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Short communication

A photoelectrochemical biosensor for *o*-aminophenol based on assembling of CdSe and DNA on TiO_2 film electrode

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ABSTRACT

A novel photoelectrochemical (PEC) biosensing platform was constructed by assembling CdSe quantum dots (QDs) and DNA on liquid phase deposited TiO_2 (DNA–CdSe/TiO_2) film electrode. The transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis indicated that CdSe QDs were homogeneously assembled on TiO_2 film. The UV–visible diffuse reflectance spectra (DRS) showed that CdSe and DNA could effectively enhance the absorption of TiO_2 film to visible light. The obtained electrode showed a sensitive PEC response to *o*-aminophenol (OAP) under visible light irradiation. Due to the interaction between DNA and OAP, the response of OAP was improved by DNA immobilized on the sensing film. Under optimized conditions, the photocurrent was linearly proportional to OAP in the concentration range from 4.0×10^{-7} to 2.7×10^{-5} mol L^{-1}, with a detection limit (3 S/N) of 8.0×10^{-8} mol L^{-1}. The novel strategy could provide a fast and sensitive method for OAP determination.

1. Introduction

Photoelectrochemical (PEC) biosensors have received increasing attention owing to their desirable sensitivity and good analytical performance (Zhang et al., 2013; Li et al., 2013). Due to its separate source for excitation and detection, the sensitivity of PEC sensors can potentially match that of electrochemiluminescence (ECL) with low background signals (Liang et al., 2008). In PEC bioanalysis, photoactive materials are usually prepared as the substrate for the subsequent immobilization of biological recognition element (Zhao et al., 2011). Some wide band-gap nanostructured semiconductors such as TiO_2, ZnO, and SnO_2 have been extensively employed as the photoactive materials because of their good stability against photocorrosion, high photoelectric and photocatalytic activities (Shi et al., 2011; Sun et al., 2008). However, wide band-gap semiconductor is disadvantageous to the absorption and use of the visible range of solar energy. To use visible light and improve the performance of photoactive materials, quantum dots (QDs), types of narrow-bandgap semiconductor nanocrystals, have been introduced as photosensitizers (Zheng et al., 2011; Wang et al., 2012) due to their broad excitation spectrum, good photostability, tunable emission spectrum, and excellent PEC properties (Cordes et al.,2006; Díaz et al., 2013). It has been reported that combining TiO_2 with QDs could greatly improve the photocurrent intensity and photostability due to the enhanced charge separation (Sant and Kamat, 2002; Nasr et al., 1998).

DNA, a biological macromolecule, can specifically bind many metal ions and organic compounds (Yaney et al., 2005). During the last decade, great progress has been achieved in developing various DNA biosensors. To fabricate DNA-based biosensors, DNA molecules are usually immobilized on various substrates, including nanoparticles which have large surface area and controllable surface properties (Tatarinova et al., 2012). The sensors have been widely applied to the detection of target DNA (Ho et al., 2008), enzymes (Fredriksson et al., 2002), ions (Zhang and Guo, 2012) and small organic molecules (Ezzati et al., 2010). The DNA biosensor also provides a useful tool to study the toxicological action of priority pollutants based on the interaction between pollutants and DNA (Wang et al., 1997).

As a phenol derivative, *o*-aminophenol (OAP) has been primarily used in various fields such as medicine, dye, petroleum, and pigment (Duan et al., 2013). However, OAP is very harmful to human body due to its easy penetration through the skin and membrane of living body. Therefore, it is important to monitor OAP in industrial wastewater and environmental waters (Xu et al., 2005). Various instrumental methods such as chromatography (Kumar and Panwar, 1993), electrophoresis (Pumera et al., 2001), and spectrophotometry (López-Cueto et al., 1996) have been established to determine OAP.

In the present work, we fabricated a highly sensitive PEC biosensor for detecting OAP in water based on a DNA–CdSe/TiO_2 electrode. In this sensor, DNA and CdSe QDs were assembled on TiO_2 film

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through the electrostatic attraction between positively charged poly (diallyldimethylammonium chloride) (PDDA) and negatively charged DNA and CdSe. Considering that herring sperm DNA has been widely used in electrochemical (Chiti et al., 2001), spectroscopic (Li et al., 2011) and ECL (Hu et al., 2009) sensors in virtue of its high affinity to some organic compounds, we selected this kind of DNA as the biological recognition element to construct the PEC sensor. Due to the interaction between DNA and OAP, OAP molecules were effectively adsorbed on the surface of the DNA–CdSe/TiO_2 electrode, and quickly oxidized by the photogenerated holes from CdSe induced under visible light irradiation, resulting in increased photocurrent. Thus, the DNA-CdSe/TiO_2 electrode was demonstrated as the visible light PEC sensor for OAP.

2. Experimental

2.1. Chemicals

Herring sperm DNA was provided by Sigma. Poly(diallyldimethylammonium chloride) (PDDA) and $(NH_4)_2TiF_6$ were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). $Cd(ClO_4)_2 \cdot 6H_2O$ was purchased from Alfa Aesar Chemical Co. Ltd. (Tianjin, China). Other reagents of analytical grade were provided by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Doubly distilled water was used throughout the investigation.

2.2. Fabrication of PEC biosensor

The TiO_2 film was deposited on FTO conducting glass (Dalian Heptachroma SolarTech Co. Ltd., China) using the liquid phase deposition (LPD) process (Ding et al., 2010) with a little modification. Before deposition, FTO substrate was cut into 1.5×1.5 cm^2 pieces and then sonicated in acetone, ethanol and water, respectively for 10 min. After being dried with a stream of high purity nitrogen gas, the substrate was soaked vertically into mixed aqueous solution of 0.025 M $(NH_4)_2TiF_6$ and 0.05 M H_3BO_3 at 55 °C for 10 h to allow the formation of TiO_2 film on the substrate surface. The as-deposited film was thoroughly rinsed by water and dried with nitrogen gas. Finally the film was calcined at 400 °C for 2 h in a muffle oven.

Water soluble CdSe QDs were synthesized by a hydrothermal method (Zhang et al., 2011). Briefly, NaHSe aqueous solution was prepared by dissolving Se powder in $NaBH_4$ solution under nitrogen atmosphere at 35 °C for 30 min. Then, 0.1 mL mercaptoacetic acid was added into 200 mL of 1.25 mmol L^{-1} $Cd(ClO_4)_2$ solution, followed by adjusting the solution pH to 11 with 2.0 mol L^{-1} NaOH. The obtained mixture was transferred into a round-bottomed flask and refluxed under constant passage of high purity nitrogen gas for 30 min, and then 5 mL freshly prepared oxygen-free NaHSe (0.05 M) was slowly injected into this mixture. After 4 h reaction, the product was collected by centrifugation, washed several times with ethanol, and dried at 60 °C.

To fabricate the PEC biosensor, the TiO_2 film electrode (exposed geometric area of ca. 0.071 cm^2) was firstly treated with 10 μL of 2% PDDA solution containing 0.5 mol L^{-1} NaCl to make the electrode surface positively charged. After thoroughly rinsed with deionized water to remove the loosely adsorbed PDDA and dried under infrared lamp, the electrode surface was coated with 5 μL of CdSe and DNA mixed solution (containing 2 mg mL^{-1} CdSe and 1.5 mg mL^{-1} DNA).

2.3. Apparatus and procedure

A TecnaiG220 transmission electron microscope (TEM) (FEI, Netherlands) and a Quanta 200 field emission scanning electron microscope (FESEM) (FEI, Netherlands) were employed for the morphology characterization. X-ray diffraction (XRD) patterns were measured using a Bruker D8 Advance X-ray diffractometer (Bruker Instruments, Darmstadt, Germany) with Cu Kα radiation. The accelerating voltage and applied current were 40 kV and 40 mA, respectively. UV–visible diffuse reflectance spectra (DRS) were measured with a UV-2550 spectrophotometer (Shimadzu, Japan). All PEC experiments were carried out on a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China) in a conventional three-electrode system. A DNA–CdSe/TiO_2 film electrode, a platinum wire, and a saturated calomel electrode (SCE) were employed as the working, auxiliary and reference electrodes, respectively. A PLS-SXE300 xenon lamp (Perfect Co., China) with an optical filter ($\lambda > 420$ nm) was used as the irradiation source and the distance between the light source and electrode surface was 10 cm.

3. Results and discussion

3.1. Characterization of film electrodes

The morphology of the fabricated PEC biosensor was observed by SEM. The LPD TiO_2 film on FTO substrate shows a rough film structure (Fig. 1A), which is beneficial to the electron transfer from CdSe to TiO_2 (Zhao et al., 2011). After CdSe (Fig. S1) or DNA–CdSe (Fig. 1B) is assembled on the TiO_2 film, many small CdSe particles are homogeneously dispersed on the film surface. By TEM characterization, the size of CdSe is estimated to be 8 nm (Fig. S2).

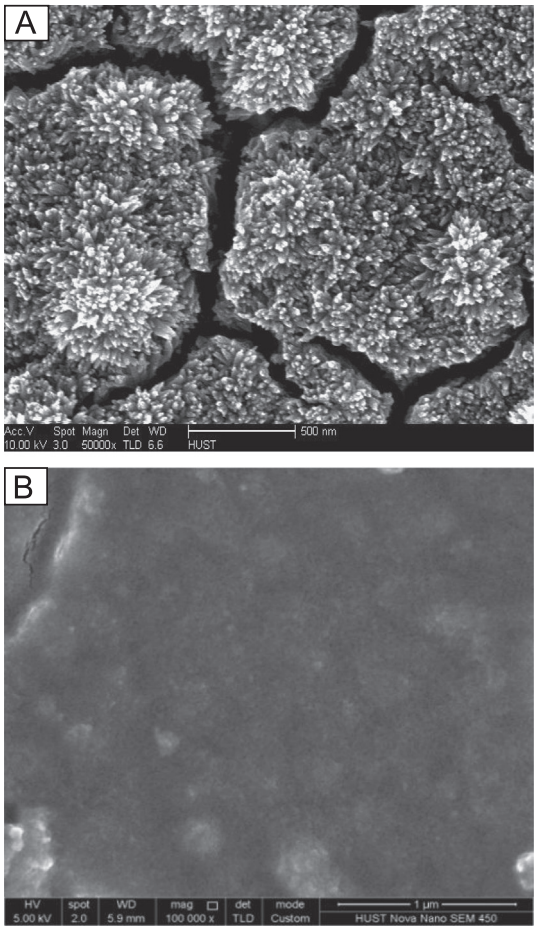

Fig. 1. SEM images of (A) TiO_2 and (B) DNA–CdSe/TiO_2 film electrodes.

At the same time, the crystalline nature of the film was investigated by XRD. As shown in Fig. 2A, the main diffraction peaks assigned to anatase phase of TiO_2 (JCPDS 21-1272) and zinc blende structure of CdSe (JCPDS 19-0191) are observed besides the peaks of FTO substrate.

On the other hand, the absorption behavior of various film-coated electrodes was studied by UV–visible DRS (Fig. 2B). It can be seen that TiO_2 film mainly absorbs UV light at a wavelength less than 400 nm but has weak absorption to visible light (curve a in Fig. 2B). While CdSe QDs are assembled on the surface of TiO_2 film, the absorption in the visible region is obviously improved (curve b in Fig. 2B). When the mixture of DNA and CdSe is coated on the film, the absorption of the electrode in visible region is further increased (curve c in Fig. 2B). This result implies that the presence of DNA in CdSe-photosensitized TiO_2 film is advantageous to fabricate the visible light-activated PEC sensor. Although DNA does not absorb visible light, it can act as an excellent dispersant to enhance the dispersibility of nanomaterials in aqueous solution (Premkumara and Geckeler, 2012). Moreover, negatively charged DNA can improve the adsorption of CdSe on positively charged electrode surface (Ma et al., 2009), which is favorable to enhance the absorption of electrode to visible light.

To evaluate the interfacial properties of the film electrodes, the electrochemical impedance spectroscopy (EIS) of various electrodes were investigated in 0.1 M KCl using $[Fe(CN)_6]^{3-/4-}$ redox probe (Fig. S3). The semicircle diameter in the Nyquist plot reflects the electron-transfer resistance (R_{et}) of $[Fe(CN)_6]^{3-/4-}$ on the electrodes. The result shows that the electron transfer of $[Fe(CN)_6]^{3-/4-}$ on FTO substrate is blocked after TiO_2 is deposited. While CdSe or DNA–CdSe is assembled on TiO_2 film, the electron transfer rate of $[Fe(CN)_6]^{3-/4-}$ is further decreased. Moreover, the R_{et} value for DNA–CdSe/TiO_2 is higher than CdSe/TiO_2, confirming that DNA molecules are effectively immobilized on the electrode surface.

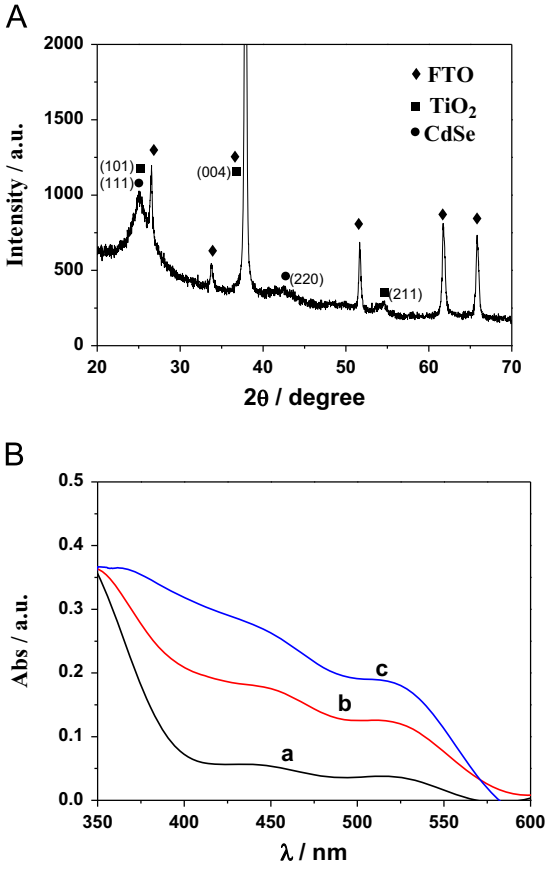

Fig. 2. (A) XRD pattern of DNA–CdSe/TiO_2 film electrode. (B) UV–visible DRS of TiO_2 film (a), CdSe/TiO_2 (b), and DNA–CdSe/TiO_2 (c) film electrodes.

3.2. PEC response of DNA–CdSe/TiO_2 film electrode

To study the PEC performance of the sensor, the photocurrents of various film-coated electrodes were recorded in 0.1 M Na_2SO_4 under visible light illumination by applying an anodic bias potential of +0.3 V. As can be seen, all the film-coated electrodes respond sensitively to the visible light irradiation. However, the TiO_2 film shows a very low photocurrent response (curve a in Fig. 3A), consistent with the weak absorption of TiO_2 to the visible light as illustrated in Fig. 2B. While the TiO_2 film is coated with CdSe, the photocurrent is increased by 10 times (curve c in Fig. 3A), due to the interparticle electron transfer between CdSe and TiO_2 semiconductor nanocluster system (Sant and Kamat, 2002). In comparison, the photocurrent of CdSe/TiO_2 film electrode is decreased by 30% when DNA and CdSe are co-assembled on the TiO_2 film (curve b in Fig. 3A). The PEC property of photoactive film electrode usually depends on its optical absorption and electrical conductivity. Since the presence of DNA is advantageous to increase the absorption of the film to visible light (Fig. 2B) but decreases the electrical conductivity (Fig. S3), the reduced photocurrent is likely attributed to the blockage of the electron transfer between CdSe and TiO_2 by nonconductive DNA biomolecules.

While 5 μmol L^{-1} OAP is present in the electrolyte, the photocurrent responses on both CdSe/TiO_2 (curve d in Fig. 3A) and DNA–CdSe/TiO_2 (curve e in Fig. 3A) film electrodes are increased. In PEC

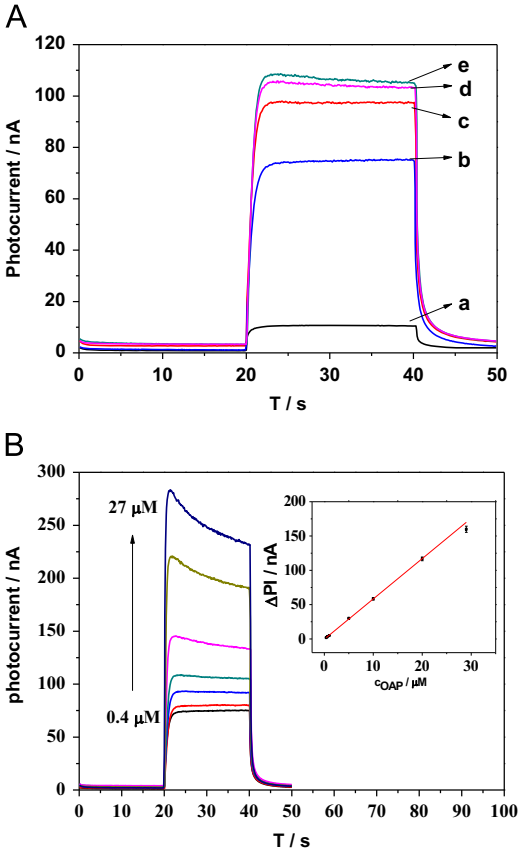

Fig. 3. (A) Photocurrent responses of TiO_2 electrode (a), CdSe/TiO_2 electrode (c, d) and DNA–CdSe/TiO_2 electrode (b, e) in 0.1 mol L^{-1} Na_2SO_4 with (d, e) and without (c, b) 5 μmol L^{-1} OAP at a bias potential of +0.3 V (vs. SCE). (B) Photocurrent responses of DNA–CdSe/TiO_2 electrode in 0.1 mol L^{-1} Na_2SO_4 in the presence of 0.4, 1, 3, 5, 10, 20, 27 μmol L^{-1} OAP at a bias potential of 0.3 V (vs. SCE). Inset: calibration curve for OAP on PEC sensor. Error bars were derived from the standard deviation of three measurements.

measurement, the applied anodic bias potential drives the photogenerated electrons to the counter electrode through an external electric circuit. It is believed that adsorbed OAP molecules on photoanode are quickly oxidized by the photogenerated holes from CdSe QDs under visible light irradiation while oxygen may be reduced at the counter electrode by photoelectrons (Kim and Anderson, 1994). Thus, the recombination of photogenerated electrons and holes is reduced, resulting in enhanced photocurrent. Interestingly, the promotion of photocurrent by OAP on DNA–CdSe/TiO_2 electrode is more significant than that on CdSe/TiO_2 electrode. This can be attributed to the fact that more OAP molecules are adsorbed on the surface of DNA–CdSe/TiO_2 due to the interaction of DNA with OAP. Thus, the DNA–CdSe/TiO_2 film electrode is utilized as the visible light PEC sensor for OAP.

3.3. Optimizing response of sensor to OAP

The influences of DNA concentration and applied potential on the PEC response of DNA–CdSe/TiO_2 electrode to OAP were investigated. The PEC response of OAP on the sensor was evaluated by the photocurrent difference (ΔPI) before and after adding OAP. As shown in Fig. S4, the PEC response of OAP is low in the absence of DNA. While 0.5 mg mL^{-1} DNA is immobilized on the electrode, the response of OAP is slightly reduced. When the concentration of DNA is increased from 0.5 mg mL^{-1} to 1.5 mg mL^{-1}, the response to OAP is markedly increased, implying that abundant DNA could adsorb more OAP onto the electrode surface. However, when the DNA concentration exceeds 1.5 mg mL^{-1}, the response decreases because superfluous DNA on the electrode blocks the electron transfer between CdSe and TiO_2 substrate. On the other hand, the applied potential also plays an important role in PEC sensing because it affects the recombination probability of the photogenerated electrons/holes (Zhang et al., 2004; Vinodgopal et al., 1996). The photocurrent response of OAP increases with increasing the bias potential from 0 to 0.3 V (Fig. S5), indicating that photogenerated electrons on electrode are effectively driven to the counter electrode by the applied anodic bias potential, which is beneficial to electron–hole separation (Ojani et al., 2012; Wen et al., 2010). Then the response becomes saturated as the potential is further increased to be higher than 0.3 V. Thus, 1.5 mg mL^{-1} DNA and +0.3 V applied potential were chosen as the optimum conditions for the PEC sensor.

3.4. Determination of OAP

The developed DNA–CdSe/TiO_2 electrode was applied to the quantitative determination of OAP. Fig. 3B illustrates the photocurrent response of sensor to OAP at different concentrations. The PEC response is found to be proportional to OAP in the concentration range from 0.4 to 27 μmol L^{-1}. The detection limit (3S/N) is estimated be 0.08 μmol L^{-1}. The linear regression equation is expressed as $\Delta PI/nA = 5.607\ C/\mu M + 0.689$ (correlation coefficient $r = 0.998$).

In order to evaluate the selectivity of the PEC biosensor, interference studies were carried out in 5 μM OAP under optimum conditions by adding two-fold concentration of some organic compounds such as *m*-aminophenol, *p*-aminophenol, *o*-chlorophenol, *p*-nitroaniline, *o*-nitrophenol, *p*-nitrophenol (Fig. S6). It was observed that *o*-chlorophenol, *p*-chlorophenol, *p*-nitroaniline did not interfere in the determination of OAP. However, the isomers of nitrophenol and aminophenol influenced the response of OAP because of their similar structure to OAP and direct oxidization by the photogenerated holes from CdSe.

4. Conclusions

In this work, we have developed a visible light PEC biosensor for OAP based on CdSe QDs, DNA and TiO_2. The as-prepared DNA-CdSe/TiO_2 electrode possesses high visible light PEC activity of CdSe QDs and excellent bioactivity of DNA molecule. The utilization of visible light could avoid the damage of UV light resource to DNA while the immobilized DNA could effectively improve the adsorption of OAP on the electrode surface. Thus, a highly sensitive DNA-based PEC biosensor for OAP determination was constructed based on the DNA–CdSe/TiO_2 electrode. Since DNA has a good affinity with many genotoxic organic pollutants, this DNA-based PEC biosensor will be useful for environmental monitoring.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.09.073.

References

Chiti, G., Marrazza, G., Mascini, M., 2001. Anal. Chim. Acta 427, 155–164.
Cordes, D.B., Gamsey, S., Singaram, B., 2006. Angew. Chem. Int. Ed. 45, 3829–3832.
Díaz, S.A., Giordano, L., Azcaárate, J.C., Jovin, T.M., Jares-Erijman, E.A., 2013. J. Am. Chem. Soc. 135, 3208–3217.
Ding, Y.B., Yang, C.Z., Zhu, L.H., Zhang, J.D., 2010. J. Hazard. Mater. 175, 96–103.
Duan, S., Zhang, X., Xu, S., Zhou, C.L., 2013. Electrochim. Acta 88, 885–891.
Ezzati, J., Dolatabadia, N., Kashanian, S., 2010. Food Res. Int. 43, 1223–1230.
Fredriksson, S., Gullberg, M., Jarvius, J., Olsson, C., Pietras, K., Gústafsdóttir, S.M., Landegren, U., 2002. Nat. Biotechnol. 20, 473–477.
Ho, H.A., Najari, A., Leclerc, M., 2008. Acc. Chem. Res. 41, 168–178.
Hu, L.Z., Bian, Z., Li, H.J., Han, S., Yuan, Y.L., Gao, L.X., Xu, G.B., 2009. Anal. Chem. 81, 9807–9811.
Kim, D.H., Anderson, M.A., 1994. Environ. Sci. Technol. 28, 479–483.
Kumar, A., Panwar, A., 1993. Microchim. Acta 111, 177–182.
Li, H.B., Li, J., Xua, Q., Yang, Z.J., Hu, X.Y., 2013. Anal. Chim. Acta 766, 47–52.
Li, P., Liu, S., Yan, S., Fan, X., He, Y., 2011. Colloids Surf. A: Physicochem. Eng. Aspects 392, 7–15.
Liang, M.M., Jia, S.P., Zhu, S.C., Guo, L.H., 2008. Environ. Sci. Technol. 42, 635–639.
López-Cueto, G., Maspoch, S., Rodríguez-Medina, J.F., Ubide, C., 1996. Analyst 121, 407–412.
Ma, L.P., Yuan, R., Chai, Y.Q., Chen, S.H., 2009. J. Mol. Catal. B: Enzymatic 56, 215–220.
Nasr, C., Kamat, P.V., Hotchandani, S., 1998. J. Phys. Chem. B 102, 10047–10056.
Ojani, R., Raoof, J.B., Zarei, E., 2012. Talanta 99, 277–282.
Premkumara, T., Geckeler, K.E., 2012. Prog. Polym. Sci. 37, 515–529.
Pumera, M., Wang, J., Grushka, E., Polsky, R., 2001. Anal. Chem. 73, 5625–5628.
Sant, P.A., Kamat, P.V., 2002. Phys. Chem. Chem. Phys. 4, 198–203.
Shi, H.J., Zhao, G.H., Liu, M.C., Zhu, Z.L., 2011. Electrochem. Commun. 13, 1404–1407.
Sun, W.T., Yu, Y., Pan, H.Y., Gao, X.F., Chen, Q., Peng, L.M., 2008. J. Am. Chem. Soc. 130, 1124–1125.
Tatarinova, O.N., Smirnov, I.P., Safenkova, I.V., Varizhuk, A.M., Pozmogova, G.E., 2012. J. Nanopart. Res. 14, 1–8.
Vinodgopal, K., Bedja, I., Kamat, P.V., 1996. Chem. Mater. 8, 2180–2187.
Wang, J., Rivas, G., Cai, X., Palecek, E., Nielsen, P., Shiraishi, H., Flair, M.N., 1997. Anal. Chim. Acta 347, 1–8.
Wang, W.J., Bao, L., Lei, J.P., Tu, W.W., Ju, H.X., 2012. Anal. Chim. Acta 744, 33–38.
Wen, D., Guo, S.J., Wang, Y.Z., Dong, S.J., 2010. Langmuir 26, 11401–11406.
Xu, H., Duan, C.F., Zhang, Z.F., Chen, J.Y., Lai, C.Z., Lian, M., Liu, L.J., Cui, H., 2005. Water Res. 39, 396–402.
Yaney, P.P., Heckman, E.M., Diggs, D.E., Hopkins, F.K., Grote, J.G., 2005. Org. Photonic Mater. Devices VII 5724, 224–233.
Zhang, B.T., Guo, L.H., 2012. Biosensors Bioelectron. 37, 112–115.
Zhang, S.Q., Zhao, H.J., Jiang, D.L., John, R., 2004. Anal. Chim. Acta 514, 89–97.
Zhang, X.R., Guo, Y.S., Liu, M.S., Zhang, S.S., 2013. RSC Adv. 3, 2846–2857.
Zhang, X.R., Li, S.G., Jin, X., Li, X.M., 2011. Biosensors Bioelectron. 26, 3674–3678.
Zhao, W.W., Yu, P.P., Xu, J.J., Chen, H.Y., 2011. Electrochem. Commun. 13, 495–497.
Zheng, M., Cui, Y., Li, X.Y., Liu, S.Q., Tang, Z.Y., 2011. J. Electroanal. Chem. 656, 167–173.