

Photoelectronic properties of horseradish peroxidase-functionalized CdSe/silica mesoporous composite and its sensing towards hydrogen peroxide

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Received: 2 March 2010 / Revised: 7 July 2010 / Accepted: 8 July 2010 / Published online: 23 July 2010
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Abstract CdSe nanocrystals were prepared within a template of mesoporous silica (MS) spheres via chemical reaction, and then the as-synthesized CdSe/MS composites were dip coated on an optically transparent electrode. The photoelectric properties of CdSe/MS composite film were examined under ultraviolet (UV) illumination at the excitation wavelength of 365 nm. The CdSe/MS composite can facilitate charge rectification and minimize charge recombination as shown by its higher photocurrent. Most importantly, horseradish peroxidase (HRP)-functionalized CdSe/MS-modified electrode (HRP/CdSe/MS) had a stronger response toward hydrogen peroxide (H_2O_2) under UV illumination than in the dark. The result demonstrates the potential application of HRP/CdSe/MS composite film as a novel biosensor for monitoring H_2O_2 under UV illumination.

Keywords CdSe/silica mesoporous · Photoelectric properties · Horseradish peroxidase · Biosensors

Introduction

In recent years, semiconductor quantum dots (QDs) attract considerable attention for their unique optical and electronic properties including extended optical absorption in the UV–VIS region, bright photoluminescence, narrow emission band, size tunable photoluminescence, and photostability [1–6]. Owing to their tailorable optical properties,

CdSe QDs have advantages in UV emission and transparent conductivity and possesses potential utilities for light-emitting/detecting devices, biological/optical probes and so on [7–12]. Recently, many uses of CdSe in biosensing and biological sensing fields were profoundly reported, such as cellular labeling and QDs assay [13–16]. For example, Jie and coworkers [14] developed an electrogenerated chemiluminescence strategy by combining CdSe QDs with carbon nanotubes and 3-aminopropyl-triethoxysilane for the sensitive immunosensing of human IgG.

However, the too low photocurrent of semiconductor QDs, resulting from the fast charge recombination, often limits the application. Fortunately, employing composite semiconductors has been an effect path towards improving the efficiency of charge separation through charge rectification [17]. Mesoporous silica (MS) is well known for its high surface area, 3D pore networks, and pore sizes in the range of 2–50 nm, which can also be used as a matrix for synthesizing conductor nanocrystals [18, 19]. Gao et al. [20] reported a photocurrent with fast-growing and decay times, as a result of photogenerated holes being trapped at the interface between ZnO and pore walls of MS film while producing the photocurrent by photogenerated electrons.

Horseradish peroxidase (HRP) belongs to the superfamily of heme-containing plant peroxidases; it catalyses the oxidation of various electron donor substrates with hydrogen peroxide [21]. The HRP immobilized on an electrode can be oxidized by H_2O_2 and then subsequently reduced by electrons provided by an electrode [22]. This process is usually slow when an electrode substitutes the electron donor substrates in a common peroxidase reaction cycle without electron donor substrates. In this article, we have fabricated CdSe semiconductor nanocomposite by chemical method using the MS sphere pores as template. The CdSe/MS nanocomposite possessed good uniformity and large

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surface areas, as well as the high porosity, which could be favorable for enzyme immobilization. The biofunctional composite CdSe/MS immobilized with HRP was also prepared and used for visible-light-assisted electrochemical detection of H₂O₂. Under UV illumination, an enhanced photocurrent was obtained from CdSe/MS film. Interestingly, we found that a higher catalytic current toward H₂O₂ was released from the HRP/CdSe/MS composite, suggesting the possibility of electrochemical detection of H₂O₂ under light irradiation. We proposed that the enhanced response to H₂O₂ was aroused from the synergy effect of the CdSe/MS composite spheres generate electron transfer, which opens up new applications on biosensor under UV light irradiation.

Experimental

Chemicals

Hexadecylamine was purchased from Fluka. HRP was obtained from Sinopharm chemical reagent company. Hydrogen peroxide (30 wt%) was purchased from Sigma-Aldrich (Germany). Ethyl silicate, isoproryl alcohol, ammonia, ethanol, and other reagents were of analytical grade. All solutions were prepared with double-distilled water.

Synthesis of CdSe/MS composite spheres

MS spheres were synthesized according to the literature [23]. Briefly, *n*-hexadecylamine was first dissolved in a mixture solution including isopropanol, H₂O, and NH₄OH to form a homogenous solution. Subsequently, tetraethyl orthosilicate (TEOS) was added into above solution under stirring with stand at ambient temperature overnight. The resulting solids were recovered by filtration of the reaction mixture, extensively washed with the deionized water. To remove the surfactants, the as-synthesized material was heated at 600 °C for 6 h. For the growth of CdSe, nanoparticles on the MS were achieved by the following strategy [24], as shown in Fig. 2. To facilitate the Cd ions adsorption onto the pores surface, the MS spheres were first impregnated with 6 mL of 0.095 M precursor CdSO₄ at room temperature for 2 h and then cooled down to -10 °C. Finally, 4 mL of aqueous sodium selenosulfate prepared by dissolution of Na₂SeSO₃, 0.2 M Se in 0.5 M Na₂SO₃, was added to the above solution to obtain final concentrations of 57 mM CdSO₄ and 80 mM selenosulfate. The CdSe nanocrystals were formed inside the MS microsphere pores by the reaction between selenosulfate and noncomplexed Cd ions, which were adsorbed in silica hydroxyl groups. The MS loaded with CdSe nanocrystals (CdSe/MS) obtained by precipitation centrifugation and washing with

double-distilled water was collected and then characterized by various analytical techniques.

Immobilization of HRP on CdSe/MS composite film

Dip-coating method was used to load the obtained CdSe/MS composite spheres on the transparent and conductive indium tin oxide (ITO) sheet. Then the as-prepared ITO was dipped for 30 min into a pH 6.8 phosphate-buffered saline (PBS) solution containing 0.2 mg·mL⁻¹ HRP; a layer of negatively charged HRP was adsorbed on the surface of CdSe/MS composite spheres, then the un-adsorption HRP was removed by immersing into the PBS. Electrochemical properties of CdSe/MS film were measured by using a CHI 660. The optically transparent electrode (OTE) were used as a working electrode, whereas Ag/AgCl (3 M KCl) and platinum wire served as the reference and counter electrodes, respectively.

Characterization techniques

Transmission electron microscopy (TEM) was measured on a JEOL JEM 100CX transmission electron microscope at 100 kV and used to examine the morphology of CdSe/MS spheres as well as the effectiveness of the coating procedure. Wide-angle X-ray (10°–80°, 40 kV/200 mA) powder diffraction (XRD) data were recorded on a Rigaku D/max 2550 VB/PC diffractometer using nickel-filtered Cu-K α radiation at wavelength $\lambda=0.154056$ nm. High-resolution electron microscopy (HRTEM) measurement was performed on the cross-section of CdSe/MS sample using JEM-2100F transmission electron microscope (JEOL, Japan). The UV photodetection measurements of CdSe/MS were recorded by an Electrochemical Workstation (CHI 660) in a dark box containing a switched UV-light (handy UV lamp, SLUV-4) at the wavelength of 365 nm.

Results and discussion

Synthesis of CdSe/MS composite spheres

Figure 1 shows a typical procedure for fabricating CdSe/MS composite. The MS spheres with approximately 10-nm pore size and 0.4-mL/g pore volume were used as the host material to incorporate the CdSe particles. In a previous work [23], we have reported the morphology and structure of 1.2- μ m synthesized MS spheres. As shown by the TEM in Fig. 2a, spherical CdSe nanocrystals were deposited uniformly on the external surface of the silica spheres. Figure 2b shows the XRD pattern of the CdSe/MS composite microspheres. The broad peaks along the (002), (110), and (112) planes are in good agreement with the

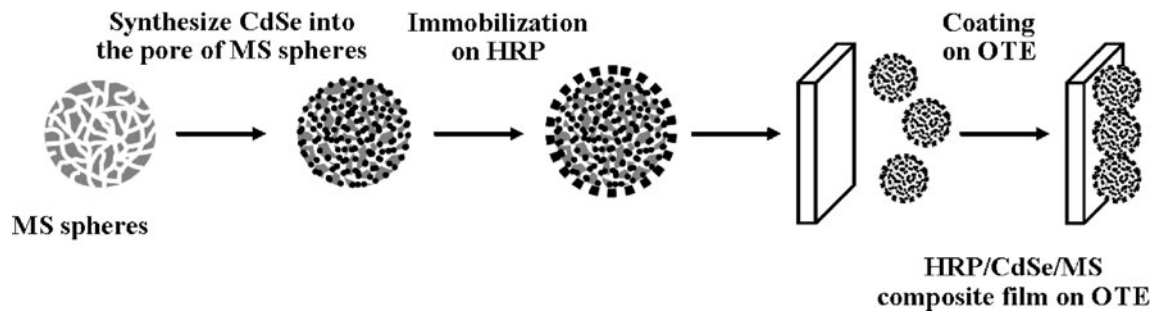


Fig 1 Schematic diagram of the fabrication procedure of the HRP/CdSe/MS composite film on OTE

results reported by JCPDS card (JCPDS no. 02–0330). These peaks were assigned to the hexagonal phase of CdSe, suggesting that the diffraction was predominantly associated to CdSe nanoparticles corresponding to an average size of about 5.38 nm calculated by Scherrer's equation.

As shown in Fig. 3, the HRTEM image and corresponding electron diffraction pattern of CdSe/MS composite

spheres displayed the distinct crystal lattice of CdSe nanocrystals, which formed a worm-like structure along the pore paths of the MS spheres. These observations indicate that the internal walls of the silica host material have been coated with CdSe particles.

Photoelectronic properties of CdSe/MS composite film

The photoelectronic properties of the MS and the CdSe/MS films were characterized on OTE by I - V response under UV illumination and in the dark. As shown by the current–voltage (I - V) curves in Fig. 4, all the CdSe/MS composite films had much higher current value than the MS films under UV excitation or in the dark. The current value of CdSe/MS composite film reached 1.8 μ A when the voltage was increased to 1 V in the dark. Upon UV illumination, the photocurrent value reached 2.4 μ A at 1 V, i.e., 0.6 μ A higher than the current value in the dark. Exposure of the

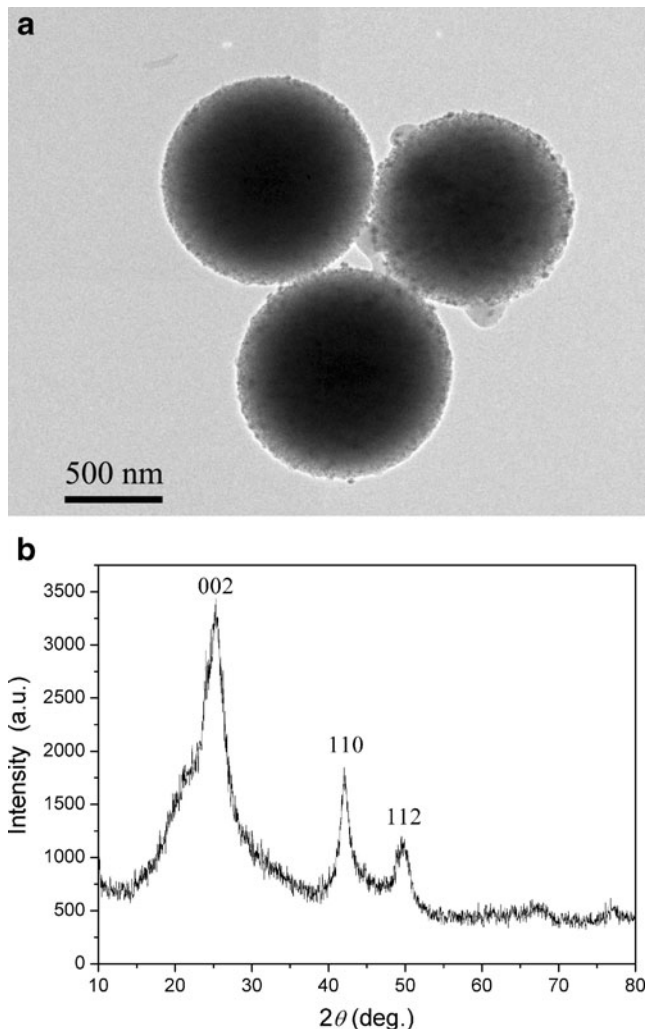


Fig 2 TEM image **a** and XRD pattern **b** for the CdSe/MS composite microspheres

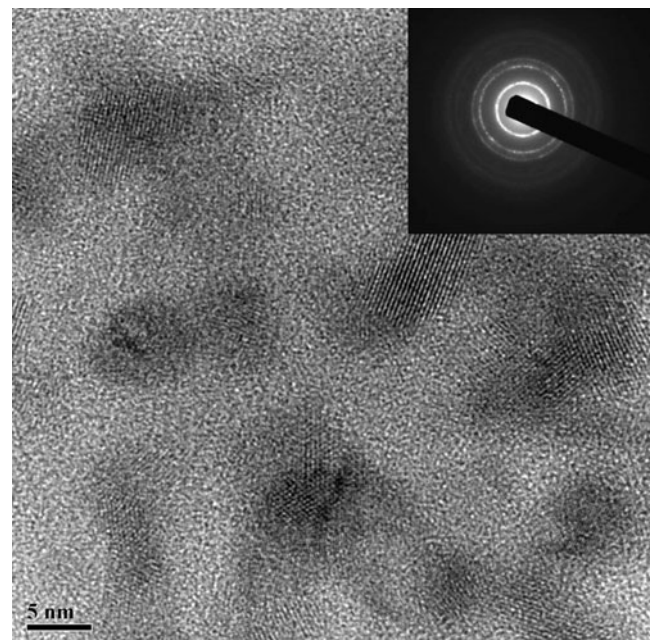


Fig 3 HRTEM image for the cross-section of the CdSe/MS composite microspheres. *Inset*: the corresponding selected area electron diffraction (SAED) pattern

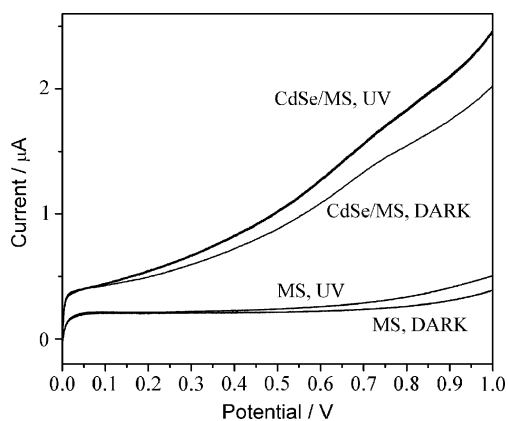


Fig 4 I - V characteristics of MS film and CdSe/MS film on OTE under UV illumination and in the dark, respectively

CdSe films to the UV light resulted in an increased conductivity. These results show that the current from CdSe/MS film has been significantly enhanced under UV illumination, thus contributing to a higher conductivity [12, 20]. Conductivity enhancement for the CdSe/MS composite film is ascribed to the CdSe nanocrystals within the MS spheres. These CdSe can absorb the UV light and then create an electron-hole pair capable of injecting excited electrons and generating photocurrent under UV illumination [26].

Figure 5 shows the photoresponses of the CdSe/MS and the MS films on the OTE at 0.2 V obtained by switching ON and OFF a UV-light at wavelength 365 nm. Compared with the MS film (data not shown), the CdSe/MS film has a distinctly higher UV response on the OTE. Under UV illumination, the CdSe/MS composite film showed a prompt increase in photocurrent. However, a sharp decrease

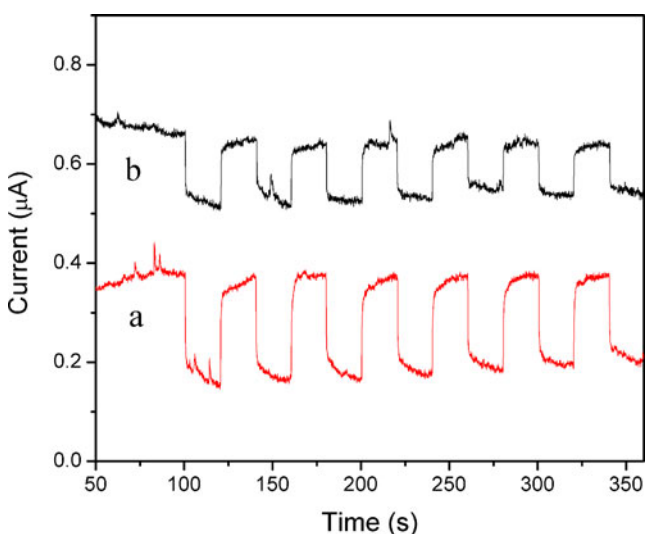


Fig 5 Photoresponse of CdSe/MS film in the presence **a** and absence **b** of H_2O_2 on OTE under UV illumination

in the short circuit current is observed, within the first illumination, before stabilization. This decaying trend of the photocurrent in the initial seconds of the UV illumination can be observed during repeated on-off cycles, in accordance with the results reported in previous study [12]. The photocurrent decay observed during the initial excitation period may result from dominant charge recombination/scattering at the CdSe/MS heterointerface or at grain boundaries within the MS network. The release of photo-generated holes from the interface occurs after the UV light is turned off [20], and this process of holes' release is easier than the process of charge rectification on the surface of CdSe/MS, because the process of charge rectification involves a carrier capturing over the potential barrier. Therefore, the easy charge-transfer procedure leads to a fast decay process. Such UV responsivity is reversible with UV light turning on and off, which suggests stable photocurrent responsivity from the CdSe/MS film, and has a potential for optical utilization.

Amperometric measurements on HRP/CdSe/MS film for H_2O_2 detection

To optimize the conditions of this enzymatic biosensor, the effect of pH and applied voltage was first studied. The highest current response was obtained at a pH of 6.8, which is in good agreement with the optimum pH value for the free HRP. For the enzyme electrode, the oxidation of the enzymatical reaction is dependant on the applied potential. In this study, the response current was obtained at the applied potential of 0.2 V versus Ag/AgCl. Therefore, we chose the pH at 6.8 and applied potential as the optimum condition for the HRP enzymatic reaction.

Figure 6 shows the typical amperometric responses of the HRP/CdSe/MS film on OTE at an applied potential of 0.2 V in the phosphate buffer-supported electrolyte (pH 6.8) containing various concentrations of H_2O_2 . The response current is linear to glucose concentration in the range of 0.8–9.8 μM . The regression equation is $i = -0.073 + 0.089 c_{H_2O_2}$ (μA , μM , $r = 0.994$, $n = 3$) under UV illumination and $i = -0.049 + 0.077 c_{H_2O_2}$ (μA , μM , $r = 0.997$, $n = 3$) in the dark, respectively. The HRP/CdSe/MS electrode shows a stronger current response to H_2O_2 under UV illumination than in the dark. It also could be seen that the response signal increases with increasing H_2O_2 concentration. For comparison, as shown in Figs. 5 and 6a, the reduction of H_2O_2 for CdSe/MS electrode was observed. The reduction current was lower than that for HRP/CdSe/MS electrode under UV illumination and in the dark. These results showed that the immobilized HRP retained its electrocatalytic activity toward H_2O_2 , and the CdSe/MS also enhanced the electrocatalytic reduction of H_2O_2 . The apparent Michaelis-Menten constant (K_M^{app}), which gives

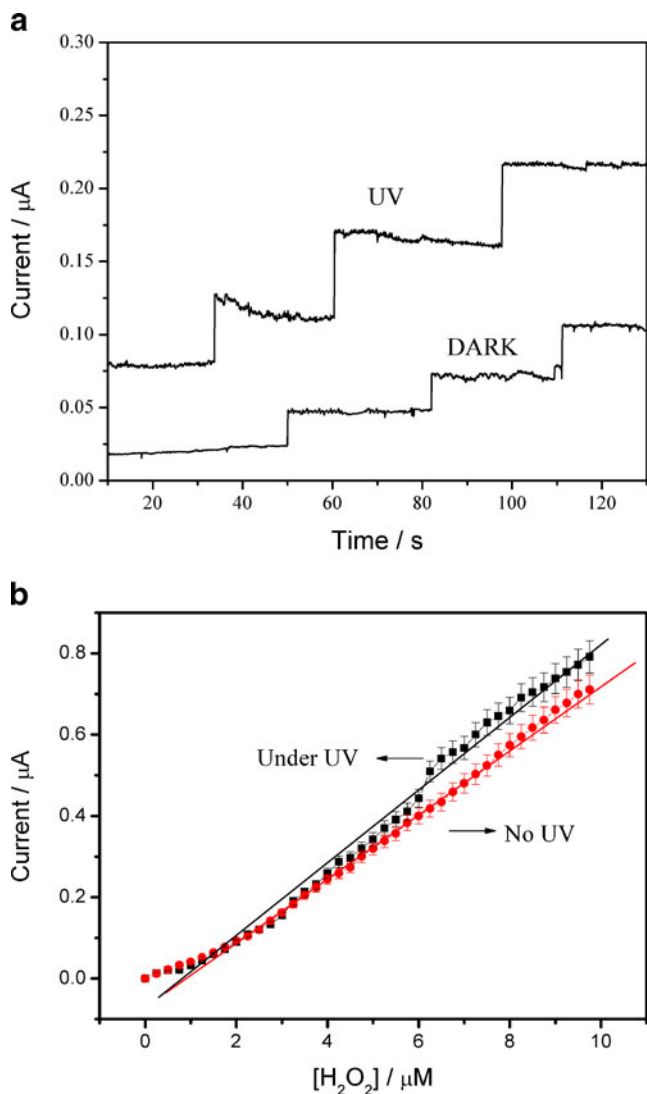
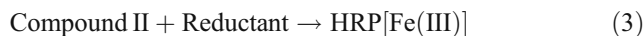
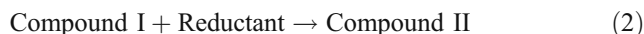
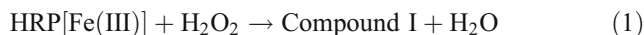


Fig 6 Amperometric response and plot of the photocurrent versus H_2O_2 concentration for HRP/CdSe/MS film on OTE under UV illumination **a** and in the dark **b** upon subsequent addition of hydrogen peroxide in PBS solution (0.05 M, pH 6.8)

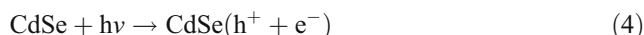
an indication of the enzyme-substrate kinetics, is generally used to evaluate the biological activity of immobilized enzyme. The apparent Michaelis-Menten constant was estimated to be 2.72 mM, according to the Lineweaver-Burk equation [27, 28], which is smaller than the reported values of polyazetidine [28]. Moreover, we investigated the reproducibility of the HRP/CdSe/MS electrode, six enzyme electrodes made at the same electrode independently showed an acceptable reproducibility with a relative standard deviation of 3.3% for the current determined at 40 μM H_2O_2 .

HRP contains the heme as the active site; in the resting state, the heme-iron oxidation state is Fe(III). The native ferric HRP can be directly reduced at the electrode surface to its ferrous state by a 1e transformation. The mechanism

of the catalytic reduction of the immobilized HRP to H_2O_2 on the electrode was exemplified by the following schemes [25]:



For the CdSe/MS film, the electron injection process is initiated when the light is absorbed by the CdSe, thus generating an electron-hole pair [26]:



Concerning the higher sensitive response to H_2O_2 , therefore, we proposed that the CdSe/MS electron-hole pairs generated under UV light can facilitate the reduction processes in the peroxidase-electrode system. In the presence of electron acceptors (H_2O_2) in solution, it could deplete the photogenerated electrons locating on the electrode and enhance the electron-hole separation of the charge transfer complex to some extent, leading to the enhancement of the steady-state photocurrent [29]. Figure 7 shows the possible charge transport process in HRP/CdSe/MS film on optically transparent electrode (OTE). When CdSe/MS composite film was used as a matrix of the peroxidase-electrode system, the electrons photogenerated from CdSe/MS composite and the electrons provided by electrode could occur simultaneously to catalyze the reduction of H_2O_2 . The holes photogenerated by CdSe are trapped at the interface between CdSe and pore walls of MS film. The result demonstrates that the HRP/CdSe/MS composite film may further be applied as an HRP biosensor for H_2O_2 monitoring under UV illumination.

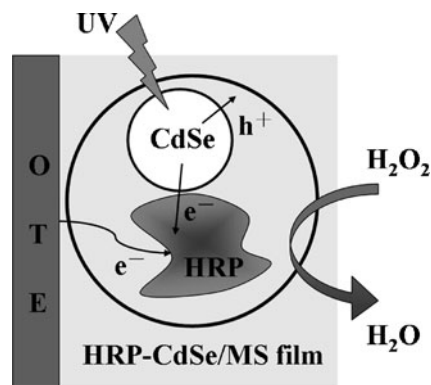


Fig 7 A scheme for charge transport in HRP/CdSe/MS film on OTE

Conclusions

In summary, the CdSe nanocrystals deposited into the MS pores efficiently improved the efficiency of charge separation through charge rectification and generated the higher photocurrent under UV illumination. Furthermore, the composite HRP/CdSe/MS film had a stronger response toward hydrogen peroxide (H_2O_2) under UV illumination than in the dark. We proposed that the electron–hole pairs of CdSe generated under UV light can facilitate the reduction processes in the peroxidase–electrode system. These results demonstrate the potential for application of such CdSe/MS film in the biosensing and photoelectronic systems.

Acknowledgment This work was supported by the National Natural Science Foundation of China (20925621, 20976054), the Key Project of Science and Technology for Ministry of Education (107045), the Innovation Program of Shanghai Municipal Education Commission (09ZZ58), the Program of Shanghai Subject Chief Scientist (08XD1401500), the Shuguang Scholar-Tracking Foundation of Shanghai (08GG09), and the Shanghai Leading Academic Discipline Project (B502).

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