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Ultrasensitive detection of 17β-estradiol using gold nanoparticle-based fluorescence immunoassay

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A novel ultrasensitive amplification immunoassay for the determination of 17β-estradiol (E_2) is reported based on the nanoparticle signal amplification platform. It involves two types of particles: magnetic microparticles (MMPs) functionalized with anti-E_2 antibody produced in rabbit as a capture probe; double-codified gold nanoparticles (DC-AuNPs) modified with both goat anti-rabbit antibody and SH-dsDNA-biotin as a signal amplifier; and the avidin-FITC was added to link to the SH-dsDNA-biotin as a tracer. The competitive reaction of the anti-E_2 antibody immobilized on magnetic microparticles with estradiol in sample solution and with the goat anti-rabbit antibody on double-codified gold nanoparticles results in a complex involving the DC-AuNPs and MMPs. Under the optimized condition, the linear range of E_2 is from 1.0×10^{-5} to 1.0 ng mL^{-1}, and the detection limit of the assay could reach to 6.37×10^{-6} ng mL^{-1}. It was applied to determine E_2 in human urine, with the mean percent recoveries in the range of 96.5%-107.4% and relative standard deviations were below 8.1%.

1. Introduction

Generally, 17β-estradiol (E_2) is the major active female sex hormone produced in premenopausal women, primarily in the ovaries, and the concentration in plasma rises just before the LH surge in the normal menstrual cycle and again mid-way through the luteal phase. Estradiol plays a crucial role in biological processes. Proper estradiol stimulates early bone growth and maintaining bone strength, and osteoporosis is a common postmenopausal disorder as estradiol levels decline.[1,2] Meanwhile, E_2 is used in the diagnosis of precocious puberty in girls, amenorrhea and to monitor follicle development in the ovary in the days prior to in vitro fertilization. E_2 is also used as oral contraceptive.[3,4] On the other side, E_2 clearly plays a role in risk for breast cancer and ovarian cancer, and there is also evidence that it plays a role in prostate cancer and in human papilloma virus (HPV) related diseases.[5-7] Since E_2 has the greatest estrogenic activity and even a very low concentration of exogenous estradiol can disturb its balance of it in vivo. So it is very necessary to establish an ultrasensitive method for the detection of E_2 in the environment. There were many analytical methods for the determination of E_2, including high performance liquid chromatography tandem mass spectrometry(GC-MS),[10,11] Fluoroimmunoassay (FIA),[12,13] chemiluminescence enzyme immunoassay (CLIA)[14,15] and electrochemical immunoassay (ECIA),[16-18] and so on. In contrast, immunoassay method[19] is highly sensitive and cost effective, so it is easy to use exhibiting good potential in widespread application. In particular, FIA has been widely applied in environmental analysis, and the major advantages of FIA, as compared with conventional immunoassay, such as RIA and ELISA, are the use of nonradioactive reagents and increased reagent stability, and the short counting time, and the wide dynamic range. So FIA has been used as an attractive analytical method in different fields, such as biotechnology, pharmacology, molecular biology, and environmental chemistries. Recently, nanosensing technologies have been widely used as versatile and sensitive tracers for the electronic, optical of different biomolecular recognition events. Gold nanoparticles (AuNPs) seem to be the most desired nanoparticles because of its unique native properties. Rapid and simple chemical synthesis, a narrow size distribution, and efficient coating by thiols or other bioligands have enabled AuNPs to be used as transducers for several biorecognition binding applications in biotechnology. The functional AuNPs that are linked to biological molecules such as proteins, enzymes, and nucleic acids, provide interesting tools for several biological systems.[20,21] The recently developed amplification assay is based on synthetic oligonucleotides, gold nanoparticles and magnetic particles, and it has attomolar (10^{-18}M) sensitivity without enzymatic amplification.[22-24] Optical sensitivity enhancement attributable to the use of gold nanoparticles as a multi-DNA-antibody carrier, which therefore amplifies the fluorescence signal, as well as the high sensitivity in the direct immunochemical detection, represents the most important achievements due to the use of this double-codified nanolabel, which can potentially be exploited in several other future applications.

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Additionally, magnetic microparticles as special biomolecule carriers via a suitable immobilization process offer promise as sensitive sensors. Magnetic microparticle labels were employed in biochemical applications like immunoassay, genetic engineering, and cell analytical techniques [25] owing to the properties of efficient preconcentration and purification of target analytes such as DNA, proteins, cells, bacteria, and viruses contained in complex samples.

In this study, a double-codified AuNPs (DC-AuNPs) which was modified with both goat anti-rabbit antibody and SH-dsDNA-biotin, and a magnetic microparticles (MMPs) functionalized with anti-E_2 antibody produced in rabbit were prepared, and a highly sensitive fluorescence immunoassay was developed for the determination of E_2, based on DC-AuNPs as a signal amplification platform, and by using functionalized MMPs as a capture probe. This method was applied to determine E_2 in human urine with satisfactory results.

2. Experimental

2.1 Reagents and apparatus

Polyclonal rabbit antibody against E_2 with no significant cross-reactivities with other chemicals was obtained from our laboratory. The oligonucleotides used in the present work were purchased from Shanghai Sangon Biotechnology Co. Ltd. (DNA1: SH-C_6-AGCTCTTCCCATACCGCTCTTCCCTGACACAG, DNA2: Bition-TACTGCTGTGTCAGGGAAGAGCGGTATGGGAAGAG).

Amino-functionalized MMPs (1.5 μm) was supplied by Bangs Laboratories, Inc.. Goat anti-rabbit IgG, estradiol, avidin-FITC, bovine serum album (BSA), ovalbumin (OVA), glutaraldehyde and dithiothreitol (DTT) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Tetrachloroauric acid trihydrate (≥49.0% Au), tris (hydroxymethyl)-aminomethane (Tris) were purchased from JK& Chemical Ltd. (Shanghai, China). All the other reagents such as NaCl, EDTA, PEG20000, K_2HPO_4, KH_2PO_4, K_2CO_3 and other common chemicals were of analytical grade and were used as received. Doubly deionized water (≥18.2 MΩ cm) was used throughout the experiments.

Fluorescence measurements were performed on the Infinite 200 auto-multifunction microplate reader (TECAN, Switzerland). Immunoreaction experiments were carried out in a 4 mL flat centrifuge tube at 37 ºC, using WDT model rocking incubator (Jinxin BS-1E, China) with thermostatic controller. Absorbance of oligonucleotide and AuNPs were collected using a UV-vis 2550 spectrometer (Shimadzu Corporation, Japan). DC-AuNPs were purified by repetitive centrifugation in high speed refrigerated centrifuge (Sigma 3-18K, Germany) at 4 ºC.

Pyridine buffer (0.01 mol L^{-1}, pH 6.0), wash buffer (0.01 mol L^{-1} Tris, 0.1% NaN_3, 0.1% w/v BSA, 0.001 mol L^{-1} EDTA, 0.15 mol L^{-1} NaCl, pH 7.4), TE buffer (5.0 mmol L^{-1} Tris, 1.0 mmol L^{-1} EDTA, 0.05 mol L^{-1} NaCl, 0.05% PEG20000, pH 7.4), Tris buffer (10 mmol L^{-1}, pH 8.0), PBS buffer (0.1 mol L^{-1} NaCl in 0.01 mol L^{-1} of phosphate buffer, pH 7.4), and PBST buffer (PBS buffer, 0.1% Tween-20, 0.10% OVA, pH 7.4) were used in the experiments, respectively.

2.2 Preparation of double-codified gold nanoparticles

Firstly, the citrate-stabilized AuNPs were prepared by following the standard methods given in the literature.[26] Briefly, 2 mL of trisodium citrate solution (1%, w/v) was added quickly to 50.0 mL of boiling $HAuCl_4$ solution (0.01%, w/v) while stirring, and the mixture was boiled up for 5 min. Gold nanoparticles were formed when the solution turned deep red and the solution was kept boiling for another 10 min. Then, the solution was cooled to room temperature under continuous stirring. For the study, the AuNPs was diluted in deionized water to an absorbance of 1 OD at 520nm.

Secondly, 1.0 mL of AuNPs solution (1 OD/1.0 mL) was adjusted to pH 8.5 with 30.0 μL of K_2CO_3 aqueous solution (0.1 mol L^{-1}). The AuNPs was functionalized with 15.0 μg of goat anti-rabbit antibody and shaken for 5min. The particles were subsequently modified with 4.0 μL of thiolated DNA (50.0 mmol L^{-1}). The mixture solution was incubated at room temperature for 1 h, after 6 h standing, followed by the addition of NaCl (2 mol L^{-1} dissolved in 0.01 M PBS, pH 7.0) to a final concentration of 0.2 mol L^{-1} NaCl. The salting process was followed by an overnight incubation step at 4 ºC. Unbound antibody and oligonucleotides were removed by repetitive centrifugation (16000×g for 30 min) at 4ºC. The clear supernatant was carefully removed and the precipitated gold conjugates were resuspended in TE buffer. Then added biotinylated DNA (complementary sequences) to gold complex solution, and hybridized with the thiolated DNA sequences from gold particles for 15 h at room temperature in dark. After that, the precipitation of DC-AuNPs was collected by repeat centrifugation, and redispersed in 0.5 mL of TE buffer and stored at 4 ºC for further use.

2.3 Functionalization of magnetic microparticles

The procedure includes two parts: activation and protein coupling. Firstly, 0.5 mL (50.0 mg mL^{-1}) of amino-functionalized MMPs was washed three times with pyridine buffer in a tube and separated magnetically. The MMPs were then activated with 3.0 mL of glutaraldehyde (5%) in PBS buffer for 3 h at room temperature under gentle shake. After that, the unreacted glutaraldehyde was separated magnetically. The activated MMPs were washed with pyridine buffer for 4 times in the magnetical field.

Secondly, 10.0 mg of anti-E_2 antibody was added to 3.0 mL of pyridine buffer (10.0 mmol L^{-1}, pH 6.0). This anti-E_2 antibody solution was then added to the glutaraldehyde-activated magnetic microparticles and the mixture was incubated for 6 h at 37 ºC under gentle shake. The solution was magnetically separated and the supernatant was saved. Wash buffer was added to the anti-E_2 antibody-functionalized MMPs and mixed vigorously. The MMPs were then separated magnetically three times. Finally, the coupled MMPs were diluted and stored in wash buffer at 2-8 ºC as a suspension.

2.4 Indirect competitive fluorescence immunoassay

40.0 μL of 1.0 mg mL^{-1} of anti-E_2 antibody coated MMPs were washed with PBST for 2 times in the magnetical field in tube. 100.0 μL of E_2 standard solution or sample were added to the above tube. 100.0 μL of DC-AuNPs was added to react competitively with the anti-E_2 antibody at 37 ºC for 40 min. After the competitive binding, the immunocomplexes (antigen/primary antibody/secondary antibody) were then magnetically separated and washed three times with PBST solution. Then 100.0 μL of avidin-FITC solution was added and incubated at 37 ºC for 1 h. The mixtures were then washed as described above and magnetically separated.

In the final step, the complexes were resuspended in 100.0 μL of freshly prepared DTT solution, and gently shaked for 80 min at 37 ºC to release the thiolated DNA sequences from the surface of the AuNPs. Magnetic particles were removed from the mixture using the magnetic separator. The supernatant containing free DNA sequences with FITC was collected to the polystyrene microtitration plates. An excitation wavelength of 485 nm and an emission wavelength of 525 nm were chosen to measure the

fluorescence intensity.

2.5 Treatment of sample

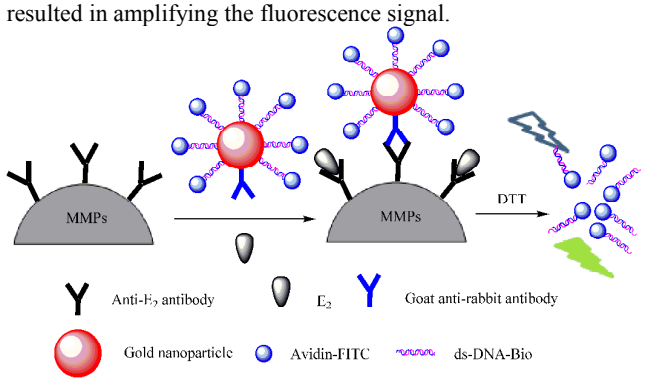
The urine samples were collected from a healthy woman's morning urine. Because the urine was packed with many other proteins, these proteins may seriously interfere with the detection of E_2, it should be necessary to remove other proteins.

The treatment process was as follows: 1mL urine was added a little distilled water, and then added 2.5 mL 2 M HCl. The mixture was heated at 100 °C for 30mins, cooled at the room temperature. The obtained urine hydrolysate was added 2.5 mL 1 M sodium carbonate solution. After eliminating carbon dioxide, added HCl or Na_2CO_3 to adjust the pH value to 7.4, and then diluted the solution to 20 mL with PBS. The volume dilution ratio of urine was 1 : 20.

3. Results and discussion

3.1 The sensing strategy of the measurement

The principle of detection was depicted in Scheme 1. In the assay, the competitive immunoreaction was typically achieved via antibody-based indirect compete with the specific binding among a secondary antibody, a primary antibody and hapten. The primary antibody served to capture the target hapten from the sample in the magnetic field, which could also bind to the secondary antibody. AuNPs were typically employed as the carrier with DNA stands labeled with FITC and secondary antibodies, which had following advantages. Competitive binding of the secondary antibody and the target hapten to the primary antibody resulted in immuno-complexes involving the DC-AuNPs and MMPs probes. When the immuno-complexes had been formed, a magnetic field was used to localize and collect them. Finally, in order to avoid the fluorescent signals quenched by AuNPs, DTT was employed to remove the thiolated DNA strands from the surface of the AuNPs. Therefore, hundreds of DNA strands labeled with FITC was released into the supernatant for each binding event by the addition of DTT, which therefore resulted in amplifying the fluorescence signal.



Scheme 1 Schematic diagram of immunoassay procedure for E_2

3.2 Characterization of DC-AuNPs

In the preparation process of the DC-AuNPs, AuNPs was used as a carrier of secondary antibody and DNA. Firstly, the antibody could easily adsorb onto the gold nanoparticle via electrostatic interaction in several minutes. Sequently, DNA strands could be directly anchored on bare gold surface via thiol bonds with high concentration salt solution, which was attributed to the molecules structure of antibody and improved stability of modified AuNPs. DC-AuNPs were finally achieved by hybridize with complementary sequences. The UV-vis spectrum of AuNPs and DC-AuNPs were recorded (Fig. S1). From Fig. S1(a), the characteristic absorbance peak of unmodified AuNPs at 520 nm, which is attributed to the typical plasmon band of the AuNPs; As can be seen from Fig. S1(b), DC-AuNPs in aqueous solution showed the absorption maxima around 526 nm, observing a small red shift, from 520 to 526 nm. A wide absorption band was revealed in 264 nm that the antibody and DNA attached to the AuNPs.

The quality of DC-AuNPs depended on amount of goat anti-rabbit antibody and thiolated ss-DNA, which were all attached to the surface of AuNPs. Therefore, it was critical to optimize the assay condition of DC-AuNPs preparation. As can be seen from Fig. S2, the DC-AuNPs preparation was executed with 15 μL (1.0 mg mL^{-1}) of goat anti-rabbit antibody and 4.0 μL of thiolated ss-DNA (50.0 μmol L^{-1}) to 1.0 mL of gold nanoparticles solution (1OD).

In addition, the quantification of immobilized goat anti-rabbit antibody and SH-dsDNA-biotin on the DC-AuNPs were determined. First, the interlinkage number of goat anti-rabbit antibody was determined using Coomassie Brilliant Blue (CBB, G 250). Antibody as the immune globulin G combined with CBB, and the maximum absorption peak of antibody-CBB compounds convert redshift to 595nm. By using this technique, the amount of unabsorbed antibodies in supernatant after centrifugation was detected, and the quantity of adsorbed antibodies was calculated. It was found that the quantities of secondary antibodies coated on the DC-AuNPs were about 8 (goat anti-rabbit antibody/AuNPs).

Second, the interlinkage number of SH-dsDNA-biotin was detected by using the fluorescence spectrometry. This amplification system relies mainly on the signal of fluorescence intensity. Therefore, when excess avidin-FITC was added to the DC-AuNPs, an amount of avidin-FITC was combined to DC-AuNPs with the biotin-avidin system. Then the amount of combined avidin-FITC was determined by fluorescence spectrum, and the amount of SH-ds-DNA-biotin of coated on the DC-AuNPs were calculated. It was indicated that the surface coverage of oligonucleotides on the DC-AuNPs were about 45 ± 2 branches (SH-dsDNA-biotin/AuNPs). Hence, the bonding ratio between secondary antibodies and SH-dsDNA-biotin is around 8 : 45.

To further prove the synthesis of the DC AuNPs, the fluorescence immunoassay was carried, which showed the antibody immobilized on the AuNPs surface had a good bioactivity.

3.3 Characterization of functionalized MMPs (anti-E_2 antibody/Fe_3O_4)

In the detection system, MMPs were utilized as the immobilized carrier in lieu of conventional polystyrene plates. Thus, it resulted in incubation and washing times shorter than those typically needed in classical ELISA tests, meanwhile amplified the fluorescence signal by the fast enrichment in the magnetic separation. For the MMPs preparation, amino-functionalize magnetic microparticles were linked to a primary antibody using glutaraldehyde-amine coupling chemistry.[27]

There were two kinds of coupling buffer used to attach primary antibody to amino-functionalized magnetic microparticles, including the phosphate buffer and the pyridine buffer. The polyvalent, negative phosphate ions in the phosphate buffer clump the positively charged amine support and resulted in the low efficiency. Moreover, the coupling buffer should be used at minimal ionic strengths according to the literature.[28] The results indicated a higher coupling efficiency if the pyridine buffer (10.0 mmol L^{-1}, pH 6.0) was chosen as the coupling buffer. Therefore, the pyridine buffer (10.0 mmol L^{-1}, pH 6.0) was selected as the optimum coupling buffer.

The coupling efficiency was calculated with pre-coupling solution and post-coupling solution and the coupling efficiency was determined to be about 70% with UV-Vis absorbance spectroscopy at 280 nm.[29]

3.4 Optimization of the assay parameters

3.4.1 Optimization of DC-AuNPs

AuNPs was carrier and quencher. The quantity of DC-AuNPs affect the sensitivity of this method. The concentration of DC-AuNPs was optimized. As shown in Fig. 1, the fluorescent intensity rapidly increased, with the increasing of the concentrated ratio. While the concentrated ratio was 10 : 1, the fluorescent intensity was highest. While the concentrated ratio was more than 10 : 1, the fluorescent intensity rapidly decreased. The results indicated that the optimal concentrated ratio was 10 : 1

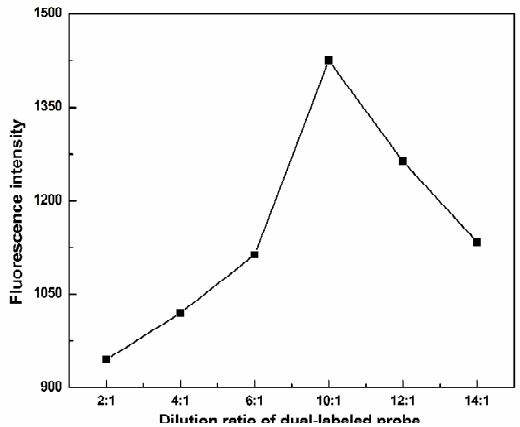


Fig. 1 The effect of DC-AuNPs

3.4.2 Optimization of anti-E_2 antibody/Fe_3O_4

Anti-E_2 antiboby was the primary antibody which served to capture E_2 and goat anti-rabbit antiboby, so E_2 and goat anti-rabbit antiboby happen to competitive immunreaction with Anti-E_2 antiboby. In addition, MMPs have properties of enrichment and separation. Thereby the quantity of anti-E_2 antibody/Fe_3O_4 could affect the sensitivity and the linear range in competition reaction. It was shown the anti-E_2 antibody/Fe_3O_4 influence fluorescent intensity in Fig. 2. With the increasing of anti-E_2 antibody/Fe_3O_4, the fluorescent intensity increased. While 40.0 μL (1.0 mg mL^{-1}) anti-E_2 antibody was added, the fluorescent intensity reached strongest .Thus, 40.0 μL (1.0 mg mL^{-1}) anti-E_2 antibody coupled MMPs was viewed as the best volume for immunoassay progress.

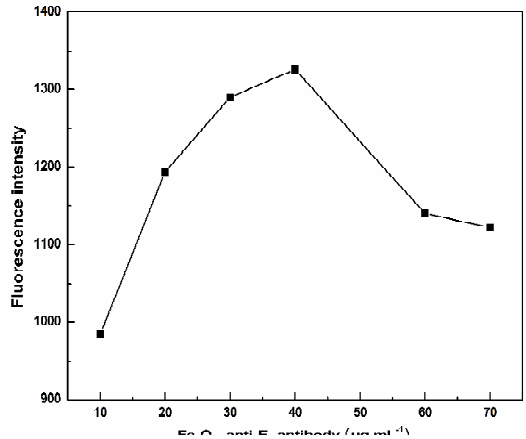


Fig. 2 The effect of functionalized MMPs

3.4.3 The effect of avidin-FITC

Fluorescence signals were performed with the binding of the biotinylated DNA sequence and avidin-FITC. The impact on the fluorescence intensity was analyzed at different concentrations of avidin-FITC. As shown in Fig. 3, fluorescence intensity increased with the increasing of avidin-FITC. The fluorescent intensity reached highest in the presence of 150.0 μg mL^{-1} of avidin-FITC. So 150.0 μg mL^{-1} of avidin-FITC was chosen.

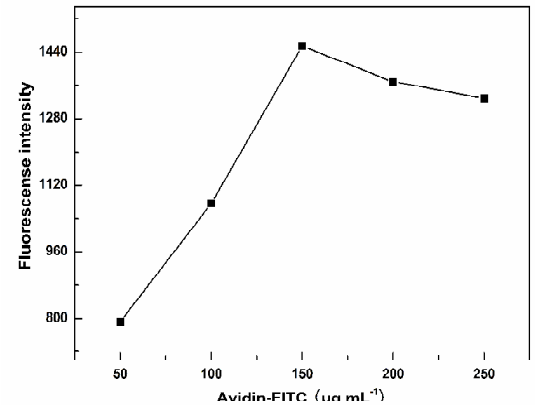


Fig. 3 The effect of avidin-FITC

3.4.4 Influence of dithiothreitol (DTT)

The interaction between donor and acceptor in fluorescence emission could result in the phenomenon of fluorescence quenching, which can cause a decrease in the fluorescence signal. The interaction extent depends on the distance between gold nanoparticles and fluorophore. In this test, DTT was used as spacers to control the distance between fluorophore (donor) and gold nanoparticles (acceptor) and quenching was inhibited, which lead to an enhanced fluorescence emission signal.[30] The proper concentration of DTT was optimized and the result was shown that 6.0 mmol L^{-1} DTT is the optimum condition to liberate the covalently attached thiolated ss-DNA from MMPs-AuNPs complex, thus to release the FITC for assay readout.

3.5 Calibration curve

With the optimized reaction conditions, nanoparticle-based fluorescence immunoassay method was applied to confirm the linear range of E_2, as shown in Fig. 4. The standard curve showed a good response for E_2 at concentrations of 1.0×10^{-5}-1.0 ng mL^{-1}. The equation was ΔI (I_0-I) = 152.83 lgC + 1284.75 for the standard curve and the linear correlation coefficient was R^2 =0.9950. According to the definition, we obtained the detection limit of 6.37×10^{-6} ng mL^{-1}.

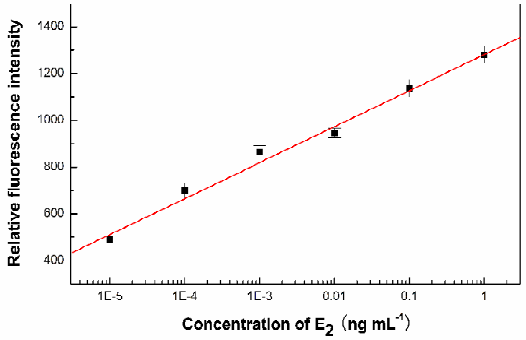


Fig. 4 Linear plots of relative fluorescence intensity versus the concentration of E_2

A detailed comparison of the analytical characteristics of this

method and other methods for E_2 are summarized in Table 1. As can be seen, the developed nanoparticle-based fluorescence immunoassay allowed one to detect E_2 concentration levels at limits of detection that were at least 2 orders of magnitude lower than the achievable assay that had been reported previously. It can be concluded that the DC-AuNP and MMPs were an excellent platform for the detection of E_2, suggesting high sensitivity and high-throughput screening of the present assay.

Table 1 Comparison of the analytical characteristics of present method and other methods for E_2

Methods	Linear range (ng mL^{-1})	Detection limit (ng mL^{-1})	Reference
LC-ESI-MS[a]	1.3×10^{-3}-5.35×10^{-2}	1.3×10^{-3}	[9]
GC-MS/MS[b]	0.1-10.0	1.84×10^{-2}	[10]
FIA[c]	1.0×10^{-2}-1.0×10^{4}	5.42×10^{-3}	[12]
CL-IA[d]	-	1.48×10^{-3}	[14]
FI-CL[e]	10.0-1000.0	3.0	[15]
EC-BS[f]	2.72×10^{-3}-2.72	2.72×10^{-4}	[16]
ECI-BS[g]	2.72×10^{-3}-2.72	5.44×10^{-4}	[17]
EC-EIA[h]	5.0×10^{-2}-0.5	2.1×10^{-2}	[18]
The present method	1.0×10^{-5}-1.0	6.37×10^{-6}	-

[a] Liquid chromatography-electrospray ionization tandem mass spectrometry
[b]Gas chromatography–mass spectrometry
[c]Fluorescence immunoassay
[d] Chemiluminescence immunoassay
[e]Flow injection chemiluminescence
[f]Electrochemical biosensor
[g]Electrochemical impedance biosensor
[h]Electrochemical enzyme immunoassay

3.6 Assay specificity

Specificity of the immunoreaction should be considered as the most important factor in the immunological analysis. The cross-reactivity of E_2 was evaluated using several endocrine disrupting compounds that structurally related to E_2 (Table 2) in this work. Specificity is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The data indicate that the specificity of this immunoreaction was acceptable in the analysis.

Table 2 Cross-reactivity with several related steroids of E_2

Compound	Cross-reactivity (%)
17β-Estradiol (E_2)	100
Estrone (E_1)	1.9
Estriol (E_3)	1.1
Ethinyl E_2	<0.02
E_2-17-glucuronide	<0.1
E_2-3-sulfate-17-glucuronide	<0.01
Progesterone	<0.1
Androstenedione	<0.03
DES	<0.01
p-NP	<0.01

3.7 Analysis of samples

In order to evaluate the applicability and reliability of the developed methodology, it was applied to the determination of E_2 in human urine sample. The urine sample spiked at 0, 0.1, 0.5, and 0.8 ng mL^{-1} were analyzed 3 times by the developed method. As shown in Table 3, the relative standard deviations (RSD) were all below 8.1%, indicating high reproducibility of the developed method. Meanwhile, the examination of recovery was carried out for spiked sample by adding defined amounts of E_2 standard solution to the sample solution. The results demonstrated that the recoveries for E_2 in spiked samples were in the range of 96.5% ~ 107.4%, suggesting that the recoveries of this method are satisfactory and the developed method was free of any matrix effect.

Table 3 The detection results of urine sample (n=3)

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
Urine	0	0.106	-	5.8
	0.1	0.211	105.1	2.1
	0.5	0.643	107.4	8.1
	0.8	0.878	96.5	6.2

4. Conclusions

A highly sensitive and specific nanoparticle-based fluorescence immunoassay for the determination of E_2 in urine was developed. In this system, a nanoparticle signal amplification platform was constructed based on two types of particles: MMPs functionalized with polyclonal anti-E_2 antibody as a capture probe; DC-AuNPs modified with both goat anti-rabbit antibody and SH-dsDNA-biotin as a signal amplifier; and the avidin-FITC was added to link to the SH-dsDNA-biotin as a tracer. This method has been shown to be advantageous over conventional immunoassay techniques in terms of high sensitivity, broader detection range, reliability and simplicity of handling. The linear range for determination of E_2 was 1.0×10^{-5}-1.0 ng mL^{-1} and the detection limit was 6.37×10^{-6} ng mL^{-1}. Since E_2 was at a low concentration in urine, high sensitivity and DC-AuNPs could contribute to achieve quantitative analysis of detecting E_2 in environmental sample, having great significance on environmental monitoring, clinical diagnosis for further study.

Acknowledgements

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Graphical Abstract

A fluorescence immunoassay based on functionalized gold nanoparticles amplification and immunomagnetic separation was constructed for E_2 detection.