Homogeneous immunoassays are becoming more and more attractive for modern medical diagnosis because they are superior to heterogeneous immunoassays in sample and reagent consumption, analysis time, portability and disposability. Herein, a universal platform for homogeneous immunoassay, using human immunoglobulin (IgG) as a model analyte, has been developed. This assay relies upon the inner filter effect (IFE) of gold nanoparticles (AuNPs) on CdTe QDs fluorescence. The immunoreaction of antigen and antibody can induce the aggregation of antibody-functionalized AuNPs, and after aggregation the IFE of AuNPs on CdTe QDs fluorescence is greatly enhanced, resulting in a decrease of fluorescence intensity in the system. Based on this phenomenon, a wide dynamic range of $1$–$100$ pg mL$^{-1}$ for determination of IgG can be obtained. The proposed method shows a detection limit of $0.3$ pg mL$^{-1}$ for human IgG, which is much lower than the corresponding absorbance-based approach and compares favorably with other reported fluorescent methods. This immunoassay method is simple, rapid, cheap, and sensitive. The proposed method has been successfully applied to measuring IgG in serum samples, and the obtained results agreed well with those of the enzyme-linked immunosorbent assay (ELISA).

Introduction

Due to the high specificity and strong affinity of many antibodies to their corresponding antigens with $K_D$ values often in the nanomolar range, immunoassays are the preferred tools for the specific detection of various analytes, especially proteins, in complex biological matrices such as blood or urine.$^1$ Most of the current immunoassays are performed in a heterogeneous format, such as the standard method of enzyme-linked immunosorbent assay (ELISA) owing to the advantage of low background and wide dynamic range. A typical heterogeneous immunoassay involves antibody immobilization, multiple steps of incubation, and washing cycles, followed by signal amplification and reading. From the initial antibody immobilization to the final reading of the assay results, the entire immunoassay can usually take several hours to complete. The heterogeneous immunoassay is rather time-consuming and labor-intensive. To overcome these problems, there is an increasing need for homogeneous immunoassays without any separation steps, especially in the field of modern diagnosis because of their simplicity, ease of automation, and high throughput.$^2,^3$ To date, several methods for homogeneous immunoassay, such as fluorescence polarization,$^4,^5$ fluorescence resonance energy transfer,$^1,^6,^7$ bioluminescence resonance energy transfer,$^8$ surface plasmon resonance$^9,^{10}$ and light scattering,$^{11,12}$ have been proposed. However, most of the homogeneous immunoassays are less sensitive than their heterogeneous counterparts because of their high background. Thus, the development of new homogeneous immunoassays with high sensitivity has become increasingly attractive.

The inner filter effect (IFE) of fluorescence refers to the absorption of the excitation and/or emission of light by absorbers in the detection system.$^13$ Although the IFE is usually considered as an annoying source of error in spectrofluorometry and should be avoided, some studies have demonstrated its application in developing novel fluorescence assays.$^{14–21}$ Generally, in the IFE-based approach, two dyes are employed, one absorbent, the other fluorescent. These two dyes must meet the requirement: the absorption band of the absorbent dye possesses a complementary overlap with the excitation and/or emission bands of the fluorophore. Because of the limited choice of dyes, it is not easy to find the suitable absorbent–fluorophore pair. Furthermore, the conventional absorbent usually has a small extinction coefficient, which restricts the sensitivity of the IFE-based fluorescent assay. Therefore, the application of this IFE-based fluorescent assay is not very extensive. To the best of our knowledge, the IFE-based fluorescent immunooassay has not been reported in the open literature.

Obviously, the key to improve the detection sensitivity of the IFE-based fluorescent assay is to enhance the inner filter...
efficiency. A general and feasible strategy is to increase the spectral overlap between fluorophore’s emission and absorber’s absorption, and/or to increase the extinction coefficient of the absorber. Gold nanoparticles (AuNPs) have tremendously larger extinction coefficient (e.g., the extinction coefficient of 13 and 30 nm diameter particles is reported to be $2.7 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$ and $3.7 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$ at 520 nm, respectively$^\text{22}$), which is much larger than that of the conventional chromophores. It is obvious that AuNPs can be used as ideal absorber in the IFE-based fluorescent assay. On the other hand, compared with conventional organic fluorescent dyes, colloidal semiconductor nanocrystals (quantum dots, QDs) have about 10–20 times brighter fluorescence and 100–200 times better photostability.$^\text{23,24}$ In particular, the emission wavelengths of QDs can be tuned by size, composition, and shape,$^\text{25}$ which results in high flexibility in the selection emission wavelength as well as maximum overlap with the absorption band of the absorbent dye. This attractive characteristic enables QDs to be a potentially powerful and ideal fluorophore in the IFE-based fluorescent assay. In our recent work,$^\text{26}$ we used AuNPs and QDs as the absorber and fluorophore, respectively, to propose a new IFE-based fluorescent assay of aminothiols. In this sensing system, the changes in the absorbance of AuNPs could be translated into the exponential changes in the fluorescence of QDs, and an enhanced sensitivity for the analytical method is reasonable with respect to the absorbance alone.$^\text{26}$ Inspired by the above work, we have designed a homogeneous and IFE-based fluorescent immunoassay for the detection of proteins. The principle of our method is shown in Scheme 1. Human immunoglobulin G (IgG) was taken as the model analyte to provide the “proof-of-principle” verification of the concept. Aqueous AuNPs (ca. 13 nm) with an intense plasmon absorption ($\lambda_{\text{max}} = 520$ nm) were prepared, and then the AuNPs were modified with goat-anti-human IgG. At the same time, the CdTe QDs with a maximum emission at ca. 520 nm were intentionally prepared. Therefore, the fluorescence of CdTe QDs could be quenched through the IFE of AuNPs. In the presence of antigen (IgG), the functionalized AuNPs can bind antigen to form dimers (or oligomers), which then leads to the increased absorption of AuNPs at ca. 520 nm. In this case, the CdTe QDs exhibit the weak fluorescence emission. The proposed immunoassay can be performed in homogeneous solution, and the fluorescence can be easily measured with a common spectrophuorimeter equipped with a 150 W high pressure Xenon lamp. The whole detection can be completed in ca. 80 min, whereas the conventional ELISA method needs more than 10 h. Furthermore, the detection limit of IgG (3$\sigma$) was estimated to be as low as 0.3 pg mL$^{-1}$, the sensitivity was increased by more than 4 orders of magnitude over that of the reported AuNPs-based colorimetric immunoassay for detection of protein.$^\text{27}$

### Experimental

#### Apparatus

All fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan) with a xenon discharge lamp at room temperature (ca. 20 °C). A 1.0 cm path length rectangular quartz cell was used for these measurements. UV-visible absorption spectra were recorded on a U-3900H spectrophotometer (Hitachi, Japan). A WH-861 vortex mixer (Huangjin Instrumental Co., Jiangsu, China) was used to blend the solutions. A HH-1 thermostatic water bath (Beijing Kewen Instrumental Factory, Beijing, China) was used to control the temperature of the immunoreactions at 0.1 °C intervals. Transmission electron microscopy (TEM) images of nanoparticles were acquired on a JEM-2100 transmission electron microscope (Tokyo, Japan). The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing).

#### Chemicals and materials

Chloroauric acid (HAuCl$_4$) was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Sodium citrate, CdCl$_3$, and NaBH$_4$ were purchased from Tianjin Chemical Reagent Company (Tianjin, China). Glutathione and Na$_2$TeO$_3$ were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Human IgG, goat-anti-human IgG, bovine serum albumin (BSA) and HRP-labeled goat-anti-human IgG were purchased from Beijing Dingguo Biotechnology Company (Beijing, China). All the measurements of IgG have been performed in 20 mM Tris–HCl buffer (pH 7.4). The human serum, provided by Shaanxi Normal University Hospital, was used as the sample to evaluate the reliability of the proposed immunoassay. Polystyrene 96-well microtiter plates were used to perform the immunoreactions.

Ultrapure water was prepared by a Milli-Q system (Millipore, France) and used in all experiments. Other reagents and chemicals were of analytical grade and used without further purification.

#### Preparation of gold nanoparticles (AuNPs)

All glassware and stirrer used in the following procedure were thoroughly cleaned in aqua regia (HNO$_3$–HCl = 1 : 3, v/v), rinsed in distilled water, and then oven-dried prior to use, to avoid unwanted nucleation during the synthesis, as well as aggregation of gold colloid solutions. The AuNPs (ca. 13 nm diameter) were prepared by the citrate reduction method according to the published protocol.$^\text{28}$ Briefly, a sodium citrate solution (1.0%, 5.6 mL) was rapidly added to a boiled HAuCl$_4$ solution (0.04%, 50 mL) under vigorous stirring. The mixed solution was maintained for 10 min at boiling and then removed from the heating mantle. Stirring was continued for another 15 min, and after cooling to room temperature, the

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**Scheme 1** Schematic illustration of fluorescence immunoassays based on the inner filter effect.
preparing AuNPs solution was stored in the refrigerator (4 °C). The synthesized AuNPs were characterized by using UV-visible spectroscopy and TEM in order to measure the particle size. The concentration of the AuNPs solution was 1.5 × 10⁻⁸ M, which was estimated by using UV-visible spectroscopy, based on an extinction coefficient of 2.7 × 10⁴ M⁻¹ cm⁻¹ at λ = 520 nm for 13 nm particles.⁵⁹

**Preparation of AuNPs-labeled goat-anti-human IgG**

The preparation of AuNPs-labeled goat-anti-human IgG was performed according to the literature procedure.⁶⁰ Briefly, after AuNPs solution was adjusted to pH value 9 with 0.1 M K₂CO₃ solution, 1.0 mL of 0.4 mg mL⁻¹ goat-anti-human IgG (10% more than the minimum amount, determination by a flocculation test according to literature⁶⁰) was added to 10 mL of pH-adjusted AuNPs solution. The mixed solution was stirred and incubated at room temperature for 0.5 h, and then added to 2.2 mL of 5% (w/v) BSA and stirred for 5 min to make AuNPs and antibody bind fully. The resulting bio-conjugate was centrifuged at 13,000 rpm at 4 °C for 30 min to get rid of the unbound goat-anti-human IgG, and the supernatant was removed. The oily ruby sediment was re-dispersed by 13 mL of 0.02 M Tris–HCl buffer (pH 7.4) containing 1% BSA. The bio-conjugate solution was stored at 4 °C. The same batch of AuNPs modified with goat-anti-human IgG was used throughout the total assay process to eliminate possible deviations between batches.

**Preparation of CdTe QDs**

The synthetic procedure of glutathione-capped CdTe QDs was modified from previous literature reports.⁶¹ 2 mL of 0.04 M CdCl₂ was diluted to 50 mL in a one-necked flask, and then we added trisodium citrate dihydrate (0.050 g). Glutathione (0.025 g), Na₂TeO₃ (2 mL, 0.01 M) and NaBH₄ (0.025 g) were added under vigorous stirring. After reaction for 2 h at room temperature, the mixture was put into a pot at 90 °C oil-bath under backflow heating conditions for 12 h. The final concentration of CdTe QDs used in our experimental was ca. 1.78 × 10⁻⁵ M according to the excitonic absorption peak value and the extinction coefficient per mole (ε) of CdTe nanoparticles.³² Then, the QDs were stored in the refrigerator at 4 °C before further use.

**Procedure of fluorescence-based immunoassay**

A typical fluorescence-based immunoassay was realized by the following steps. First, 200 μL of the AuNPs modified with goat-anti-human IgG (1.14 × 10⁻⁹ M) was added to 200 μL of human IgG with different concentrations or diluted serum samples. Afterward, 100 μL of 0.005 M NaCl was added. The mixtures were blended by the vortex mixer and incubated for 1 h at 37 °C water bath. Second, 50 μL of the above obtained solution was mixed with 200 μL of the diluted CdTe QDs (1.78 × 10⁻⁷ M), and then 350 μL Tris–HCl (pH 7.4) and 400 μL water were added into the mixture. The resulting solution was incubated for 20 min at room temperature before spectral measurements. Finally, the instrument excitation and emission slits were set at 10 nm, and the fluorescent spectrum of the resulting solution were obtained with excitation wavelength of 360 nm at room temperature. The fluorescence intensity of the system at 522 nm was recorded. The concentration of IgG was quantified by \( F_0/F \), where \( F_0 \) and \( F \) were the fluorescence intensity of the system at 522 nm in the absence and presence of IgG, respectively.

**Results and discussion**

**Optical characteristics of AuNPs and CdTe QDs**

Fig. 1 shows the absorption spectrum of AuNPs and the fluorescence emission spectrum of CdTe QDs. Aqueous AuNPs (ca. 13 nm) have a surface plasma resonance absorption peak at about 520 nm and appear pink, which is consistent with literature.³³ The fluorescence emission wavelength of CdTe QDs could be easily tuned by changing the refluxing time. When the refluxing time was 12 h, we obtained the glutathione-capped CdTe QDs with a maximum emission at 522 nm, which was just near the absorption maximum (520 nm) of AuNPs (Fig. 1). It can also be seen that the width of the fluorescence spectrum is narrow (the width at half-maximum is about 40 nm), showing that the QDs are monodisperse and uniform. The mean size of the QDs is estimated from the adsorption peak by Peng et al. empirical equation³² to be 1.78 nm. As shown in Fig. 1, it is obvious that the emission spectrum of CdTe QDs overlaps nicely with the absorption spectrum of AuNPs, which laid a foundation as possible IFE between CdTe QDs and AuNPs.

**IFE of AuNPs on the fluorescence of CdTe QDs**

The experimental results show that AuNPs (ca. 13 nm, \( \lambda_{\text{max}} = 520 \) nm) could effectively decrease the fluorescence intensity of glutathione-capped CdTe QDs with a maximum emission at 522 nm. Wang and Guo demonstrated that the positively charged QDs could form the fluorescence resonance energy transfer (FRET) donor–acceptor assemblies with negatively charged AuNPs by electrostatic interactions because the electrostatic interaction could shorten the distance between the QDs donor and the AuNPs acceptor, and the efficient FRET process could not occur between the negatively charged QDs and the negatively charged AuNPs.³⁴ In this work, the AuNPs prepared by the citrate reduction method were capped with citrate. So, AuNPs possess negative charge, which is supported by the zeta potential of AuNPs. The zeta potential of the citrate-capped

![](image-url)

**Fig. 1** Absorption spectra of AuNPs (a), and fluorescence emission spectra of GSH-capped CdTe QDs (b).
AuNPs was $-36.6$ mV due to the ionization of the $-\text{COOH}$ group in citrate. At the same time, the zeta potential of the glutathione-capped CdTe QDs was also negative in pH 7.4 because of the ionization of the $-\text{COOH}$ group in glutathione (the $pK_a$ of $-\text{COOH}$ group in glutathione is 3.6). Obviously, an efficient FRET process could not occur between the negatively charged QDs and the negatively charged AuNPs in this present system. Furthermore, the absorption spectrum of AuNPs did not change in the presence of CdTe QDs, and the fluorescence spectrum of CdTe QDs also remained unchanged in the presence of AuNPs, which suggest that the complex did not form between QDs and AuNPs in this present system. Therefore, the observed fluorescence decrease should be mainly to the IFE of AuNPs on the fluorescence of CdTe QDs. On the other hand, we measured the fluorescence lifetime of CdTe QDs in the absence and presence of AuNPs. The average lifetime of the CdTe QDs in the absence of AuNPs was 44.7 ns, and the average lifetime was 43.3 ns in the presence of AuNPs. The lifetime hardly changed, which suggests that the QDs did not form the complex with AuNPs via hydrogen bonding or electrostatic forces. Fluorescence lifetime measurements provide additional proof confirming an efficient IFE of AuNPs on the fluorescence of CdTe QDs.

With increasing the AuNPs concentration, the absorbance of the absorber increased, which would shield the emission light form CdTe QDs. As a result, the emission intensity of CdTe QDs decreased correspondingly. Therefore, the fluorescence of CdTe at 522 nm can be turned by the absorbance of AuNPs at 520 nm via IFE. Compared to the complicated and costly QDs–AuNPs FRET system, in which it is necessary for the AuNPs to be modified or engineered so as to directly contact with or be indirectly linked to, special modified QDs, this IFE strategy is very simple.

**Effect of IgG on absorption spectrum of antibody-functionalized AuNPs**

AuNPs are one of the most widely used labels because of several advantages, such as easy preparation, chemically tailorable physical properties and good biocompatibility. In this work, we used AuNPs to label the antibody (goat-anti-human IgG). As shown in Fig. 2, the slight red-shift of spectra can be observed after the introduction of antibody to AuNPs. The maximum absorption wavelength ($\lambda_{\text{max}}$) was increased (Fig. 2), and this absorbance shift was considered as the confirmation of the protein absorption onto the particle surface. In the presence of antigen (IgG), the absorption intensity of antibody-functionalized AuNPs at ca. 520 nm increased, which suggests the occurrence of the immunoreaction. AuNPs (ca. 13 nm) in aqueous solution have a surface plasma resonance absorption peak at 520 nm and appear pink. It is well known that the surface plasmon absorption of AuNPs is very sensitive to their interparticle distance and surface state. When human IgG is mixed with the antibody-functionalized AuNPs, the functionalized AuNPs are crosslinked and aggregated through the immunoreaction. In this system, the linked AuNPs exhibit only a single absorption peak. The results are similar to the case where the aggregation of AuNPs is induced by the crosslink of DNA, or alkylne–azide click reaction, but the results are different from the case where the aggregation of AuNPs is induced by salt or small molecules. The aggregated AuNPs induced by salt or small molecules (such as melamine) often show two evident absorbance maxima. The UV-visible spectra of aggregated AuNPs depend on the particle size and the interparticle distance, and when the interparticle distance is more than about four-fold the particle radius, the aggregated AuNPs show one peak, located near the resonance peak for single particles. In this system, the interparticle distance is the size of antibody–antigen–antibody immunocomplex, and the size of IgG is about 30–40 nm. For this AuNPs (ca. 13 nm) system, the ratio of interparticles distance to particles radius is calculated to be 15, which can explain the reason for only one peak (ca. 520 nm) in UV-visible spectra.

TEM images were also used to explore the change of AuNPs induced by the immunoreaction in this system. Compared with the bare AuNPs (Fig. 3A), the goat-anti-human IgG functionalized AuNPs (Fig. 3B) were larger. Before the immunoreaction with human IgG, AuNPs were highly dispersed (Fig. 3A and B), and when human IgG was added into the goat-anti-human IgG functionalized AuNPs solution, the immunoreaction between human IgG and the goat-anti-human IgG resulted in crosslinking of the functionalized AuNPs and led to form dimers (or oligomers) (Fig. 3C). The results are consistent with the change of the UV-visible absorption spectra (Fig. 2). Furthermore, compared with that of the single gold particle, the absorption intensity of the linked AuNPs (dimers or oligomers) at ca. 520 nm increased significantly (Fig. 2), which was similar to Lu et al. results. The absorption intensities at 520 nm increase with increasing the human IgG concentration in a wide range. Therefore, the fluorescence emission of CdTe QDs at 522 nm can be turned by the IgG–AuNPs system via IFE, which provides a new strategy for fluorescent detection of IgG.

**Fluorescence detection of IgG through the IFE of AuNPs on the fluorescence of CdTe QDs**

We designed a fluorescent immunoassay based on the antigen–antibody reaction, which then decreases the fluorescence of the fluorophore (CdTe QDs). As shown in Fig. 4, the fluorescence intensity of AuNPs–QDs system decrease gradually with increasing the concentration of IgG. Compared with the significant change of fluorescence intensity, no considerable change in absorbance was observed.
The control experiments showed that IgG could not quench the fluorescence intensity of the CdTe QDs in the absence of AuNPs. Thus, the decreased fluorescence should originate from the interaction between IgG and the AuNPs coated with goat-anti-human IgG, which then affected the emission of the CdTe QDs.

The performance of the fluorescence immunoassay is strongly influenced by the assay conditions such as media pH, AuNPs concentration and incubation time. We investigated the effect of these factors. Both the immunoreaction and the aggregation of the AuNPs involve charged species and electrostatic interaction. The media pH would affect the charge distribution of antigen, antibody and AuNPs surface, which consequently affect the aggregation of the AuNPs and the fluorescence intensity of the system. The experimental results showed that the decreased fluorescence intensity reached a maximum at pH 7.4. So, we chose 0.02 M Tris–HCl (pH 7.4) as the immune reaction media. Ionic strength (mean the concentration of NaCl added to the Tris–HCl buffer) also influenced the fluorescence response of the system. When the added NaCl concentration was greater than 0.005 M, the background of the system was rather high. The possible reason is that the high slat itself can induce the aggregation of AuNPs. In this system, 100 μL of 0.005 M NaCl was used to control the ionic strength in the solution for the immunoassay.

The concentration of the antibody-functionalized AuNPs influenced the fluorescence response of the system. When the antibody-functionalized AuNPs concentration was low, it was very sensitive for detecting a smaller amount of IgG because of the low background. But the antibody-functionalized AuNPs concentration was too low, the aggregation rate of AuNPs was very slow. Therefore, the 1.14 × 10⁻⁸ M antibody-functionalized AuNPs were used in all experiments. For 1.14 × 10⁻⁸ M the antibody-functionalized AuNPs, the optimum concentration of CdTe QDs was 1.78 × 10⁻⁷ M. The effect of the incubation time on the immunoassay was also investigated by monitoring the fluorescence response of the system. The experimental results show that at room temperature, the fluorescence response remained constant when the incubation time of IgG and the antibody-functionalized AuNPs was over 50 min, which illustrates that the immune reaction completes already. Thus, 60 min was selected as the optimum time for the incubation time of IgG and the antibody-functionalized AuNPs.

Under the optimized experimental conditions, the experiments were carried out by adding increasing amounts of IgG into the system to examine whether the fluorescence change could be used for IgG quantification. As shown in Fig. 4, the fluorescence intensity decreases with increasing IgG concentration, to reveal a linear relationship in the IgG concentration range 1–100 pg mL⁻¹. Fig. 5A and B show the linear regression equation (Y is the fluorescence intensity ratio (F/F₀), X is the concentration of IgG, and R is the correlation coefficient) in 1 × 10⁻⁹ mg mL⁻¹ to 10 × 10⁻⁹ mg mL⁻¹ range and 1 × 10⁻⁸ mg mL⁻¹ to 10 × 10⁻⁸ mg mL⁻¹ range, respectively. The detection limit (3σ) was 0.3 pg mL⁻¹. The increase in sensitivity of this system is more than 4 orders of magnitude more than that of the reported AuNPs-based colorimetric immunoassay for protein. The changes in the absorbance of the absorber translate into exponential changes in the fluorescence of the fluorophore, an enhanced sensitivity for the analytical method is reasonable with respect to the absorbance alone. Thus, using IFE-based fluorescence strategy, we achieved the purpose of the signal amplification to detect IgG. The low detection limit is 10-fold lower than that obtained in the reported AuNPs-based electrochemiluminescence immunoassay for IgG. The detection limit is also much lower than that in the reported QDs-based FRET for IgG detection. To the best of our knowledge, the present limit of detection for this present system is superior to all previously reported immunoassays for IgG. A series of eleven repetitive measurements of 2 pg mL⁻¹ human IgG were used for estimating the precision, and the relative standard deviation (RSD) was less than 4%, indicating an acceptable level of precision for the immunoassay.

Control experiments were conducted to evaluate the specificity of the fluorescence immunoassay. When using BSA (10 ng mL⁻¹) instead of human IgG (10 pg mL⁻¹) for the fluorescence immunoassay, the fluorescence intensity was similar to the blank. The results indicated that the AuNPs coated with goat-anti-human IgG can specifically recognize the human IgG and the specificity
of the AuNPs-based immunoassay has an acceptable level. On the other hand, the interference of some common substances in human serum was investigated for the determination of IgG. The experimental results show that Na+, K+, Ca2+, Mg2+, Cl−, HCO3−, glucose, lactate and uric acid at normal physiological concentrations could not interfere with the determination of IgG.

In order to evaluate the reliability and application of the proposed immunoassay to clinical diagnostics, human serum samples were analyzed simultaneously with the proposed method and the ELISA. The fresh human blood samples from some volunteers were collected from Shaanxi Normal University Hospital and used as testing samples. Before the test, the samples were diluted appropriately step by step to be in the linear range of the proposed method. At the same time, the conventional ELISA method was used as the standard method, and the ELISA test procedures were according to the operational manuals of the ELISA kits. The experimental results (Table 1) show that the values obtained by the proposed method were comparable with those obtained by the ELISA method and the relative deviation between the two methods was over the range ±5%. Therefore, the developed method is applicable for the determination of IgG in real sample, human serum.

### Conclusion

In this work, we have demonstrated the feasibility of developing a new fluorescence homogeneous immunoassay using the inner filter effect of AuNPs on fluorescence of CdTe QDs for the first time. Compared with previous fluorescent immunoassay methods, the method is more simple, time-saving and economical, since no modification step of the fluorophore (CdTe QDs) was needed. The proposed method allows the detection of IgG as low as 0.3 pg mL⁻¹, which is, to the best of our knowledge, the lowest ever reported for IgG detection with fluorescence-based immunoassay method. The remarkably high sensitivity could be attributed to the choice of AuNPs (as the absorber) and CdTe QDs (as the fluorophore), a good spectral overlap of absorber AuNPs with the emission spectra of CdTe QDs. In addition, the present study suggests that AuNPs could be a favorable choice to be powerful absorbers in the IFE-based assays due to their extremely large extinction coefficient and tunable plasmon absorption feature. Therefore, the proposed immunoassay has great potential for protein assay in the fields of molecular biology and clinical diagnostics.

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