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CdS/TiO_2–fluorescein isothiocyanate nanoparticles as fluorescence resonance energy transfer probe for the determination of trace alkaline phosphatase based on affinity adsorption assay

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ABSTRACT

The CdS/TiO_2–fluorescein isothiocyanate (FITC) luminescent nanoparticles (CdS/TiO_2–FITC) with the particle size of 20 nm have been synthesized by sol–gel method. CdS/TiO_2–FITC could emit the fluorescence of both FITC and CdS/TiO_2. The fluorescence resonance energy transfer (FRET) occurred between the donor CdS/TiO_2 and the acceptor FITC in the CdS/TiO_2–FITC. Taking advantages of the excellent characteristics of FRET, a new CdS/TiO_2–FITC FRET labeling reagent and a CdS/TiO_2–FITC–wheat germ agglutinin (CdS/TiO_2–FITC–WGA) fluorescent probe have been developed. The FRET occurring between the donor CdS/TiO_2 and the acceptor FITC in the labelled product CdS/TiO_2–FITC–WGA–AP, formed in the affinity adsorption reaction between the WGA in this CdS/TiO_2–FITC–WGA fluorescent probe and alkaline phosphatase (AP), sharply enhanced the fluorescence signal of FITC and quench the fluorescence signal of CdS/TiO_2. Moreover, the ΔF (the change of the fluorescence signal) of FITC and CdS/TiO_2 were proportional to the content of AP, respectively. Thus, a new method that CdS/TiO_2–fluorescein isothiocyanate nanoparticles for the determination of trace AP based on FRET-affinity adsorption assay has been established. The limit of quantification (LOQ) of the method was 1.3×10^{-17} g AP mL^{-1} for CdS/TiO_2 and 1.1×10^{-17} g AP mL^{-1} for FITC, respectively. This sensitive, rapid, high selective and precise method has been applied to the determination of AP in human serum and the prediction of human disease with the results agreed well with enzyme-linked immunosorbent assay (ELISA) in Zhangzhou Municipal Hospital of Fujian Province. Simultaneously, the reaction mechanism for the determination of AP was also discussed.

1. Introduction

AP is mainly used to identify and diagnose liver cancer as well as skeleton cancer [1]. Abnormal content of AP could lead to some tumor diseases like liver cancer, skeleton cancer [2–5], and the structure of AP varies with the different structures of N carbohydrate chain of glycoprotein in pathological tissues and serum [6]. So far, lectin affinity sedimentation method has been used to determine the carbohydrate chain structure of AP and identify different liver diseases in clinic [7]. Obviously, AP is a sensitive physical and chemical index in clinical diagnosis [8]. There have been many methods used to determine the content of AP in serum, such as surface-enhanced raman spectroscopy (LOQ: 3.3×10^{-8} g mL^{-1}) [9], electrochemical method (LOQ: 2.2×10^{-11} g mL^{-1} [10]; LOQ: 2.3×10^{-11} g mL^{-1} [11]), affinity adsorption solid substrate-room temperature phosphorimetry (LOQ: 3.3×10^{-15} g mL^{-1} [12]; 9.2×10^{-14} g mL^{-1} [13]; 3.6×10^{-13} g mL^{-1} [14]; 1.2×10^{-15} g mL^{-1} [15]; 1.2×10^{-15} g mL^{-1} [16]; 1.2×10^{-13} g mL^{-1} [17]; 3.2×10^{-15} g mL^{-1} [18]; 1.2×10^{-14} g mL^{-1} [19]; 2.0×10^{-17} g mL^{-1} [20]) nitrophenylphosphate plastic membrane sensor (LOQ: 2.1×10^{-3} g mL^{-1} [21]) and fluorescence method (LOQ: 24.6 mU L^{-1} [22]; 24.6 mU L^{-1} [23]). However, the sensitivity of surface-enhanced Raman spectroscopy, nitrophenylphosphate plastic membrane sensor and fluorescence method is low, the reproducibility of electrochemical method is poor and the luminescent nanoparticles used in affinity adsorption solid substrate-room temperature phosphorimetry needs surface modification.

FRET is most widely studied of energy transfer fluorescence analysis which is one of the important development directions of modern molecular luminescence spectroscopy studies. According to the Förster energy transfer theory [24–25], when energy discrepancy of vibrational level from ground state and first excited state between donor molecule and receptor molecule is

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equivalent, or even when emission spectrum of donor molecule and excitation spectrum of receptor molecule effectively overlappes, non-radiative energy transfer could still carry out from donor molecule to receptor molecule. In recent years, FRET technology [26] has been successfully applied in the studies of the structure, properties, reaction mechanism and quantitative analysis of biological macromolecule, which plays a key role in the study of protein and nucleic acid [27]. For example, effective FRET was carried out between some organic dyes (calcein-safranine T [28], eosinY-rhodamine B [27] and acridine orange-rhodamine 6 G [29], etc). However, the FRET between CdS/TiO_2 and FITC and CdS/TiO_2–FITC FRET affinity adsorption assay for the determination of trace AP and the forecast of human disease were rarely reported.

In this paper, the FRET between the donor CdS/TiO_2 and acceptor FITC in the CdS/TiO_2–FITC luminescent nanoparticles and their fluorescence characteristics were studied, and a new CdS/TiO_2–FITC FRET labeling reagent and CdS/TiO_2–FITC–WGA fluorescence probe has been developed. Simultanously, a new method for the determination of trace AP based on CdS/TiO_2–FITC FRET affinity adsorption assay and the forecast of human diseases has been established. The detection limit of this method was lower than that of Refs. [12–21] showing high sensitivity. CdS/TiO_2–FITC could label WGA without any surface modification and the sepecificity of CdS/TiO_2–FITC–WGA was high, showing high selectivity. Besides, AP could be determined at the fluorescence excitation/emission wavelength of either FITC or CdS/TiO_2, exhibiting flexibility of this method. Furthermore, the method has been applied to forecast of human diseases. This research widened the application of nanomaterial and FRET as well as provided a new technique for the determination of trace AP and the forecast of human diseases in clinic.

2. Experimental

2.1. Apparatus and reagents

Fluorescence measurements were carried out on a LS-55 luminescence spectrophotometer (Perkin Elmer Corporation, U.S.A.). The instrument's main parameters are as follows: excitation (Ex) slit 10 nm, emission (Em) slit 8 nm, and scan speed 1500 nm min^{-1}. An AE240 electronic analytical balance (Mettler–Toledo Instruments Company Limited) was also used.

AP solution: 1.00 mg mL^{-1} AP (Sigma Company, Santa Clara, CA, USA) was prepared as stock solution. Then, it was respectively diluted to 10.00 ng mL^{-1} and 1.00 ng mL^{-1} with Na_2CO_3–$NaHCO_3$ buffer solution of 0.10 mol L^{-1} (pH=9.12), and stored in the refrigerator at 4 °C for use. 10.00 mg mL^{-1} WGA (Sigma Company, Santa Clara, CA, USA) was diluted to 20.00 ng mL^{-1} with of KH_2PO_4–Na_2HPO_4 buffer solution 0.067 mol L^{-1} (pH=7.40). Preparation of EDC–NHS coupling agent solution: the mixture of 5 mM 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC, Alfa Company) and 5 mM *N*-hydroxysucci-nimide (NHS, Alfa Company) was prepared with 40% ethanol. 1.0×10^{-4} mol L^{-1} FITC, CdS/TiO_2 (synthesized according to the method in Ref. [30]), CdS/TiO_2–FITC (synthesized by ourselves) HS–CH_2–COOH, C_2H_5OH, $CdCl_2\cdot2.5H_2O$, $Na_2S\cdot9H_2O$, $Ti(OC_4H_9)_4$ and HNO_3 were used in the experiment. All the reagents were A.R. grade expect that AP and WGA were biological reagents. The water was prepared by thrice quartz sub-boiling distillation.

2.2. Synthesis of CdS/TiO_2 and CdS/TiO_2–FITC

Synthesis of CdS/TiO_2 [30]: the transparent TiO_2 solution was obtained by slowly adding 6.00 mL $Ti(OC_4H_9)_4$ into 200.00 mL water at (0 ± 0.5) °C, and the solution was dialysed with 4 L water for 8 h to obtain TiO_2 sol of pH within 2–3. 10.00 mL of 1.0×10^{-2} mol L^{-1} HS–CH_2–COOH was added into 50.00 mL of 0.20 g L^{-1} TiO_2 sol with rapid stirring, and then 1.00 mL of 5.0×10^{-2} mol L^{-1} $CdCl_2$ and 5.00 mL of 1.0×10^{-2} mol L^{-1} Na_2S were added. At last, the buff transparent 0.75 g L^{-1} CdS/TiO_2 solution was obtained and kept at 4 °C for 72 h (pH=4).

Synthesis of CdS/TiO_2–FITC: 6.00 mL $Ti(OC_4H_9)_4$ was dropped into 12.00 mL anhydrous ethanol, and then 0.50 mL of 1.0×10^{-4} mol L^{-1} FITC was added into the mixture. The following steps were carried out according to the steps of synthesis of CdS/TiO_2, and the salmon pink transparent CdS/TiO_2 solution was obtained without any precipitation at 4 °C for 72 h. At the same time, for the system containing 1.00 fg AP mL^{-1} and 1.00 mL of 20.00 ng WGA mL^{-1}, the effects of the synthetic conditions of CdS/TiO_2–FITC luminescent nanoparticles on the ΔF of the system were examined. Results show that the ΔF of the system reached the maximum when 2.00 mL $Ti(OC_4H_9)_4$, 4.00 mL C_2H_5OH, 10.00 mL of 1.0×0^{-2} mol L^{-1} HS–CH_2–COOH and 0.50 mL of 1.0×10^{-4} mol L^{-1} FITC were used.

2.3. Characterization of CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP

Firstly, to a 10-mL color comparison tube, 1.00 mL CdS/TiO_2–FITC and 1.00 mL of 20.00 ng WGA mL^{-1} were added and then diluted to 10 mL with anhydrous ethanol and mixed homogeneously. The mixture was kept at 50 °C for 45 min and cooled by flowing water for 5 min. At last, the CdS/TiO_2–FITC–WGA solution was obatined. Secondly, to a new 10-mL color comparison tube, 1.00 mL of 1.00 fg AP and 1.00 mL CdS/TiO_2–FITC–WGA were added and then diluted to 10 mL with anhydrous ethanol and mixed homogeneously. The mixture was kept at 50 °C for 45 min and cooled by flowing water for 5 min. Then, CdS/TiO_2–FITC–WGA–AP was obtianed. Proper CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP solution were taken out and their particle sizes were measured by a JEM-2000EX transmission electron microscope (TEM, Japan Electric Co.), respectively. Simultaneously, proper CdS/TiO_2, CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP solution were taken out and dried to power, then the corresponding power was scanned by X′Pert PRO X-ray diffractometer (XRD, made by PANalytical Co. in Holand) to obtain their XRD spectra.

2.4. Measurement of fluorescence

To a 10-mL color comparison tube, proper volume of AP, 1.00 mL CdS/TiO_2–FITC, 1.00 mL WGA, 1.00 mL EDC–NHS and 3.00 mL of 0.10 mol L^{-1} Na_2CO_3–$NaHCO_3$ (pH=9.12) were added, then diluted to 10 mL with anhydrous ethanol and mixed homogeneously. The system was kept at 50 °C for 45 min, and cooled by flowing water for 5 min, the fluorescence signals of CdS/TiO_2–FITC–WGA–AP testing solution (F) and CdS/TiO_2–FITC–WGA blank solution (F_0) were recorded at 452/520 nm (λ_{ex}max/λ_{em}max) for FITC or 213/372 nm for CdS/TiO_2. Then, ΔF was calculated, which was equal to (F–F_0) for FITC but (F_0–F) for CdS/TiO_2.

2.5. Sample treatment

1.00 mL of human serum from nine persons (A, B, C, D, E, F, G, H and I) were diluted with Na_2CO_3–$NaHCO_3$ buffer solution (pH=9.12) to 1000 mL. Then, 1.00 mL solution was taken out and diluted to 1000 mL. 1.00 mL of serum solution was taken and the content of AP was determined according to experimental method. Meanwhile, a standard addition recovery experiment was also conducted.

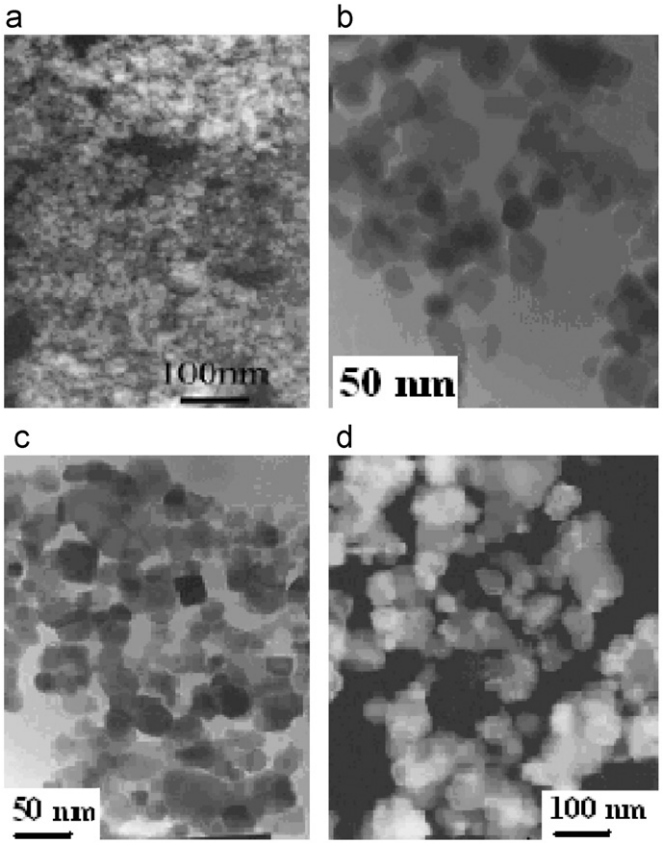


Fig. 1. TEM images of CdS/TiO_2, CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP.

3. Results and discussion

3.1. Morphology characterization of CdS/TiO_2, CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP

In XRD spectra, positions of the strongest diffraction peak (2θ) were 25.2°, 25.3°, 25.4° and 25.5° for CdS/TiO_2, CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP, respectively, which were basically consistent. According to the peak width of XRD, the grain diameters calculated by Scherre formula ($D=k\lambda P\beta\cos\theta$) were 9.7 nm for CdS/TiO_2, 14.9 nm for CdS/TiO_2–FITC, 19.8 nm for CdS/TiO_2–FITC–WGA and 24.5 nm CdS/TiO_2–FITC–WGA–AP, and the results were agreed well with TEM images (Fig.1, a–d).

3.2. Fluorescence spectra of CdS/TiO_2–FITC FRET

FITC and CdS/TiO_2 could emit strong and stable fluorescence at 458.2/504.6 nm and 375.2/431.8 nm (λ_{ex}max/λ_{em}max, Fig. 2, Table 1), and F values were 815.0 (curve c) and 226.5 (curve d), respectively. In the system of CdS/TiO_2–FITC, the fluorescence signal of acceptor FITC (λ_{ex}max/λ_{em}max=452.6/517.6 nm, F=909.6, curve b) was stronger than the individual FITC, the ΔF was 94.6, with λ_{em}max red shifting for 13 nm; while the fluorescence signal of donor CdS/TiO_2 (λ_{ex}max/λ_{em}max=375.4/427.7 nm, F=179.1, curve e) was weaker than the individual CdS/TiO_2, the ΔF was 47.4, with a blue shift of λ_{em}max for 4.1 nm. Moreover, there was overlap between the fluorescence emission spectrum (curve e) of donor CdS/TiO_2 and the fluorescence excitation spectrum (curve a) of acceptor FITC (Fig. 2), implicating that FRET could occur between CdS/TiO_2 and FITC in CdS/TiO_2–FITC system [24,25]. The non-radiative energy transferring from the donor molecule to acceptor decreased the non-radiative energy of CdS/TiO_2 and increased the non-radiative energy of FITC in FRET course, the fluorescence signal of CdS/TiO_2 quenched while the fluorescence signal of FITC enhanced in the CdS/TiO_2–FITC system, which further indicated that CdS/TiO_2–FITC system was a new FRET system.

Efficiency (η) of FRET could be calculated by $\eta=1-(F_d/F_{d0})$ [31], here F_{d0} and F_d represented the fluorescence intensity of donor CdS/TiO_2 in presence and in absence of FITC, respectively, and the FRET system of CdS/TiO_2–FITC was 20.9% ($\eta=1-(179.1/226.5)$, which further proved the FRET phenomenon occured between FITC and CdS/TiO_2 molecule.

When 20.0 ng WGA was added to the CdS/TiO_2–FITC system, CdS/TiO_2–FITC reacted with WGA to form CdS/TiO_2–FITC–WGA, which caused the fluorescence signal of the acceptor FITC to sharply quench (λ_{ex}max/λ_{em}max=452.3/520.0 nm, F=663.9, Tables 2, ΔF=245.7) and the fluorescence signal of the donor CdS/TiO_2 to sharply enhance (λ_{ex}max/λ_{em}max=213.2/371.8 nm, F=420.1, ΔF=241.0, Fig. 3, Table 2), indicating that there was no FRET occurring between the donor CdS/TiO_2 and the acceptor FITC. There was stipple overlap between the fluorescence emission spectrum (curve h) of the donor CdS/TiO_2 and the fluorescence excitation spectrum (curve f) of the acceptor FITC (Fig. 3). The distance between CdS/TiO_2 and FITC was too far to take place FRET [32]. Obviously, CdS/TiO_2–FITC could be used as the fluorescent probe for WGA.

When there was 100.0 fg AP in the CdS/TiO_2–FITC–WGA system, CdS/TiO_2–FITC–WGA took a specific affinity adsorption reaction with AP to producted CdS/TiO_2–FITC–WGA–AP, which sharply enhance the fluorescence signal of the acceptor FITC (λ_{ex}max/λ_{em}max was 452.4/520.2 nm, F=938.2, Tables 1, ΔF=274.3) and sharply quench the fluorescence signal of the donor CdS/TiO_2 (λ_{ex}max/λ_{em}max was 213.0/371.6 nm, F=200.6, Tables 2, ΔF=219.5). Besides, there was overlap between the fluorescence emission spectrum (curve i) of donor CdS/TiO_2 and the fluorescence excitation spectrum (curve g) of acceptor FITC (Fig. 3) and η was 11.4% ($\eta=1-200.6/226.5$), indicating that the fluorescence resonance energy transfered from CdS/TiO_2 to FITC. Thus, CdS/TiO_2–FITC–WGA could be used as fluorescent probe for the determination of trace AP, and working wavelengthes were 213/372 nm for CdS/TiO_2 and 452/520 nm for FITC, respectively.

3.3. Optimum conditions

In order to improve the sensitivity, precision, repeatability and accuracy of this method, for the system containing 1.00 fg AP mL^{-1}, the effects of the concentration and the volume of reagents, reaction time and temperature, acidity for reaction and standing time on the ΔF of the system were examined in a univariate approach (Table 3), respectively. Results show that the ΔF reached the maximum and remained stable when 1.00 mL of 20.00 ng mL^{-1} WGA and 3.00 mL Na_2CO_3–$NaHCO_3$ were used, the reaction temperature was 50 °C and the time was 45 min, pH was 8.11–10.20. The ΔF of the CdS/TiO_2–FITC–WGA–AP system almost remained unchanged within 15–50 min after being cooled by flowing water for 5 min, indicating the method had good repeatability. At this time, the pH value of the system was 9.20.

3.4. Linear range, working curve and LOQ

Under the optimal conditions above, the ΔF of CdS/TiO_2–FITC–WGA–AP FRET system was linearly related to the content of AP. When AP was detected with CdS/TiO_2 and FITC, the regression equations of working curve were $\Delta F=0.09725+22.61C$ AP (fg mL^{-1}, $n=7$) and $\Delta F=0.2253+27.30C$ AP (fg mL^{-1}, $n=7$) with the correlation coefficients of 0.9988 and 0.9987, respectively, and the corresponding linear range was 0.010–10 (fg mL^{-1}). The LOQ (calculated by 10 Sb/k, Sb referred to the standard

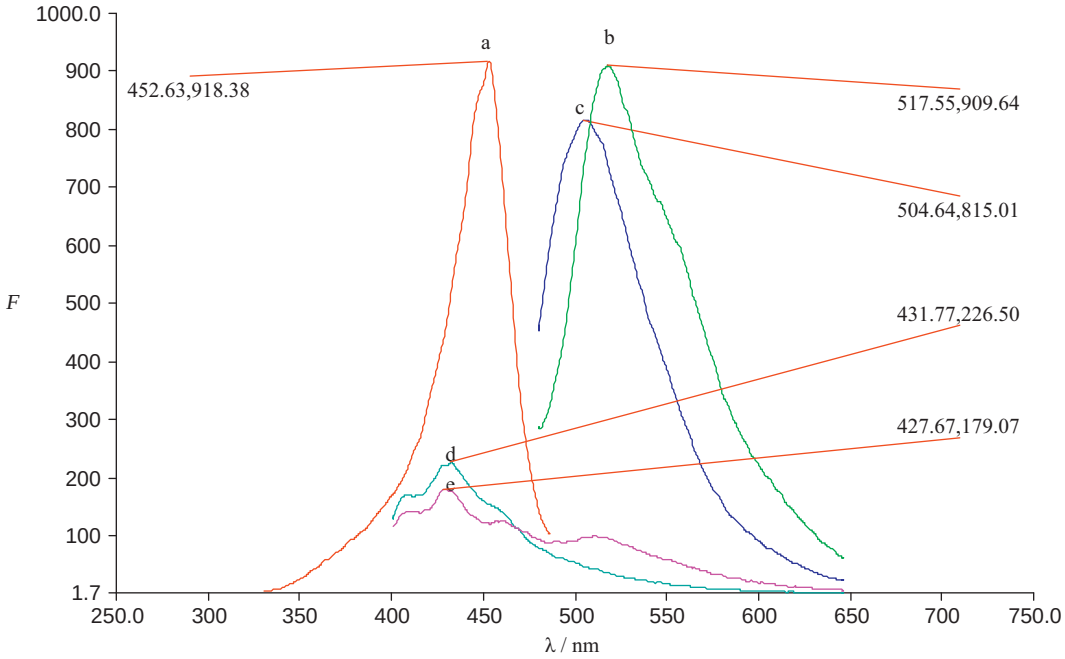


Fig. 2. Excitation and emission spectra of CdS/TiO_2–FITC FRET system (Curve a was excitation (Ex.) spectra, and curves b, c, d, e were emission (Em.) spectra. There was overlap between the emission spectrum (e) for CdS/TiO_2 and.the excitation spectrum for acceptor FITC (a). 0.75 g L^{-1} CdS/TiO_2; 3.0×10^{-6} mol L^{-1} FITC).

Table 1
Fluorescence FRET characteristics of CdS/TiO_2–FITC system.

System	λ_{ex}max for FITC	λ_{em}max for FITC	λ_{em}max for CdS/TiO_2	*F* for FITC	*F* for CdS/TiO_2	*F* for FITC	*F* for CdS/TiO_2	Ex. and Em. spectra
0031.00 mL FITC+3.00 mL Na_2CO_3–$NaHCO_3$		504.6 (curve c)		815.0				
1.00 mLCdS/TiO_2 +3.00 mL Na_2CO_3–$NaHCO_3$			431.8 (curve d)		226.5			
1.00 mL CdS/TiO_2–FITC+3.00 mL Na_2CO_3–$NaHCO_3$	452.6 (curve a)	517.6 (curve b)	427.7 (curve e)	909.6	179.1	Enhanced	Quenched	Overlapped (curves a, e)

Table 2
FRET characteristics of CdS/TiO_2-FITC-WGA-AP system.

System	λ_{ex}max for FITC	λ_{em}max for FITC	λ_{em}max for CdS/TiO_2	F for FITC	F for CdS/TiO_2	F for FITC	F for CdS/TiO_2	Ex. and Em. Spectra
1.00 mL FITC +3.00 mL Na_2CO_3–$NaHCO_3$		504.6 (curve k)		815.0				
1.00 mL CdS/TiO_2+3.00 mL Na_2CO_3–$NaHCO_3$			431.8 (curve j)		226.5			
1.00 mL CdS/TiO_2–FITC + 20.0 ng WGA	452.3 (curve f)		373.2 (curve h)	663.9	420.1	Quenched	Enhanced	Overlapped (curves h, f)
1.00 mL CdS/TiO_2–FITC + 20.0 ng WGA+100.0 fg AP	452.4 (curve g)		371.6 (curve i)	938.2	200.6	Enhanced	Quenched	Overlapped (curves g, i)

deviation of 11 parallel analysis of the blank reagent, *k* is the slope of the working curve, $n=11$), relative standard deviation (RSD%, 8 times of repeated measurements for the systems containing 0.010 and 10.0 fg mL^{-1} AP, respectively) and labelling reagents of this method were compared with those of Refs. [12–21]. Results are listed in Table 4.

Seen from Table 4, this method had lower LOQ, and higher sensitivity than those in Refs. [12–21]. When the molecular weight of AP was 100,000 [33], LOQ were 1.3×10^{-17} mol L^{-1} AP for CdS/TiO_2 and 1.1×10^{-17} mol L^{-1} AP for FITC, respectively. Compared with above methods, the operation of this method was easy because CdS/TiO_2–FITC could label WGA without any surface modification and the sepecificity of CdS/TiO_2–FITC–WGA was high, showing high selectivity. Besides, the content of AP was at ng level [34] in human serum. Thus, this method could be used to determine AP, and had wide application prospect. Simultaneously, a new phosphorescence labelling reagent was also exploited, which provided a new way for the application of CdS/TiO_2 and FITC.

3.5. Interference test

10.0 fg AP mL^{-1} was determined by both this method and the method in Ref. [12], respectively. The allowed concentration of coexistence materials (relative error (Er) was within ±5%) are listed in Table 5.

Results show that the allowed concentrations of coexistence materials and familiar ions of this method was higher and those

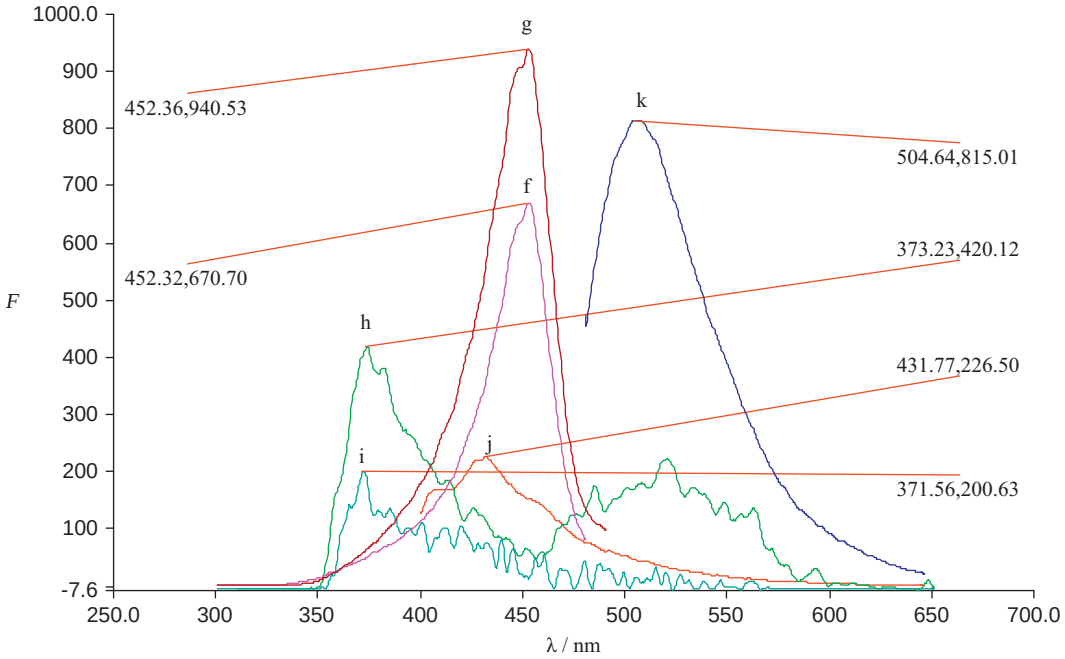


Fig. 3. Excitation and emission spectra of CdS/TiO_2–FITC–WGA–AP FRET system (Curves g, f were the Ex. spectra, and curves h, i, j, k were the Em spectra. There was overlap between the emission spectrum (curve i) for CdS/TiO_2 and the excitation spectrum (curve g) for acceptor FITC.).

Table 3
Optimum conditions for the determination of trace AP (n=5).

Single factor	Factor variables	The ΔF in CdS/TiO_2–FITC1–WGA–AP system	Optimal
WGA (ng mL^{-1})	5.00, 10.00, 15.00, 20.00, 30.00, 40.00	6.3, 11.0, 17.1, 20.7, 16.4, 12.3 (FITC) 4.4, 5.1, 10.2, 16.4, 15.3, 8.6 (CdS/TiO_2)	20 ng mL^{-1}
WGA (mL)	0.25, 0.50, 0.75, 1.00, 1.50, 2.00	7.1, 10.2, 17.3, 20.8, 16.2, 12.6 (FITC) 4.9, 8.8, 10.1, 16.3, 14.5, 8.5 (CdS/TiO_2)	1.00 mL
Na_2CO_3–$NaHCO_3$ (mL)	1.50, 2.00, 2.50, 3.00, 3.50, 4.00	8.3, 11.2, 15.6, 20.5, 14.8, 13.4 (FITC) 6.2, 9.2, 11.0, 16.3, 13.2, 9.7 (CdS/TiO_2)	3.00 mL
T °C	4.0, 25, 40, 50, 60,	10.3, 13.1, 17.6, 20.4, 7.9 (FITC) 6.2, 7.8, 14.5, 17.0, 9.2 (CdS/TiO_2)	50 °C
t/min	5, 15, 20, 30, 45, 50	8.4, 11.9, 14.3, 17.1, 20.6, 15.2 (FITC) 6.1, 7.8, 9.7, 13.4, 16.5, 8.8 (CdS/TiO_2)	45 min
pH	7.02, 8.11, 9.40, 10.20, 11.22	15.9, 20.4, 20.5, 20.5, 9.4 (FITC) 11.8, 16.6, 16.7, 16.7, 7.1 (CdS/TiO_2)	8.11–10.20
Standing time (min)	5, 15, 25, 35, 45, 50	17.5,21.6, 21.8, 21.5, 21.6, 15.9 (FITC) 12.8, 7.1, 16.8, 16.9, 17.2, 14.3 (CdS/TiO_2)	15–50 min

Table 4
Analytical parameters comparison of the methods mentioned above (LOQ: limit of quantification; RSD: relative standard deviation; ref: References; FITC: fluorescein isothiocyanate; WGA: wheat germ agglutinin; AP: alkaline phosphatase; FRET: fluorescence resonance energy transfer; AA-SS-RTP: affinity adsorption solid-substrate room-temperature phosphorimetry; R-D-SiO_2: rhodamine 6 G-dibromoluciferin luminescent nanoparticle; F-ol: fullerenol; DBS: dodecylbenzenesulfonic acid sodium salt; ESOR: self-ordered ring of eosin; DMA: *N*, *N*-dimethylaniline; D-SiO_2: dibromoluciferin luminescent nanoparticle; 4G D: 4.0-generation dendrimer; MWNTs-B's: multi-wall carbon nanotubes; RTP: room temperature phosphorescence; 8-QBA-PMS: 8-quinolineboronic acid phosphorescent molecular switch.).

Method	LOQ (g mL^{-1})	RSD (%)	Labelling reagents	Ref.
CdS/TiO_2-FITC-WGA-AP-FRET	1.3×10^{-17} Sb was 0.030 k was 22.61 1.1×10^{-17} Sb was 0.030 k was 27.30	4.5–3.3 3.7–2.6	CdS/TiO_2-FITC	The method
AA-SS-RTP for the WGA-AP-WGA-R-D-SiO_2 system	3.4×10^{-15}	4.9–3.6	R-D-SiO_2	[12]
WGA-AP-WGA-F-ol-DBS	8.6×10^{-13}	4.5–3.6	F-ol-DBS	[13]
ESOR-WGA-AP	3.3×10^{-12}	3.6–1.3	ESOR	[14]
AA-SS-RTP for the WGA- AP-WGA-F-ol-FITC-DMA	1.2×10^{-15}	4.1–1.3	F-ol –FITC-DMA	[15]
AA-SS-RTP for the R-SiO_2-WGA-AP system	1.2×10^{-15}	3.6–4.2	D-SiO_2	[16]
WGA-AP-WGA-4G D-Triton-100X	1.2×10^{-13}	3.3–4.8	4G D-Triton-100X	[17]
AP-WGA- DMA-F-ol- $(FITC)_n$	3.2×10^{-15}	3.4–4.2	DMA-F-ol-$(FITC)_n$	[18]
SS-RTP by AP enhancing MWNTs-B's RTP	1.2×10^{-14}	4.4–1.2	MWNTs-B'	[19]
WGA-AP-WGA-8-QBA-PMS	2.0×10^{-17}	2.1–2.4	8-QBA-PMS	[20]
Nitrophenylphosphate plastic membrane sensor	2.0×10^{-3}			[21]

of Ref. [12] indicating better selectivity. The interference content order of non-pole amino acid to ALP was: L-Isoleucine > L-Valine > L-Leucine > L-Alanine. In pole amino acid, the interference content of L-Cys is the highest. In acid and alkaline amino acid, the interference content order was: L-Aspartic acid > L-Glutamic acid > L-Arginine > L-Lysine > L-Histidine, which indicates that the interference content of acid amino acid was higher than that of alkaline amino acid. Interference content order of aromatic amino acid was: L-Tryptophane > L- Phenylalanine. These results indicated that the selectivity was good.

3.6. Sample analysis

Results of this method were compared with those obtained by ELISA in Zhangzhou Municipal Hospital of Fujian Province and the results are listed in Table 6 (*Note*: (+) stands for have pathology,

Table 5
Effect of coexistent materials (CM stands for coexistent materials; AC (pg mL^{-1}) stands for the allowed concentration; M stands for multiple (Allowed concentration/10.0 fg AP mL^{-1}); HAS is human serum albumin; BSA is bovine serum albumin; IgG is human immunoglobulin; Lyso is lysozyme; OVA is ovine albumin; Chy is α-chymotrypsin.).

This method				Ref. [12]	This method				Ref. [12]
CM	AC	M	Er	M	CM	AC	M	Er	M
L-Alanine	3000	3.0×10^5	2.8	2.7×10^4	L-Threonine	3000	3.0×10^5	−1.7	2.7×10^4
$C_2O_4^{2-}$	850	8.5×10^4	2.5	8.0×10^3	SCN^-	850	8.5×10^4	3.2	8.0×10^3
Sr^{2+}	850	8.5×10^4	2.3	8.0×10^3	Ti(IV)	850	8.5×10^4	−1.5	8.0×10^3
L-Proline	850	8.5×10^4	2.5	8.0×10^3	L-Serine	850	8.5×10^4	−1.2	8.0×10^3
L-Leucine	850	8.5×10^4	2.4	8.0×10^3	L-Valine	800	8.0×10^4	2.3	7.0×10^3
L-Histidine	800	8.0×10^4	2.0	7.0×10^3	Cr(IV)	600	6.0×10^4	2.9	5.0×10^3
As(III)	600	6.0×10^4	3.1	5.0×10^3	As(V)	600	6.0×10^4	3.6	5.0×10^3
L-Isoleucine	600	6.0×10^4	−2.2	5.0×10^3	L-Cysteine	10.0	1000	−2.9	500
ClO_4^-	20.0	2000	−1.5	800	BrO_3^-	20.0	2000	2.6	800
Na^+	300	3.0×10^5	3.2	2.7×10^4	K^+	3000	3.0×10^5	1.5	2.7×10^4
Cl^-	3000	3.0×10^5	4.2	2.7×10^4	Br^-	3000	3.0×10^5	2.2	2.7×10^4
NO_2^-	3000	3.0×10^5	1.7	2.7×10^4	NO_3^-	3000	3.0×10^5	2.6	2.7×10^4
SO_3^{2-}	3000	3.0×10^5	−3.3	2.7×10^4	S^{2-}	3000	3.0×10^5	3.4	2.7×10^4
SO_4^{2-}	3000	3.0×10^5	4.1	2.7×10^4	F^-	900	9.0×10^4	1.4	8.0×10^3
Al^{3+}	900	9.0×10^4	2.0	8.0×10^3	Ni^{2+}	900	9.0×10^4	2.6	8.0×10^3
Ba^{2+}	900	9.0×10^4	4.4	8.0×10^3	Zn^{2+}	900	9.0×10^4	−3.1	8.0×10^3
Bi^{3+}	850	8.5×10^4	2.3	7.0×10^3	Cd^{2+}	800	8.0×10^4	2.1	7.0×10^3
Cu^{2+}	800	8.0×10^4	1.6	5.0×10^3	Fe^{3+}	500	5.0×10^4	2.3	3.0×10^3
Fe^{2+}	500	5.0×10^4	−3.5	3.0×10^3	L-Glutamic acid	25.0	2500	1.7	2000
L-Tryptophane	20.0	2000	1.9	1500	Co^{2+}	20.0	2000	−2.9	600
Mn^{2+}	20.0	2000	1.2	600	L-Aspartic acid	20.0	2000	1.6	1400
L-Phenylalanine	30.0	3000	1.8	2500	L-Lysine	50.0	5000	3.6	4000
Hg^{2+}	20.0	2000	−4.2	600	Casein	10.0	1000	−4.1	400
HSA	10.0	1000	3.6	500	BSA	10.0	1000	2.9	400
IgG	10.0	1000	−1.8	500	Lyso	10.0	1000	−3.3	400
OVA	10.0	1000	4.3	500	Chy	10.0	1000	1.6	400
L-Arginine	40.0	4000	3.7	3000					

while (−) stands for have no pathology. B-TUT: is B-type ultrasonic testing; CT is computer tomography X. BDD is B-diasonagraph detection. CXFPD is computer-X-ray-faultage-photography determination.).

Table 6 showed that the results of this method were tallied with ELISA, which indicated that the AP content of A, B and C was within normal range of 13.1–49.2 ng mL^{-1}, the AP content of D, E, F was lower than 13.1 ng mL^{-1}, and the AP content of G, H, I was higher than 49.2 ng mL^{-1}. According to the decrease of AP content in liver patient serum and the increase of AP content in osteopathy patient serum, we could forecast that D, E, F might be liver patients, while G, H, I might be osteopathy patients. According to results of clinical detection and diagnosis, treatment on patients D, E, F, G, H, I was carried out. The content of ALP was 13.1–49.2 ng ml^{-1} in four weeks using this method, indicating in the healthy level. Meanwhile, no diseases were found after B-TUT and CT detection.

This new method could be used to determine AP by fluorescence excitation/emission wavelength of either FITC or CdS/TiO_2, respectively, and it has been applied to forecast human diseases, which not only improved the flexibility of the method but also widened its applicability.

3.7. Discussion of the mechanism for the determination of AP

Based on the synthesis mechanism of CdS/TiO_2 nanoparticles with $Ti(OC_4H_9)_4$, $CdCl_2$, HS–CH_2–COOH and Na_2S reported in the Ref. [30], it was believed $Ti(OC_4H_9)_4$ could be firstly hydrolyzed to TiO_2 sol in the acid solution when FITC and $Ti(OC_4H_9)_4$ coexisted, and FITC was dispersed in TiO_2 sol, then FITC–TiO_2 sol (A) was formed [30] (Scheme 1); the dehydration reaction could be carried out between the active–OH on the surface of TiO_2 sol and the –COOH of HS–CH_2–COOH to form the compound B [30] (Scheme 1) which reacted with Cd^{2+} to get the compound C (Scheme 1); the compound C reacted with Na_2S and finally CdS/TiO_2–FITC luminescent nanoparticles D was formed (Scheme 1).

In the system containing WGA, EDC firstly coupled with the –COOH of CdS/TiO_2–FITC to form CdS/TiO_2–FITC–COO–EDC which reacted with NHS to generate CdS/TiO_2–FITC–COO–NHS [35] (Scheme 2), and at last CdS/TiO_2–FITC–COO–NHS reacted with the –NH_2 of WGA to produce the labelling product CdS/TiO_2–FITC–CONH-WGA, which led the fluorescence signal of the donor CdS/TiO_2 to intensely enhance (ΔF=247.9) and the fluorescence signal of the acceptor FITC to quench sharply (ΔF=245.8) due to there was no FRET occurring between the CdS/TiO_2 and FITC in CdS/TiO_2–FITC–WGA. When AP was added into the system, affinity adsorption reaction could be carried out between the –NH_2 of WGA in CdS/TiO_2–FITC–WGA and the –COOH of AP to form CdS/TiO_2–FITC–WGA–AP [36] (Scheme 2):

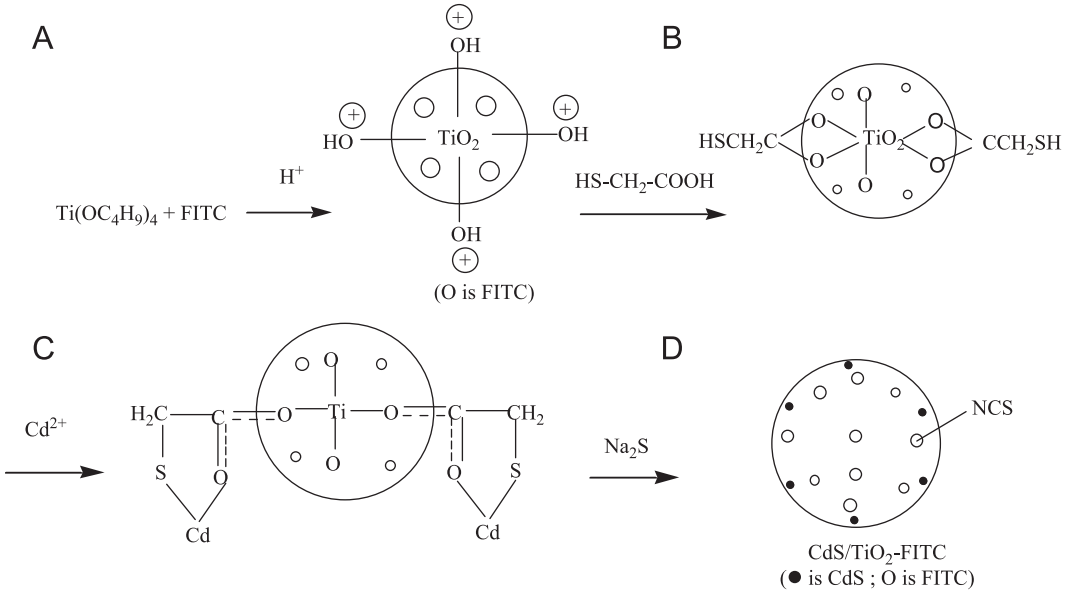
As shown in Fig. 1, their appearances were different from each other and their particle size presented in the increasing trend. The reason might be that CdS/TiO_2, CdS/TiO_2–FITC and CdS/TiO_2–FITC–WGA could react with FITC, WGA and AP to produce CdS/TiO_2–FITC, CdS/TiO_2–FITC–WGA and CdS/TiO_2–FITC–WGA–AP, respectively.

Similar to intermediate acceptor [37], CdS/TiO_2–FITC–WGA (intermediate acceptor) was excited by the FRET1 energy tranferring from the excited CdS/TiO_2 (donor). Thus, CdS/TiO_2–FITC–WGA–AP was excited to emit fluorescence based on the energy of the excited CdS/TiO_2–FITC–WGA by the FRET2 transferred to the CdS/TiO_2–FITC–WGA–AP (final acceptor) (Scheme 3).

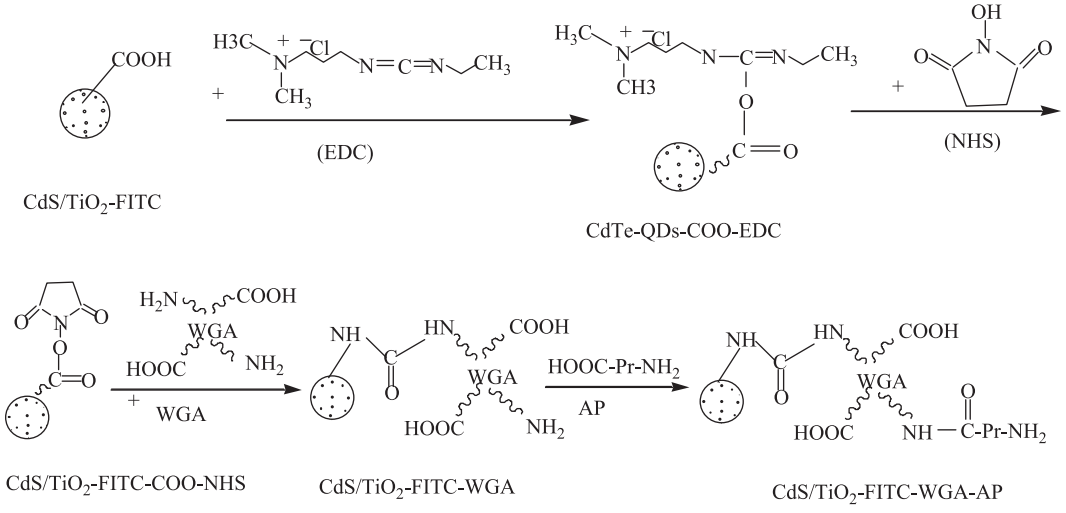
Due to the overlap occurring between the fluorescence emission spectrum (curve i) of the donor CdS/TiO_2 and the fluorescence excitation spectrum (curve g) of the acceptor FITC in the CdS/TiO_2–FITC–WGA–AP system (Fig. 3), the non-radiative energy transferring from donor molecule CdS/TiO_2 to the acceptor molecule FITC caused the non-radiative energy of CdS/TiO_2 to decrease but the non-radiative energy of FITC to increase, which

Table 6
Analytical results of AP in human serum (n=6).

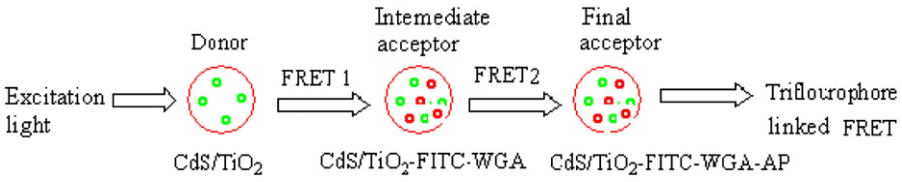
Sample	Obtained (ng m L^{-1})	λ_{ex}max/λ_{em}max (nm)	RSD (%)	Added (ng m L^{-1})	Obtained (ng m L^{-1})	Recovery (ng m L^{-1})	Percent recover (%)	ELISA (ng m L^{-1})	Disease	BDD	CXFPD
Serum A	34.2	213/372	2.0	3.40	37.7	3.50	103	35.0	–	–	–
	34.5	452/520	1.2	3.40	38.0	3.40	103	35.7	–	–	–
Serum B	19.0	213/372	1.5	1.90	20.9	1.90	100	19.6	–	–	–
	18.8	452/520	1.3	1.90	20.7	1.90	100	19.0	–	–	–
Serum C	27.2	213/372	1.0	2.70	30.0	2.80	104	27.8	–	–	–
	27.4	452/520	1.7	2.70	30.2	2.80	104	28.2	–	–	–
Serum D	11.2	213/372	3.5	1.10	12.2	1.10	90.1	10.9	+	+	+
	10.8	452/520	2.7	1.10	11.8	1.00	90.1	10.3	+	+	+
Serum E	12.2	213/372	3.3	1.25	13.5	1.30	104	12.6	+	+	+
	12.4	452/520	2.8	1.25	13.7	1.30	104	12.9	+	+	+
Serum F	10.3	213/372	3.7	1.05	11.4	1.00	105	10.7	+	+	+
	10.5	452/520	3.4	1.05	11.6	1.10	105	11.0	+	+	+
Serum G	51.7	213/372	1.7	5.20	56.9	5.20	100	52.4	+	+	+
	52.1	452/520	0.6	5.20	57.3	5.20	100	52.7	+	+	+
Serum H	57.1	213/372	1.1	5.70	62.9	5.80	102	57.8	+	+	+
	57.2	452/520	0.7	5.70	63.0	5.80	102	57.5	+	+	+
Serum I	55.0	213/372	1.2	5.50	60.7	5.70	104	56.2	+	+	+
	55.2	452/520	0.9	5.50	60.9	5.70	104	55.9	+	+	+



Scheme 1. Synthesis procedure of CdS/TiO_2 –FITC luminescent nanoparticles .



Scheme 2. Labelling reaction of WGA and affinity adsorption reaction between CdS/TiO_2–FITC–WGA and AP .



Scheme 3. Scheme of tri-flourophore linked FRET system .

further caused the fluorescence signal of the donor CdS/TiO_2 to quench (ΔF=219.5) and the fluorescence signal of the acceptor FITC to enhance (ΔF=274.3). Based on the fact that the ΔF of both CdS/TiO_2 and FITC were proportional to the content of AP, trace AP could be detected by affinity adsorption assay using CdS/TiO_2–FITC as FRET fluorescent probe.

4. Conclusion

In this study, we synthesized luminescent nanoparticles CdS/TiO_2–FITC with the particle size of 20 nm by sol–gel method and found the FRET phenomena occurring between the donor CdS/TiO_2 and the acceptor FITC in the CdS/TiO_2–FITC. The CdS/TiO_2–FITC–WGA fluorescent probe which could directly take affinity adsorption reaction with AP without surface modification was developed by labelling WGA with CdS/TiO_2–FITC, and the product CdS/TiO_2–FITC–WGA–AP could keep the excellent characteristics of FRET. Thus, the CdS/TiO_2–FITC–WGA probe for the determination of trace AP in serum and the detection of human diseases in clinic based on FRET affinity adsorption assay has been proposed. The research has higher academic research value and wider application prospect in the biological analysis and clinical diagnosis, which will promote the progress of the luminescence analysis, bio-analytical science and nanotechnology.

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