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ORIGINAL PAPER

Preparation and Characterization of CdHgTe Nanoparticles and Their Application on the Determination of Proteins

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Abstract CdHgTe nanoparticles (NPs) with the emission in the near-infrared regions were prepared in aqueous solution, and were characterized by transmission electron microscopy, X-ray diffraction spectrometry, spectrofluorometry and ultraviolet-visible spectrometry. Based on the fluorescence quenching of CdHgTe NPs in the presence of proteins, a novel method for the determination of proteins with CdHgTe NPs as a near-infrared fluorescence probe was developed. Maximum fluorescence quenching was observed with the excitation and emission wavelengths of 500 and 693 nm, respectively. Under the optimal conditions, the calibration graphs were linear in the range of 0.04×10^{-6}–5.6×10^{-6} g ml^{-1} for lysozyme (Lyz) and 0.06×10^{-6}–6.1×10^{-6} g ml^{-1} for bovine hemoglobin (BHb), respectively. The limits of detection were 13 ng ml^{-1} for Lyz and 27 ng ml^{-1} for BHb, respectively. Four synthetic samples were determined and the results were satisfied.

Keywords CdHgTe nanoparticles · Near-infrared · Fluorescence probe · Protein · Determination

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Introduction

The determination of proteins as a reference for the measurements of other components in biological systems is a basic requisite in biochemistry. Upon till now, many techniques have been developed for detection of proteins, such as spectrophotometric methods [1, 2], fluorometric methods [3–5], calorimetry [6], and capillary electrophoresis [7].

Recent studies suggest that semiconductor NPs are excellent fluorophores due to their continuous excitation peaks, narrow and tunable emission peaks, and high resistance toward photobleaching [8]. There have been increasing interests in the development of bioactive and biocompatible nanomaterials for a variety of applications [9–12]. Wang et al. used CdS capped with 2-acrylamidoglycolic acid to detect proteins [13]. Lysozyme was determined using functionalized $CdSe_2$/CdS and CdTe as the probes, respectively [14, 15]. Functionalized nano-ZnS was employed for the determination of proteins [16].

Currently, the emission wavelengths of NPs fluorophores used for protein detection are mostly at the range of 400–600 nm. However, comparing with the measurements made in the ultraviolet and visible regions, near-infrared (NIR, about 600–1,000 nm) fluorescence has many advantages [17]. In this low-energy region, the level of background interference is low, which is especially beneficial for bioanalytical and other complex samples, where background fluorescence in the visible region is a source of interference. Scattering (Raman and Rayleigh) can be a large contributor of noise at low concentration of the analyte. However, it can be reduced at longer wavelength due to its dependence on the wavelength of detection by $1/\lambda^4$. Since the NIR fluorescence can effectively penetrate through skin and overlaying tissue, there is potential for the development of a noninvasive clinical diagnosis. From the

above mentioned features, NIR fluorescence is especially suitable for the studies of biological samples [18].

Here, CdHgTe NPs were synthesized by combining the virtue of the stable and high fluorescence intensity of CdTe NPs and the NIR wavelength tuning of HgTe NPs. The CdHgTe NPs conquered the demerit of HgTe NPs which is not possible to reflux the initial solution of HgTe NPs to accelerate the ripening process without rapid loss of luminescence [19, 20]. CdHgTe NPs show much promise because their NIR wavelength can be tuned by the composition and the incubation temperature. The fluorescence intensity of the prepared CdHgTe NPs at a NIR emission wavelength of 693 nm is quenched in the presence of proteins. Based on this phenomenon, a novel method has been developed for the determination of proteins using CdHgTe as the NIR fluorescence probe.

Experimental

Reagents and chemicals

All the chemicals used in the synthesis were of analytical grade or better. All the proteins and amino acids were of B. R. grade. All stock solutions of chemicals were prepared in doubly distilled water which was obtained from a SZ-93A automatic double pure water distillator (Yarong Biochemistry Intrument Factory, Shanghai, China). $CdCl_2 \cdot 2.5\ H_2O$ (Shuanghuan Chemical Reagent Factory, Beijing, China). Thioglycolic acid and $NaBH_4$ (Guangfu Fine Chemical Research Institute, Tianjin, China). Tellurium powder (Beilian Fine Chemical Factory, Tianjin, China). $Hg(NO_3)_2$ (Tongren Chemical Reagent Factory, Guizhou, China). Lysozyme and bovine hemoglobin were purchased from Lanji Technological Company, Shanghai, China. All stock protein standard solutions were prepared by directly dissolving them in doubly distilled water and stocked at 0–4 °C.

Apparatus

A transmission electron microscopy (TEM) image of the NPs was acquired on a Philips Tecnai G2 20 S-TWIN transmission electron microscope (Philips, Holland). Powder X-ray diffraction (XRD) spectrum was collected on a Rigaku D/max-2500 X-ray diffractometer (Rigaku, Japan). Fluorescence measurements were performed using a F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 × 1 cm). UV absorption spectra were recorded with a UV-3600 UV-VIS-NIR spectrophotometer (Shimadzu, Japan). The pH values were measured with a model Orion 420+ pH meter (Thermo electro Corporation, USA). All optical measurements were performed at room temperature under ambient conditions.

Synthesis of CdHgTe NPs

The aqueous colloidal solutions of thiol-stabilized CdTe NPs were prepared following standard techniques [21]. Colloidal solutions of CdHgTe NPs stabilized by thioglycolic acid were prepared as follows.

Preparation of sodium hydrogen telluride

$NaBH_4$ was used to react with Te powder under N_2 protection in water to produce sodium hydrogen telluride (NaHTe) by a molar ratio of 20:1. Briefly, 0.1898 g of $NaBH_4$ was

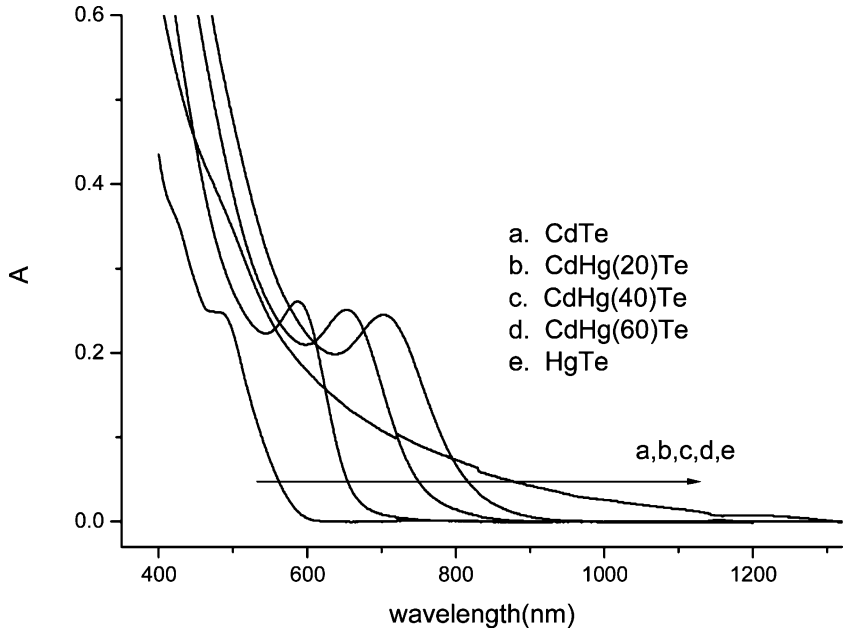

Fig. 1 Absorption spectra of CdTe, CdHg(X)Te and HgTe NPs. Incubation temperature, 60 °C; incubation time, 11 h

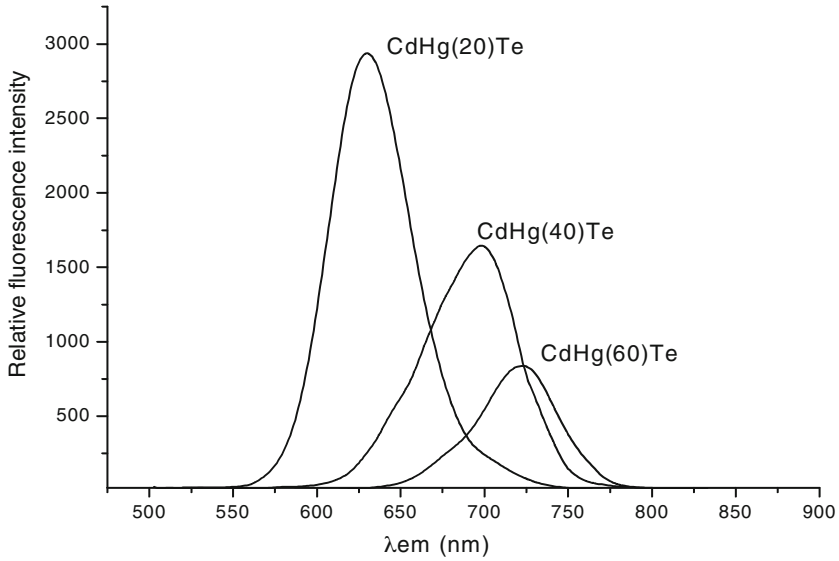

Fig. 2 Fluorescence spectra of CdHg(X)Te NPs. Incubation temperature, 60 °C; incubation time, 11 h

transferred to a small flask; then 4 ml of doubly distilled water was added. After 0.0319 g of Te powder was added in the flask, the reacting system was stirred under the protection of N_2. During the reaction, a small outlet connected to the flask was kept open to discharge the pressure from the N_2 and the resulting H_2. After Te powder was completely reduced, the resulting NaHTe solution was obtained.

Preparation of CdTe NPs

Freshly prepared oxygen-free NaHTe solution was added to a N_2-saturated aqueous solution of $CdCl_2$ and thioglycolic acid at pH 9.43 to prepare CdTe NPs solution. The molar ratio of Cd^{2+}/Te^{2-}/thioglycolic acid was 1:0.5:2.4. Then the above solution was refluxed after stirring for 1 h. The reflux was stopped when the emission maximum of CdTe NPs reached 550 nm with the excitation of 450 nm.

Preparation of CdHgTe NPs

The composite CdHg(X)Te NPs were synthesized through the chemical modification of CdTe NPs precursors by mercury [22, 23]. Under stirring, certain amounts of Hg^{2+} were added to CdTe colloidal solutions. Here, X is the molar percentage of the Hg to the Te contained in the CdHg (X)Te NPs. After stirring for 1 h, the above solution was incubated in a water bath. The incubation was stopped when the needed emission wavelength of CdHg(X)Te was reached with the excitation of 500 nm.

Procedures for spectrofluorometric determination of proteins

To a series of 10-ml calibrated test tubes, certain amounts of CdHg(40)Te NPs solution, 1.0 ml of KH_2PO_4–Na_2HPO_4

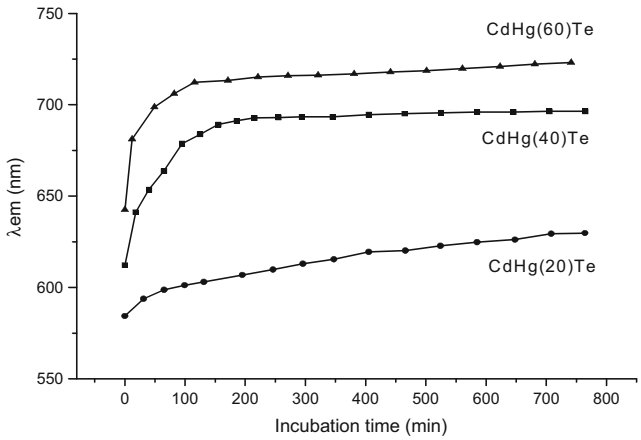

Fig. 3 Dependence of fluorescence emission wavelength of CdHg(X)Te NPs on incubation time. Incubation temperature, 60 °C

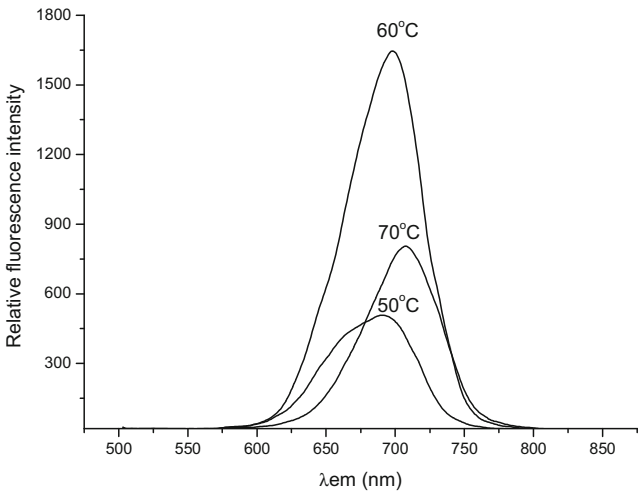

Fig. 4 Fluorescence spectra of CdHg(40)Te NPs prepared at different incubation temperatures. Incubation time, 11 h

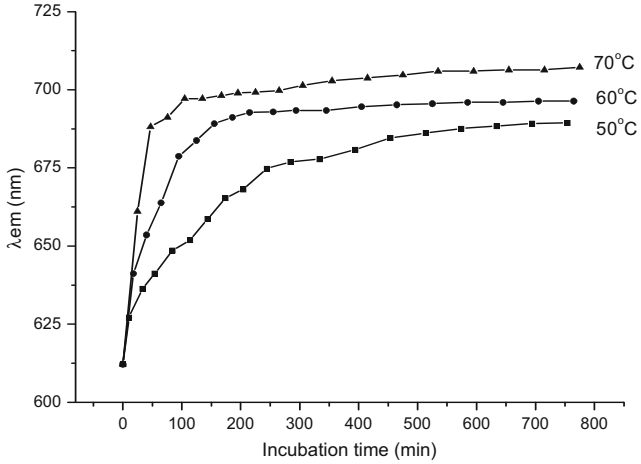

Fig. 5 Dependence of fluorescence emission wavelength of CdHg (40)Te NPs on incubation time at different incubation temperatures

buffer solution (pH 5.56) and appropriate volume of a protein working solution were sequentially added. The mixture was then diluted to volume with doubly distilled water and mixed thoroughly. The fluorescence intensities of the mixed solution (F) and the reagent blank (F_0) were recorded at 693 nm with the excitation wavelength of 500 nm. Plot a calibration curve of the change in normalized fluorescence intensity (F_0-F) vs. the concentration of the protein.

Results and discussion

Preparation of the CdHgTe NPs

The absorption spectra of CdTe, CdHg(X)Te and HgTe NPs were given in Fig. 1. The absorption edge of colloidal CdTe was observed at about 615 nm. With the increasing of the contents of Hg(II), the absorption edge of CdHg(X)Te shifts to longer wavelengths and finally to NIR spectral region. These results are attributed to the formation of HgTe on the surface of CdTe NPs. Because the bandgap of HgTe is small, the more the added Hg^{2+} is, the smaller the bandgap of CdHg(X)Te NPs becomes. Thus, we conclude that the electronic structure of the CdTe particles is strongly influenced by HgTe.

Effect of different contents of Hg(II)

Due to the much lower solubility of HgTe compared with CdTe, the addition of Hg^{2+} to CdTe colloidal solution resulted in an exchange of Cd^{2+} by Hg^{2+}. Figure 2 showed a set of fluorescence spectra of colloidal CdHg(X)Te solutions with different contents of Hg(II). With the increasing of the contents of Hg(II), the emission wavelengths of CdHg(X)Te NPs shift to longer wavelengths. This luminescence is attributed to the HgTe formed on the surface of the CdTe particles. It can be seen from Fig. 2 that the difference of fluorescence emission wavelength and intensity between CdHg(20)Te and CdHg(40)Te NPs is remarkable, whereas these differences between CdHg(40)Te and CdHg(60)Te NPs are smaller. The more the content of Hg(II) is, the fewer the Cd(II) which on the surface of NPs is. Therefore, with the addition of Hg^{2+} to the NPs system, the exchange between Cd^{2+} and Hg^{2+} becomes difficult and its influence on the fluorescence spectra becomes weak. Meanwhile, it can be seen from Fig. 3 that the red moving rates of the emission wavelength of CdHg(40)Te and CdHg(60)Te NPs were faster than that of CdHg(20)Te NPs. The emission wavelengths of CdHg(40)Te and CdHg(60)Te NPs reached stable in 100 min. However, CdHg(20)Te NPs needed to expend more time to achieve steady.

Effect of incubation temperatures

After mixing the CdTe NPs and Hg^{2+}, the fluorescence intensity of CdHg(40)Te NPs increased slowly. However, the increasing rate of this intensity can be accelerated by

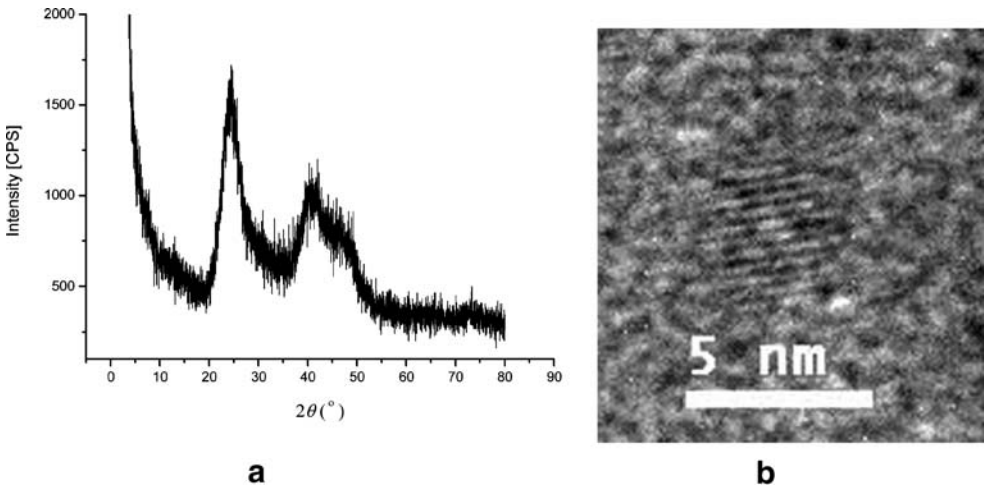

Fig. 6 XRD spectra (**a**) and TEM image (**b**) of CdHg(40)Te NPs

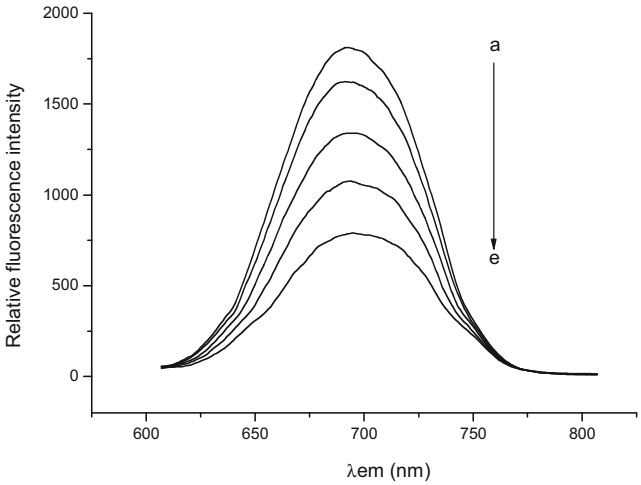

Fig. 7 Fluorescence spectra of CdHg(40)Te NPs alone and in the presence of Lyz. Lyz (from **a** to **e**): 0, 0.6, 1.6, 2.6, 3.6 μg ml^{-1}; pH 5.56; CdHg(40)Te NPs, 2.5×10^{-5} mol l^{-1}

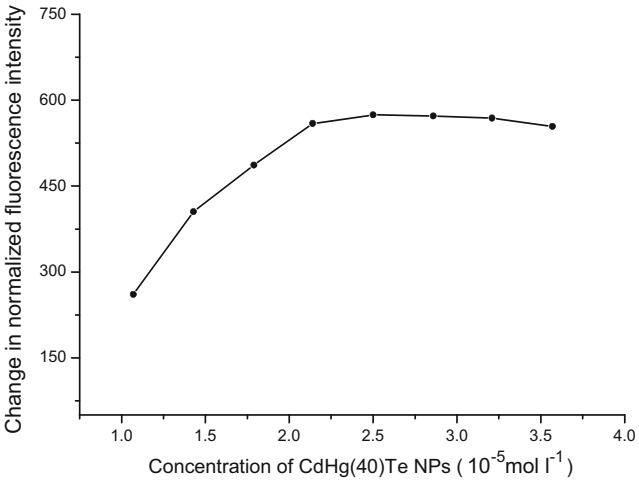

Fig. 9 Effect of concentration of the CdHg(40)Te NPs on the change in normalized fluorescence intensity. pH 5.56; Lyz, 2.0 μg ml^{-1}

incubation [24]. The fluorescence spectra of CdHg(40)Te NPs prepared at different incubation temperatures were shown in Fig. 4. It can be seen from Fig. 4 that the fluorescence intensities of CdHg(40)Te NPs synthesized at different incubation temperatures are different.

The dependence of fluorescence emission wavelength of CdHg(40)Te NPs prepared at 50, 60 and 70 °C on the incubation time was given in Fig. 5. It can be seen from Fig. 5 that a shift of the fluorescence emission wavelength of CdHg(40)Te NPs to longer wavelength was achieved by incubation. But the rates of shifts are different at different incubation temperatures. At the first stage of incubation, the tendence of shift is quick. After about 200 min, the fluorescence emission wavelengths of CdHg(40)Te NPs prepared at 60 and 70 °C tend to unchanged. But at 50 °C, the fluorescence emission wavelength of CdHg(40)Te NPs is difficult to approach stable until the incubation time is above 500 min. Therefore, 60 °C is the proper incubation temperature since the fluorescence emission wavelength is suitable and the fluorescence intensity of CdHg(40)Te NPs is the highest at this temperature.

We synthesized CdHgTe NPs by combining the virtue of CdTe (stable and high fluorescence intensity) and HgTe (potentially huge wavelength tuning range). By tuning the mercury content and the incubation temperature, we can synthesize CdHgTe NPs with the NIR emission wavelength we need. On one hand, the tuning of the emission wavelength of CdHgTe NPs is easier than that of single material NPs (CdTe or HgTe) by controlling the synthetic conditions; on the other hand, CdHgTe NPs are stable than HgTe NPs.

The longer the detection wavelength is, the less the interference of the scattering light and background fluorescence are. However, the wavelength range of our F-4500

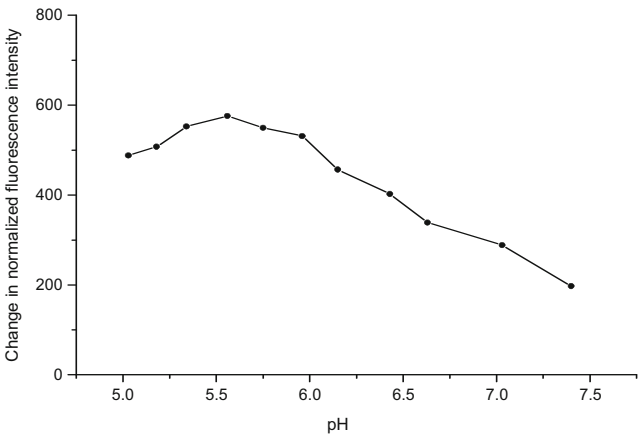

Fig. 8 The effect of pH on the change in normalized fluorescence intensity. CdHg(40)Te NPs, 2.5×10^{-5} mol l^{-1}; Lyz, 2.0 μg ml^{-1}

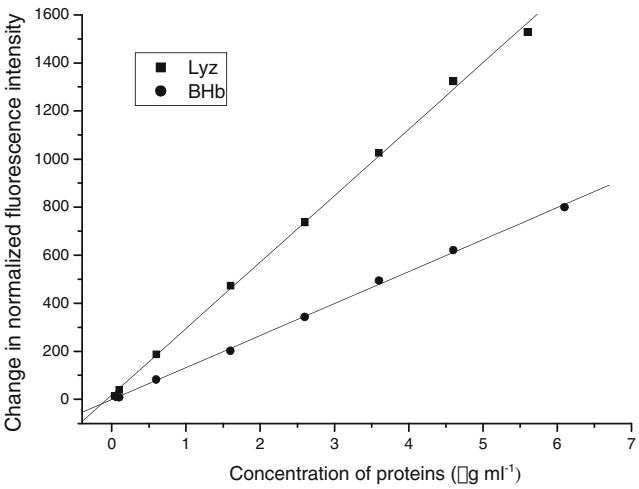

Fig. 10 The calibration graphs for Lyz and BHb

Table 1 Tolerance of foreign substances

Substances	Concentration (μg ml^{-1})	Relative error (%)	Substances	Concentration (μg ml^{-1})	Relative error (%)
Mg(II)	1.0	−3.0	L-aspartic acid	38	−3.8
Fe(III)	0.4	3.4	DL-alanine	72	4.9
Ca(II)	1.0	−4.4	L-tyrosine	50	4.4
Mn(II)	2.0	2.1	L-lysine	80	−3.6
Co(II)	0.1	−3.5	Glycin	22	−3.4
Zn(II)	0.4	−3.5	Benzedrine	20	2.7
Pb(II)	0.1	−2.4	D-fructose	10	−4.2
D-glucose	34	4.8	Sucrose	10	−3.8
L-histidine	2.5	4.1	Lactose	68	2.9
L-glutamic acid	25	−5			

pH 5.56; Lyz, 2.0 μg ml^{-1}; CdHg(40)Te NPs, 2.5×10^{-5} mol l^{-1}

fluorescence spectrophotometer is 200–730 nm. Therefore, we employed CdHg(40)Te NPs with the NIR emission wavelength of 693 nm as the fluorescent probe in the determination of proteins.

Characterization of the prepared CdHgTe NPs

The XRD spectra of CdHgTe

The lattice structures of both the CdTe and HgTe NPs are the same, and their lattice constants are very close to each other (the lattice constants of CdTe and HgTe are 0.648 and 0.646 nm, respectively) [25]. The XRD spectra of the prepared CdHg(40)Te NPs are presented in Fig. 6a. As shown in Fig. 6a, the XRD peaks of CdHg(40)Te NPs are well matched with those of the CdTe and HgTe crystals [25]. The broadening of the XRD peaks observed in the XRD pattern originates from the nanometer-scaled diminution of the crystallite sizes [25].

TEM image of CdHgTe NPs

The morphology of the prepared CdHg(40)Te NPs was studied by TEM. Figure 6b showed a typical image for the prepared CdHg(40)Te NPs. The shape of these NPs is close to spherical single-crystalline and their average size is about 5 nm in diameter.

Application on the determination of proteins

Spectral characteristics

The fluorescence spectra of the composite CdHg(40)Te NPs alone and in the presence of various concentrations of Lyz were shown in Fig. 7. The maximum emission wavelength of CdHg(40)Te NPs with the excitation wavelength of 500 is 693 nm. The presence of Lyz resulted in a significant decrease of the fluorescence intensity but without obvious shift of the maximum emission wavelength. Therefore, an excitation wavelength of 500 nm and an emission wavelength of 693 nm were chosen in the subsequent study.

Effect of pH

The effect of pH on the change in normalized fluorescence intensity was studied. The concentrations of CdHg(40)Te NPs and Lyz were maintained at 2.5×10^{-5} mol l^{-1} and 2.0 μg ml^{-1}, respectively. The results indicated that the maximal and constant change occurred in the pH range of 5.34–5.96 and the values of the change decreased at other

Table 2 Analytical results for the detection of Lyz in the synthetic samples

Protein content in sample (μg ml^{-1})	Main interferences	Found (μg ml^{-1})	Recovery (%, n=5)	RSD (%)
2.00	Mg(II), Mn(II), lactose, benzedrine	2.01	98.3–101.3	1.4
2.00	Ca(II), Zn(II), D-glucose, glycin	2.01	98.2–101.8	1.7
2.00	Fe(III), D-fructose, BSA, L-tyrosine	1.97	98.7–103.2	1.9
2.00	Ca(II), sucrose, BSA, DL-alanine	2.02	98.3–101.8	1.8

Mg(II) 5.0×10^{-7} g ml^{-1}, Mn(II) 1×10^{-6} g ml^{-1}, lactose 7.0×10^{-5} g ml^{-1}, benzedrine 1×10^{-5} g ml^{-1}, D-glucose 2.0×10^{-5} g ml^{-1}, Ca(II) 4×10^{-7} g ml^{-1}, Zn(II) 4.0×10^{-7} g ml^{-1}, glycin 1.0×10^{-5} g ml^{-1}, Fe(III) 4.0×10^{-7} g ml^{-1}, D-fructose 5.0×10^{-6} g ml^{-1}, BSA 1.0×10^{-6} g ml^{-1}, L-tyrosine 5.0×10^{-6} g ml^{-1}, sucrose 1.0×10^{-5} g ml^{-1}, DL-alanine 4.0×10^{-5} g ml^{-1}

pH values outside this range (Fig. 8). In the subsequent studies, a pH of 5.56 was recommended as acidity condition on the determination of proteins.

Effect of the concentration of the CdHg(40)Te NPs

The influence of the concentration of CdHg(40)Te NPs on the change in normalized fluorescence intensity was investigated with constant concentration of Lyz (2.0 μg ml^{-1}) at pH 5.56. The results showed that the maximal and constant change was reached when the concentration of the colloidal CdHg(40)Te solution was in the range of 2.1×10^{-5}–3.6×10^{-5} mol l^{-1} (Fig. 9). Thus, 2.5×10^{-5} mol l^{-1} of CdHg(40)Te colloidal solution was chosen for use.

Calibration graph and limit of detection

The calibration graphs for Lyz and BHb were constructed from the results obtained under the optimal conditions (Fig. 10). The calibration graphs were linear in the range of 0.04×10^{-6}–5.6×10^{-6} g ml^{-1} for Lyz (r=0.9994) and 0.06×10^{-6}–6.1×10^{-6} g ml^{-1} for BHb (r=0.9995), respectively.

The limit of detection (LOD) is given by the equation $LOD=KS_0/S$, where K is a numerical factor chosen according to the confidence level desired, S_0 is the standard deviation of the blank measurements (n=9), and S is the sensitivity of the calibration graph. Here a value of 3 was used for K. The limits of detection were 13 ng ml^{-1} for Lyz and 27 ng ml^{-1} for BHb, respectively.

Tolerance of foreign substances and the determination of synthetic samples

The interference of foreign substances was tested, and the results were presented in Table 1. According to the tolerance of foreign substances, four synthetic samples were made. The results of the determination using the standard procedure were given in Table 2. The results in Table 2 show that this NIR fluorescent probe method is reliable, sensitive and reproducible.

Conclusions

CdHgTe NPs were prepared in aqueous solution. The synthesized CdHgTe NPs show much promise because their NIR wavelength can be tuned by the composition and the incubation temperature. CdHg(40)Te NPs as a novel NIR fluorescence probe for detection of proteins endowed with a high sensitivity was developed. Besides, the NIR emission wavelength of 693 nm and the large Stokes shift ($\Delta\lambda$= 193 nm) could allow this method to avoid the interference of the background efficiently in biological systems.

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