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Ultrasensitive electrochemiluminescence immunosensor for determination of hepatitis B virus surface antigen using CdTe@CdS-PAMAM dendrimer as luminescent labels and Fe_3O_4 nanoparticles as magnetic beads

Bahareh Babamiri [a], Rahman Hallaj [a,b,*], Abdollah Salimi [a,b,*]

[a] Department of Chemistry, University of Kurdistan, 66177-15175, Sanandaj, Iran
[b] Research Center for Nanotechnology, University of Kurdistan, 66177-15175, Sanandaj, Iran

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ABSTRACT

Here, a sandwich-type electrochemiluminescence (ECL) immunosensor for ultrasensitive determination of HBsAg was designed based on signal amplification with dendrimeric-quantum dots structures. First, the primary antibody of HBs (Ab_1) was immobilized on the surface of the carboxyl-modified magnetic nanoparticles (MNPs). Then, the PAMAM dendrimer with many amine functional groups was employed as the carrier for immobilizing CdTe@CdS quantum dots (QDs) and the secondary antibody (Ab_2) which can amplify the ECL signal of QDs significantly and improve the sensitivity of the proposed method. Dendrimer increasing loading of CdTe@CdS-Ab_2 labels, while ECL response from CdTe@CdS QDs enhanced 4 folds compared to the unamplified protocol. Furthermore, MNPs enhances the electron transfer between the nanoprobe and the electrode and also, their large surface area leads to immobilize more Ab_1. Under the optimal conditions, the constructed immunosensor showed a wide linear range from 3 fg mL^{-1} to 0.3 ng mL^{-1} with the detection limit of 0.80 fg mL^{-1} (S/N ratio of 3) for HBsAg detection. The proposed ECL immunosensor exhibited good analytical performance and excellent stability and it was applied for HBsAg detection in human serum samples with satisfactory results.

1. Introduction

Hepatitis B virus (HBV) is a common pathogen that causes cirrhosis, chronic hepatitis, and primary liver cancer and HBV surface antigen (HBsAg) is the most important diagnostic marker for the clinical diagnosis of hepatitis B virus infection [1]. The HBsAg antigen is a secreted cover protein that continuously enters into the blood by virus liver cells so that the detection of HBsAg in serum is one of the basic steps in the early diagnosis of hepatitis B [2]. Thus, the accurate and sensitive detection of low level quantities of HBsAg is a significant step for the diagnosis of HBV infection. During recent years, the conventional diagnostic methods, such as enzyme-linked immunosorbent assay (ELISA) [3], chemiluminescence immunoassay (CLIA) [4], time-resolved fluoroimmunoassay (TRFIA) [5] and capillary electrophoresis (CE)-electrochemical immunoassay [6] have been used as the main methods for detection of hepatitis B virus (HBV). However, despite the reliability of these methods, they are often time-consuming, complicated and expensive which leads to the limitation of these methods. Therefore, the development of sensitive analytical techniques for the detection of HBsAg is an obvious demand.

The electrochemiluminescence (ECL), or electrogenerated chemiluminescence is an optical radiation process in which the excited species generate at the surface of electrodes undergo high-energy electron transfer reactions [7]. This method is a combination of the controllability and simplicity of electrochemistry with inherent sensitivity, low background and wide linear range of chemiluminescence (CL) method [8]. In comparison to the conventional immunoassays such as enzyme linked immune sorbent assay (ELISA), the electrochemiluminescence (ECL) immunoassay technique shows high sensitivity, fast detection, high stability, wide dynamic detection range, simple controllability and good flexibility [9–11]. So, as an important and powerful analytical tool, this

* Corresponding authors at: Department of Chemistry, University of Kurdistan, 66177-15175, Sanandaj, Iran.
E-mail addresses: Rhallaj@uok.ac.ir (R. Hallaj), absalimi@uok.ac.ir, absalimi@yahoo.com (A. Salimi).

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technique has attracted much attention for the quantitative detection of the trace amounts of different types of targets. However, the bioanalysis based on those conventional luminescent has some limitations, and ruthenium-complex derivatives labeling at multiple sites may result in the loss of biological activity of molecules [12], and the ECL signals of luminol are weak in the neutral solution [13]. ECL sensors have been developed with QDs as ECL emitters after the first application of Si QDs [14]. Recently the QD-based ECL analytical technique has been scrutinized in many fields because of its superiorities such as simplified setup, low background signal, high sensitivity, low cost and fast sample analysis [15,16]. Quantum dots (QDs), with the numerous advantages, such as strong fluorescence, surface effects, small size effects, feasibility for surface modification, broad absorption and narrow emission for multicolor imaging have been widely used as luminescence labels for bioassay and bio imaging [17–20]. Various QDs including CdSe [21], CdS [22], CdTe [23] etc., have been synthesized and used as ECL emitters in the aqueous systems. In comparison to the conventional molecular emitters, QDs have several distinctive merits such as size/surface-trap controlled luminescence, good stability against photobleaching, better biocompatibility, and also they could be more easily bio labelled and stabilized at the surface of electrode, so they are more promising for photoelectrocatalytic processes and ECL bioassays [24–29]. Despite these advantages, the ECL signals of QDs are weaker than that of conventional luminescent reagents such as luminol or $Ru(bpy)_3^{2+}$, therefore the quantitative applications of QDs ECL may be limited [30]. In order to achieve the lower detection limit and higher sensitivity, signal amplification techniques are critical needs for developing ultrasensitive electrochemiluminescence immunoassay methods based on QDs. The bandgap engineering by adjusting the CdS shell thickness on CdTe core QDs emit strong luminescence peaking in the spectral range of 550–800 nm [31]. Furthermore, due to promising applications of various QDs in photovoltaic devices as the near-infrared (NIR) emitters, CdTe/CdS and CdTe/CdSe core/shell QDs have become to be more considered [32]. Then, shelling of CdTe with CdS would make CdTe@CdS core/shell QD with highly luminescent and compared to the CdTe or CdS, the shell model not only largely decreased the surface traps of CdTe@CdS QDs but also highly improved their brightness and stability, providing the potential possibility as ECL emitters.

Polyamidoamine (PAMAM) dendrimers are hyper-branched and three-dimensional macromolecules with many substantial tertiary amine groups, which have high capacity for QDs labeling that could greatly amplify the electrochemical and ECL signals [33–36]. Furthermore, the functionalized magnetic nanoparticles (MNPs) with high specific surface area, was used for effective separation of the immobilized biomolecules from a reaction mixture and as a carrier of antibodies for amplify the sensitivity and also improve the detection limit of the renewable immunosensors [37–39]. Furthermore, the application of MNPs based nanocomposites as electrochemical platform and catalysis for accelerate the electron transfer from analyte to electrode and development of electrochemical sensors and biosensors were reported [40–43].

In this study, magnetic nanoparticles (MNPs) and dendrimer-CdTe@CdS nanocluster were prepared for dual signal amplification in the electrochemiluminescence immunoassay (Scheme 1). Magnetic nanoparticles were employed for HBs antibody (Ab_1) immobilization and the simplification of the separation procedures. PAMAM were employed as carriers for the immobilization of the QDs and the secondary antibodies (Ab_2) and the CdTe@CdS QDs were used as the luminescent labels probe to generate ECL signals. The proposed ECL immunosensor based on the magnetic nanoparticles (MNPs) and PAMAM-CdTe@CdS extraordinarily sensitive to fg mL^{-1} of HBsAg with good reproducibility and ability of the immunosensor for HBsAg analysis in human serum was also evaluated.

2. Experimental

2.1. Materials

Hepatitis B virus surface antigen (HBsAg) and anti-HBs antibodies (Ab_1 and Ab_2) were purchased from Dia., Pro Diagnostic Bioprobes Co., Ltd. Polyamidoamine dendrimer (PAMAM) (generation 4, G4) were purchased from Sigma-Aldrich. $CdCl_2{\cdot}2.5H_2O$ (99.0%), $NaBH_4$ (96.0%), tellurium powder (99.9%), thioglycolic acid (TGA, 99%), tri-*n*-propylamine (TPrA), bovine serum albumin (BSA), N- hydroxysuccinimide (NHS), and 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDC), Ferric chloride, ferrous chloride, Citric acid (CA), were obtained from Merck or Fluka. All other common solvents and salts were of analytical grade and used as received.

2.2. Apparatus

All electrochemical experiments were carried out at room temperature with a computer-controlled potentiostat, Autolab electrochemical analyzer model PGSTAT30 (Eco Chemie, Utrecht, The Netherlands) driven with GPES software (Eco Chemie) using a conventional three-electrode system. Electrodes were a modified magnetic Au working electrode (4 mm diameter), a platinum wire as the auxiliary electrode and an Ag/AgCl electrode as the reference electrode. PL spectra were obtained at different excitation wavelengths using a spectrophotometer (Varian Cary Eclipse Fluorescence Spectrophotometer, Agilent, USA). ECL measurements were carried out by homemade analyzer. Ultraviolt-visible (UV-vis) absorption spectra (200–800 nm), (0.5 nm resolution) were acquired using UV–vis spectroscopy (Varian Cary 5000 UV-vis-NIR absorption spectrometer, Agilent Technologies, USA), utilizing quartz cuvette with optical path lengths of 10 mm. The FT-IR spectra were obtained using the KBr pellet technique by a Vctor-22 BRUKER spectrophotometer (Switzerland). Electrochemical impedance spectroscopy (EIS) measurements were done 0.1 M KCl solution containing 2.5 mM $Fe(CN)_6^{3-/4-}$. SEM, TEM and AFM imaging were used for characterization of QDs and magnetic nanoparticles.

2.3. Preparation of CdTe@CdS QDs

TGA-capped CdTe@CdS QDs were directly prepared in aqueous solution using the method described previously [44]. Briefly, 2.5×10^{-4} mol of $CdCl_2$ $2.5H_2O$ was dissolved in the 50 mL of water, and 38 μL of TGA was added under stirring, followed by adjusting the pH to 12 by adding dropwise 1.0 M NaOH. The solution was deaerated by bubbling high purity (99.99%) of N_2 gas for 30 min. Then, 250 μL of the freshly prepared NaHTe solution (0.04 M) quickly injected to the above solution. After the injection NaHTe, instantaneously yellow color was observed. The solution was aged at 4 °C for 24 h and the small CdTe cluster solution was obtained. The CdTe@CdS QDs were synthesized by further aging small CdTe cluster solution at 90 °C for 10 h and the color of the solution changed from yellow to orange.

2.4. Synthesis of Fe_3O_4 nanoparticle

Magnetic Fe_3O_4 nanospheres were prepared by a hydrothermal route [45]. Briefly, 4.44 g of $FeCl_3$ and 1.732 g of $FeCl_2$ were dissolved in the 80 mL of water were placed in a 500 mL round flask and the temperature was slowly increased to 70 °C in the refluxing condition under the nitrogen atmosphere with the constant