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ORIGINAL PAPER

A fluorescent probe for detecting thiamine using the luminescence intensity of nanoparticles

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Abstract Determination of molecules and biomolecules using nanoparticles is promising in the development of analytical techniques. Modified Eu-doped Y_2O_3 nanoparticles (Y_2O_3:Eu NPs) by captopril have been used as a probe for thiamine (vitamin B_1) determination. According to the fluorescence enhancement of modified Eu-doped Y_2O_3 nanoparticles caused by thiamine, a simple and sensitive method were proposed for its detection. The increase in modified Y_2O_3:Eu NPs fluorescence signal as a function of thiamine concentration was found to be linear in the concentration range of 0–44 μM. The limit of detection (LOD) of thiamine by this method was 0.144 μM. All the measurements were performed in natural pH, at the room temperature under ambient conditions. Possible interaction mechanism was discussed.

Keywords Nanoparticle · Luminescence probe · Thiamine · Spectroscopy

Introduction

The development of new methods and novel techniques for the determination of molecules and biomolecules is very significant in chemical and biochemical analysis, biodiagnostics and biotechnology. Because of high sensitivity and selectivity, spectral methods are widely used in these areas [1, 2]. The light-scattering properties of new nanomaterials with different composition, size and shape have attracted great attention recently [3]. Application of these materials for analytical purposes has grown dramatically. There are well-developed methods for the preparation of nanomaterials which have found real applications in practice [4–9] such as; optoelectronic devices [10], photo catalysts [11], electrochemical and chemical sensors [12, 13]. Due to unique advantages, nanoparticles (NPs) are applicable as optical probes. The aim of most scientific investigations is developing new technologies and re-inventing existing ones to gain lower concentration levels of various analytes in complex environment. With the improvement of nanotechnology, novel sensing procedures for different kind of substances have been exploited. In comparison with traditional materials, rare earth doped nanoparticles have high emission quantum yield, size-dependent wavelength tenability, broad excitation with narrow and symmetrical emission spectrum and excellent photostability [14]. The analytes affect the luminescence intensity of NPs through electrostatic or van der Waals interactions, hydrogen bonds, hydrophobic and steric contacts within the binding site [15, 16]. The measurement of luminescence signals provides an applicable method for monitoring the chemical and biochemical environment of a fluorophore.

Vitamin B_1 also called thiamine is sulfur containing vitamin of the B complex and it is a colorless compound with chemical formula $C_{12}H_{17}N_4OS$. Its structure is shown in Fig. 1, contains an amino pyrimidine and thiazole rings with methyl and hydroxymethyl side chains linked by a methylene bridge. In the human metabolism and proper brain functioning thiamine is essential. This vitamin is used in the synthesis of neurotransmitter acetylcholine and gamma amino butyric acid. The coenzyme in catabolism of amino acids and sugars is thiamine pyrophosphate which is the best characterized form of that. This is only synthesis in bacteria, fungi and plants, so it

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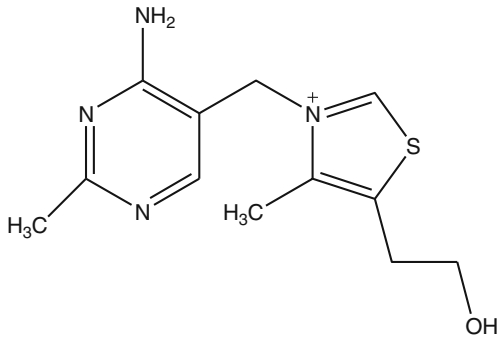

Fig. 1 Structure of vitamin B_1

is an essential nutrient in human diet. In creatures, deficiency results some disease such as korsakoff's syndrome, beriberi and optic neuropathy can lead to metabolic coma and death [17, 18].

Recently, luminescent nanostructures such as CdSe quantum dots [19], Mn-doped ZnS quantum dots [20], CdSe/ZnS quantum dots [21], CdTe quantum dots [22], CdS nanoparticles [23] and silica nanoparticles doped with europium coordination compound [24] have been applied as a probe for determination of various chemical and biological molecules. There are quite a few reports on thiamine sensing based on the fluorescence response of nano probes. For the first time Sun and coworkers reported a luminescent method for the vitamin B_1 by using of CdSe quantum dots with 70 ng mL^{-1} detection limit and linear rang of 5–40 μg mL^{-1} [19]. Afterward, Li and coworkers developed this method for determination of vitamin B_1 based on the enhancement effect of analyte on the luminescence of water-soluble CdTe nanorods modified with thioglycolic acid and cysteine. The linear range was obtained from 0.1 to 3 μmol L^{-1} and 0.03 μmol L^{-1} limit of detection [25]. In this work the analytical parameters is improved and we used a novel probe to its detection. The Y_2O_3:Eu NPs was dispersed in phosphate buffer solution in pH 7.4 and modified by captopril. Then the fluorescence intensities of these suspensions after addition of analyte were used as a probe for thiamine assay. To the best of our knowledge, this is the first study demonstrating the quantitative analysis for vitamin B_1 based on the luminescence properties of Y_2O_3:Eu NPs.

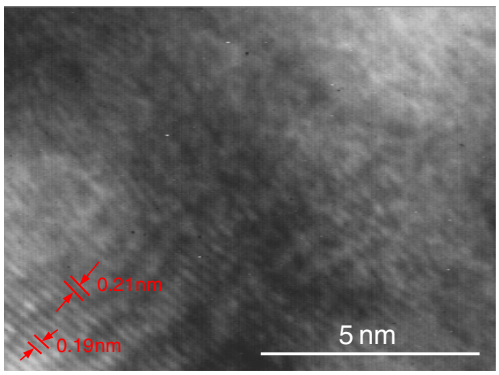

Fig. 2 TEM image of Y_2O_3:Eu NPs

Experimental

Materials

All chemicals were obtained from Merck without further purification. The fluorescence spectrophotometric studies were obtained using a Fluorescence Spectrophotometer CARY Eclipse. The absorption spectra were recorded on a Shimadzu UV-Vis spectrophotometer. Transmission electron microscopic (TEM) studies were performed with the help of a Phillips TEM.

Synthesis and characterization of Eu-doped Y_2O_3 NPs

Y_2O_3:Eu NPs was prepared by solution combustion method according to the method described previously [26, 27]. The typical procedure is as follows: Europium and yttrium nitrates were prepared by the reaction between their oxides with nitric acid. The fresh solution was heated up to dryness on a hot plate. Initially, the solution boiled till getting the gel and underwent the extra acid was removed. Deionized water was added to nitric salts to prepare the nitrates solution. Known stoichiometric aqueous solutions of europium and yttrium nitrates were mixed together. Then urea as a fuel was added to the mixture. The molar ratio of urea/cation ions was 2.5.

The resultant gel was heated in order to evaporate the free water and combustion of the mixture abruptly by using a furnace. Afterward the synthesized powder was thermally treated at 600 °C to increase the crystallinity.

The TEM image obviously shows the atomic layers of NPs and distance of these layers are about 0.2 nm (Fig. 2). Also, the Y_2O_3:Eu particles were characterized by X-ray diffraction (XRD). Figure 3 demonstrates the X-ray diffraction patterns of Y_2O_3:Eu NPs which was in agreement with single phase of cubic Y_2O_3 with Ia-3 space group. The crystallite size, D_{XRD} was calculated using the Scherrer's equation as follows [28]:

$$D_{XRD} = \frac{0.9\lambda}{B\cos\theta} \quad (1)$$

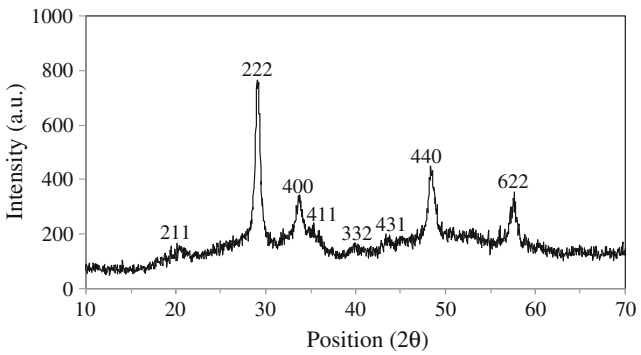

Fig. 3 XRD pattern of Eu-doped Y_2O_3 NPs

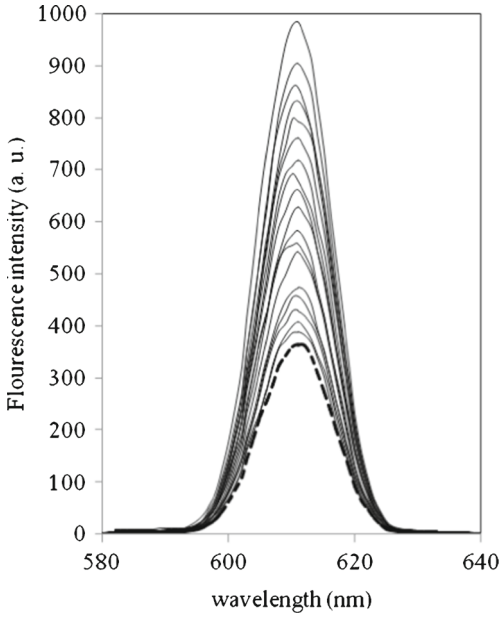

Fig. 4 Fluorescence spectra of modified Y_2O_3: Eu NPs containing of thiamine with different concentrations. From down to up: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.2, 1.5, 2, 3, 4, 5, 7, 9, 14, 19, 29 and 44 μM, respectively

Where λ is the wavelength of the radiation, θ the diffraction angle and B is the corrected half-width of the diffraction peak. The value of B is expressed as:

$$B^2 - B_m^2 - B_s^2 \quad (2)$$

Where B_m is the full-width at half maximum of the diffraction peak and B_s is the instrumental broadening. The crystallite size determined from the broadened peak was about 18 nm in europium-doped Y_2O_3 luminescence NPs.

Spectrophotofluorimetric determination

0.05 g Y_2O_3:Eu NPs were dispersed in 100 mL phosphate buffer solution (0.25 M) in pH 7.4. 5 mL of the clear suspension was transferred into a calibrated 10 mL test tube and captopril added to the solution to modification of NPs. The prepared stock solutions of thiamine were added to the prepared solution. The fluorescence intensity was recorded at 612 nm.

Results and discussions

Y_2O_3:Eu NPs are potentially useful in sensor design, as they possess a significant response in fluorescence so it can be used as an indicator in monitoring molecules and biomolecules. Figure 4 shows that the increasing concentration of analyte efficiently enhance the luminescence band of modified Y_2O_3:Eu NPs, with no optical shift of emission which shows that the modified NPs didn't aggregate or become smaller after adding analyte [29]. Moreover, the narrow and symmetric fluorescence emission spectrum in 612 nm indicates that as prepared NPs are nearly monodisperse and homogeneous. The results indicated that enhancement of the fluorescence intensity reflected the analyte concentration. The increase of Y_2O_3:Eu fluorescence intensity was plotted as a function of thiamine concentration. To illustrate the increasing effects of thiamine on the fluorescence intensity of modified Y_2O_3:Eu NPs, Langmuir binding isotherm equation is used to describe the relationship between the PL intensity of NPs and the concentration of analyte. Based on the Langmuir equation

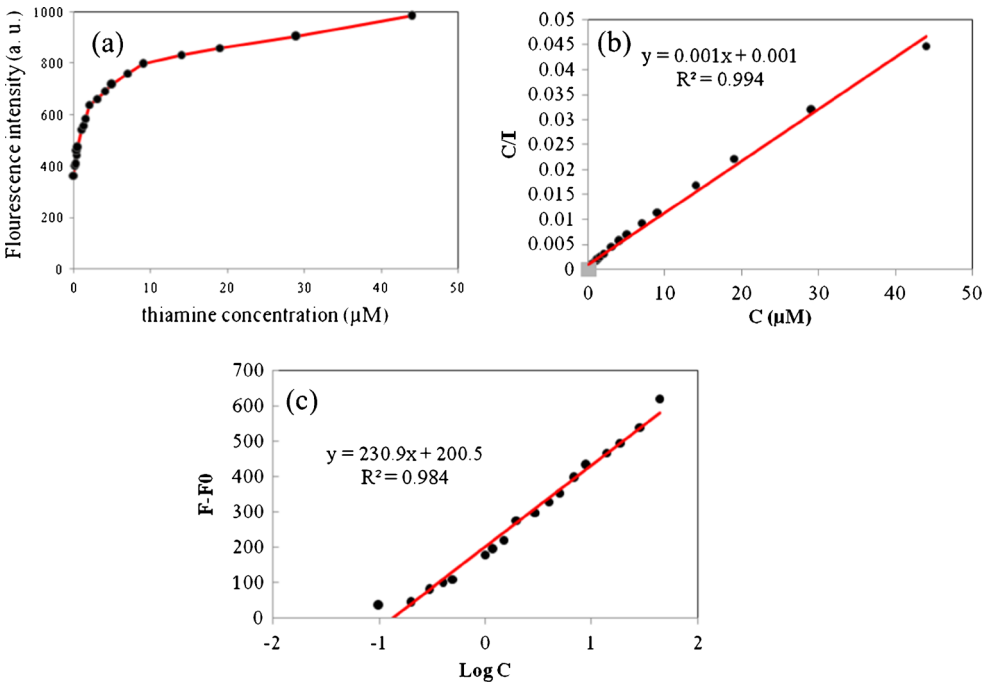

Fig. 5 **a** Fluorescence intensity of the modified Y_2O_3:Eu NPs versus the thiamine concentration from 0 to 44 μM, **b** Langmuir binding isotherm for the added thiamine from 0 to 44 μM with a correlation coefficient of 0.995 and **c** Enhanced fluorescence intensity against the logarithm of analyte concentration from 0 to 44 μM

Table 1 Analytical parameters of luminescent NPs in optical detection of different analytes

NPs	Analyte	*B* value	LOD (mol L^{-1})	Linear range (mol L^{-1})	RSD (%)	Ref.
CdTe	Mercury(II) ion	1.547	1.55 nM	2–14 nM	–	[31]
CdTe	Glyphosate	0.069	0.0725 nM	1–25 nM	0.1048	[32]
CdTe	Methomyl	0.41	0.08×10^{-6}	$(0.1–50)\times10^{-6}$	–	[32]
CdSe	Edaravone	0.3	0.09×10^{-6}	$(0.008–0.1)\times10^{-6}$	2	[33]
CdTe	Acetamiprid	–	3.4×10^{-8}	$0–10^{-3}$	3.9–4.5	[34]
CdSe-Psca	Tyrosine	0.02	0.012	$5\times10^{-8}–10^{-5}$	–	[35]
CdSe-Psca	Cysteine	0.31	0.032	$10^{-8}–10^{-4}$	–	[35]
Y_2O_3	Thiamine	1	0.144×10^{-6}	$0–44\times10^{-6}$	0.05	This work

the surface of the NPs consists of limited binding sites. Every single of these binding sites could absorb one analyte from the sample solution. Occupied site of NPs is defined as θ. So available binding site is 1-θ and the amount of binding of thiamine to the surface of modified NPs is depend on the thiamine concentration, C. Amount of binding of analyte to the surface of NPs, R_b is mentioned as:

$$R_b = K_b C(1-\theta) \tag{3}$$

The rate of desorption of the bound molecules from the surface of NPs just dependence on the fraction of occupied binding sites and is expressed as:

$$R_d = K_d\theta \tag{4}$$

The rate of binding is equal to the rate of desorption at equilibrium:

$$K_d\theta = K_b C(1-\theta) \tag{5}$$

The equilibrium can be solved for θ as a function of the ratio $B=K_b/K_d$:

$$\theta = (BC)/(1+BC) \tag{6}$$

The fraction of occupied binding sites, θ, is related to the ratio between the signal obtained at given thiamine concentration I and the maximum intensity I_{max}.

$$\theta = I/I_{max} \tag{7}$$

Therefore an expression that relates the thiamine concentration, *C*, to the signal intensity can be written as:

$$I/Imax = (BC)/(1+BC) \tag{8}$$

This equation can be linearized to take the form of Langmuir binding isotherm equation that is given in the following form:

$$\frac{C}{I} = \left(\frac{1}{BI_{max}}\right) + \left(\frac{1}{I_{max}}\right)C \tag{9}$$

As mentioned before, in this Equation B is the equilibrium binding constant and C is the analyte concentration. I and I_{max} represent the signals found at given concentration of analyte and the maximum intensity, respectively. Therefore, if the Langmuir theory for the binding of thiamine on the surface of modified NPs is correct, a plot of C/I as a function of C must be linear. From Fig. 5 it can be seen that the plot displays

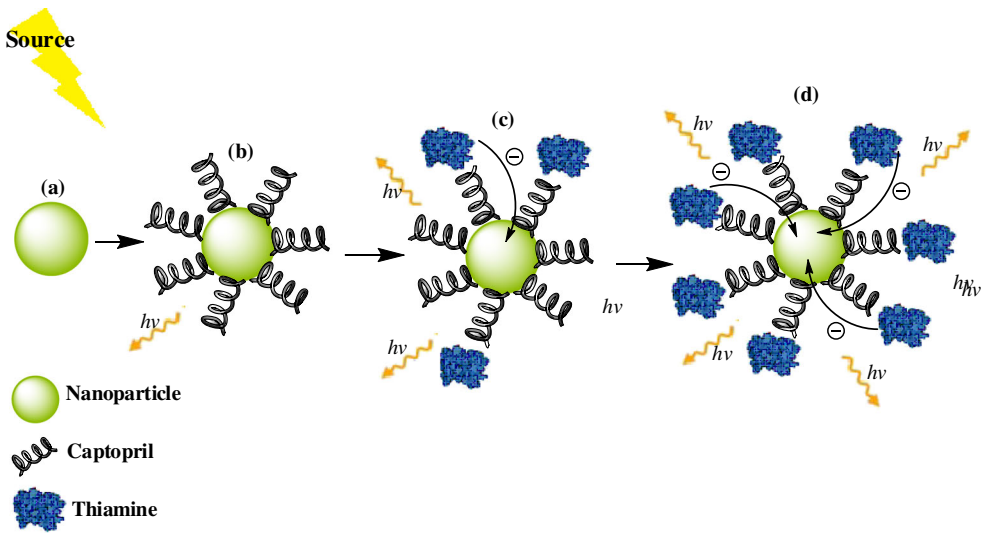

Fig. 6 Schematic illustration of a possible mechanism for fluorescence enhancement of Y_2O_3: Eu NPs by thiamine

a good linearity in the concentration range of 0.1–44 μM and the value of correlation coefficient (R) displayed 0.9946. Bullen and coworkers suggested that the equilibrium binding constant (B) represent the surface affinity of analyte and NPs [30]. So, if the B value is high the corresponding surface affinity is strong. From Table 1 several analytical parameters of recent reports are summarized.

It was deduced that thiamine might interact with the mentioned NPs through electrostatic forces (Fig. 6). Since the Y_2O_3:Eu were modified by captopril, the surface of them contain sulfur and carboxyl groups which appear as negative charge, while the thiamine contain positive charge. So thiamine interacts with the surface of NPs via electrostatic forces and creates more radiative centers and new radiative centers at the system and decrees the nonradiative recombination of electron-hole [26, 35].

Conclusions

We have demonstrated that the modified Y_2O_3:Eu NPs can be used as a reliable agent for determination of thiamine concentration. A novel method for analysis of thiamine has been proposed based on the enhancement of the fluorescence intensity of modified Y_2O_3:Eu NPs. Under the optimum condition, the modified Y_2O_3:Eu NPs exhibit good linear response in fluorescence intensity when thiamine with concentration ranging from 0.1 to 44 μM was analyzed. Because of their rapid and high sensitive detection, modified Eu doped Y_2O_3 NPs can be apply as a novel probe in biological systems.

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