$ $2.5–50\times10^{-6}$	420 890	[27]
		$1.8–30\times10^{-6}$	160	
Flow injection chemiluminescence	Luminol–hydrogen-peroxide	$0.15–24\times10^{-6}$	10	[14]
HPLC	Post-column photochemical derivatization,	$0.1–30\times10^{-6}$	3.8	[28]
Non-immediate fluorescence methods	Iron(III) tetrasulfonatophthalocyanine	$0.01–100\times10^{-6}$	1.3	[10]
Electrochemistry	Keineckate liquid membrane electrode	$10^{-6}\times10^{-3}$	0.3	[29]
Electrochemical luminescence	Rhodamine B	$0.3–5\times10^{-6}$	80	[30]
Non-immediate fluorescence methods	Silica nanoparticles, tetra-substituted carboxyl iron phthalocyanine	$5.0\times10^{-9}–1.0\times10^{-6}$	0.6	[31]
RRS method	Gold nanoparticles	$0–2.8\times10^{-7}$	0.9	[21]
Immediate luminescent method	CdSe quantum dots	$1.5–12\times10^{-5}$	70	[22]
Optical sensor method	Lipophilic esters of tartaric acid	$1.0\times10^{-5}–0.1$	–	[32]
Immediate luminescent method	CdTe nanorods	$1.0\times10^{-7}–3.0\times10^{-6}$	10	Present work

interference, which can be eliminated by means of necessary separation strategy such as mercapto cotton treatment [26]. In our case, the interference from Cu^{2+}, Mn^{2+} and Hg^{2+} was neglected, considering the content of vitamin B_1 is far more than that of them coexisted in the samples.

It should be pointed out that vitamin B_2 is an intensely fluorescent compound with the emission wavelength at 532 nm. The spectral interference may occur due to the spectral overlap partially, when the excitation wavelength is 400 nm and emission wavelength of CdTe nanorods is below 665 nm (see Fig. 2a). However the interference can be effectively eliminated when employing the long wavelength emission CdTe nanorods with the excitation wavelength at 530 nm and emission wavelength at 665 nm (see Fig. 2b), displaying the advantage of low spectral background interference by means of long wavelength detection.

Analytical performance

Under optimum conditions, the luminescence enhancement is proportional to the concentration of vitamin B_1. The linear regression equation is $I-I_0=338.6+102C$ (C: mol L^{-1}) in the range of 0.1 and 3.0 μmol L^{-1} and the correlation coefficient is 0.998. The relative standard deviation is 1.3% for 2.0 μmol L^{-1} vitamin B_1 (n=6). From Table 1 it can be seen that the method displays a medium linear range and detection limit.

Table 2 Determination of vitamin B_1 in the commercial vitamin B_1 and vitamin B complex tablets

Sample	Labeled values [a] ($10^{-7}molL^{-1}$)	VB_1 found [b] ($10^{-7}molL^{-1}$)	R.S.D % (n=6)	Relative error (%)	VB_1 added ($10^{-7}molL^{-1}$)	Total found [b] ($10^{-7}molL^{-1}$)	Recovery (%)
VB_1 tablet	24.5 (VB_1)	24.1	1.6	1.6	10	34.3	102
VB_1 tablet+VB_2	24.5 (VB_1) 5.0 (VB_2)	25.1	1.5	2.4	10	34.7	96
VB_1 tablet+VB_2	24.5 (VB_1) 10 (VB_2)	24.8	1.5	1.2	10	35.2	104
VB complex tablet	6.5 (VB_1) 3.2 (VB_2)	6.4	1.3	1.5	10	16.3	99
21 Super VITA tablet	6.2 (VB_1) 5.9 (VB_2) 1.1(VB_6) 0.0003(VB_{12})	6.0	1.5	3.2	10	15.9	99

[a] Based on the content of vitamin given by the manufacturer

[b] Mean of six determinations

Analytical applications

Vitamin B_1 in commercial vitamin B complex tablets and vitamin B_1 tablets in the present of different concentration of vitamin B_2 were determined by the established method. The determination results are listed in Table 2. A satisfactory recovery of 96–104% was obtained. The results indicate that the present method is reliable and practical.

Conclusion

Water-soluble CdTe nanorods have been used to investigate their luminescence responses to vitamin B_1. An immediate luminescence enhancement method for the determination of vitamin B_1 has been proposed, based on the intense passivation effect of vitamin B_1 on the surface of the CdTe nanorods observed. The relative long wavelength emission at 665 nm of the CdTe nanorods has proven to be suitable for this assay in terms of analytical reproducibility, sensitivity and selectivity. The proposed method without derivative treatment to sample can be performed with satisfactory recovery in the presence of vitamin B_2.

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