Electrochemistry Communications 40 (2014) 84–87

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Short communication

Electrochemical detection of DNA damage through visible-light-induced ROS using mesoporous TiO_2 microbeads

Roghayeh Imani [a,b], Aleš Iglič [b], Anthony P.F. Turner [a], Ashutosh Tiwari [a,*]

[a] Biosensors and Bioelectronics Centre, Institute of Physics, Chemistry and Biology (IFM), Linköping University, S-58183 Linköping, Sweden
[b] Biophysics Laboratory, Faculty of Electrical Engineering, University of Ljubljana, SI-1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 18 December 2013
Accepted 19 December 2013
Available online 28 December 2013

Keywords:
DNA damage
TiO_2 microbeads
Reactive oxygen species
Electrochemical detection

ABSTRACT

Rapid detection of DNA damage could serve as a basis for genotoxicity studies of new bio-nanoconjugations. A novel TiO_2 bio-nanoconjugation, consisting of mesoporous TiO_2 microbeads, dopamine (DA) and ss-DNA, was constructed on fluorine-doped tin oxide-coated glass (FTO) and used for the detection of DNA damage in the photocatalytic reaction of TiO_2 under visible light. Stable mesoporous TiO_2 microbead films were coated on FTO by the doctor-blade method; dopamine with oxygen containing ligands, was tightly coupled to the titanium surface prepared under phase coordination. Specific single-strands of DNA were electronically linked to TiO_2 by using a dopamine bridge. DNA damage, caused by reactive oxygen species (ROS) that were photogenerated through the photocatalytic reaction, was detected with square wave voltammetry (SWV) by recording the catalytic oxidation current of $[Ru(NH_3)_6]^{3+}$, an intercalated electroactive probe. The ability of antioxidant to protect DNA against damage in the photocatalytic reaction was also tested.

1. Introduction

The detection of DNA oxidation damage by oxidative stress and evaluation of the protective effects of antioxidant against such kind of damage is an important requirement [1]. Various methods have been developed for DNA oxidation damage detection, but there is considerable interest in electrochemical sensors [2]. One key feature for the design of such sensors, is the efficient attachment of DNA onto the electrode surface through a specific linkage [3]. TiO_2-oligonucleotide nanoconjugates were first described nearly a decade ago and have since been extended to a broad range of applications for TiO_2-DNA bio-nanoconjugates [4-6]. One drawback of such bio-nanoconjugations on TiO_2, however, is the damage to the biomolecules that occurs because of photocatalytic reactions.

Our research is aimed towards developing an ultra-sensitive detection of reactive oxygen species (ROS) mediated DNA oxidation damage that can be applied to screen the genotoxicity of new bioconjugations and monitor oxidative damage using antioxidant. Dopamine (DA) was used for the construction of the bio-TiO_2 conjugation. It also helped in the harvesting of visible light in the complex. Dopamine, due to its two −OH groups in the ortho position makes a strong bidentate complex with coordinatively unsaturated titanium at the surface of microbeads that results in irreversible binding of dopamine molecules to the electrode. The amine group of dopamine was linked covalently to a specific single-strand of DNA having a carboxyl group at the 5′-end [7]. When DNA or proteins were covalently bound to dopamine, it was found that dopamine acts as a bridge between TiO_2 nanocrystallites and biomolecules. Hence, in the current study, we fabricated a novel bioconjugated electrode (DNA/DA/TiO_2/FTO) based on mesoporous TiO_2 microbeads for the electrochemical detection of DNA oxidation damage by visible light-mediated ROS in a photocatalytic reaction. It was also observed that the antioxidant protects against DNA damage in the photocatalytic reaction.

2. Experimental

2.1. Materials and instrumentation

Dopamine hydrochloride (DA, >99%), ascorbic acid (AA, >99%), hexaammineruthenium(III) chloride (>98%), potassium ferrocyanide (≥98.5%) and potassium ferricyanide (≥98.5%) were purchased from Sigma-Aldrich, USA. ss-DNA was procured from Eurofins MWG Operon, Germany with 5′-terminal carboxyl group (HPLC grade, 50 mer, 5′-GGGCCTGGTCTACCAAGCAAACTCCAGTACAGCCAGGGAACATGAGAG GG-3′) and stored in a 1.0 μM stock solution with phosphate buffer at pH 7.4.

The particle morphology was examined with a Hitachi S4700 field-emission scanning electron microscope (SEM, Hitachi, Japan). The crystal structure properties of the mesoporous TiO_2 microbeads were obtained from hard X-ray low-angle one reflectivity measurements, using a Philips PW1710 powder diffractometer (Philips, The Netherlands). The electrochemical response was measured in a conventional three-electrode system using bare or modified fluorine-doped tin oxide coated glass (FTO, TEC15, Hartford Glass) electrodes as the working electrode, a platinum wire as the counter electrode, and a Ag/AgCl(3 M KCl) electrode

* Corresponding author. Tel.: +46 1328 2395; fax: +46 1313 7568.
E-mail address: ashutosh.tiwari@liu.se (A. Tiwari).

1388-2481/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.elecom.2013.12.027

as the reference electrode. The electrochemical signals were recorded in a phosphate buffer solution at pH 7.4 using an IviumStat (Ivium, The Netherlands). All potentials cited in the text are referred to the reference potential.

2.2. Preparation of the modified electrode and photooxidative damage of the ss-DNA

A mesoporous TiO_2 microbead paste was produced according to a previously reported procedure [9]. Mesoporous TiO_2 microbead paste was coated by the doctor-blade method on the FTO electrodes with a 5 mm × 5 mm active area [10]. After drying in air, the electrodes were sintered at 500 °C for 30 min. The thickness of the fabricated mesoporous TiO_2 microbead film was measured using a Diktak profilometer (VEECO/SLOAN DEKTAK 3, New York, US) and found to be at 4 μm, following the first step; this electrode was denoted as TiO_2/FTO. The TiO_2 modified electrode was rinsed carefully in deionised water and then dipped in a freshly prepared 10 mM dopamine aqueous solution. The dopamine covered electrode was then rinsed carefully several times with deionised water to remove excess dopamine. At this step, the electrode was denoted as DA/TiO_2/FTO. A condensation reaction through intermediate *N*-hydroxy-succinimide ester was used to bind the carboxyl group of the oligonucleotide to the amino group of dopamine by an amide bond. In the final step, the DA/TiO_2/FTO modified electrode was immersed in a ss-DNA solution (10 μM in pH 7.4 phosphate buffer) overnight at 4 °C for DNA adsorption. The electrodes were then washed with water, dried at room temperature, and were then ready for use. Following the last step, the electrode was denoted as DNA/DA/TiO_2/FTO (Fig. 1a).

2.3. DNA oxidation damage and ascorbic acid activity measurements

To study the photocatalytic reaction, the DNA/DA/TiO_2/FTO electrode was immersed in deionised water and illuminated at 420 nm. The light was 5 cm above the modified electrode. After illumination the electrode was washed with deionised water and immersed in 10 mM $[Ru(NH_3)_6]^{3+}$ (in phosphate buffer at pH 7.4). DNA oxidation damage was evaluated using square wave voltammetry (SWV) measurement of the $[Ru(NH_3)_6]^{3+}$ oxidation current and compared with the electrochemical signal recorded with the nonirradiated DNA/DA/TiO_2/FTO-modified electrodes. The effect of AA (as an antioxidant) was investigated on DNA protection in the photocatalytic reaction by adding various concentrations of AA to the above described set up.

3. Results and discussion

3.1. SEM and XRD of mesoporous TiO_2 microbeads

Fig. 1b (i) shows scanning electron microscopy (SEM) images of mesoporous TiO_2 microbeads prepared by the solvothermal method. Monodisperse TiO_2 microbeads with a diameter of 600 ± 50 nm have rough surfaces. As illustrated by the high magnification SEM image, pores could be observed over the surface of the beads and these TiO_2 beads contained ~14 nm sized nanocrystals. Fig. 1b (ii) illustrates the

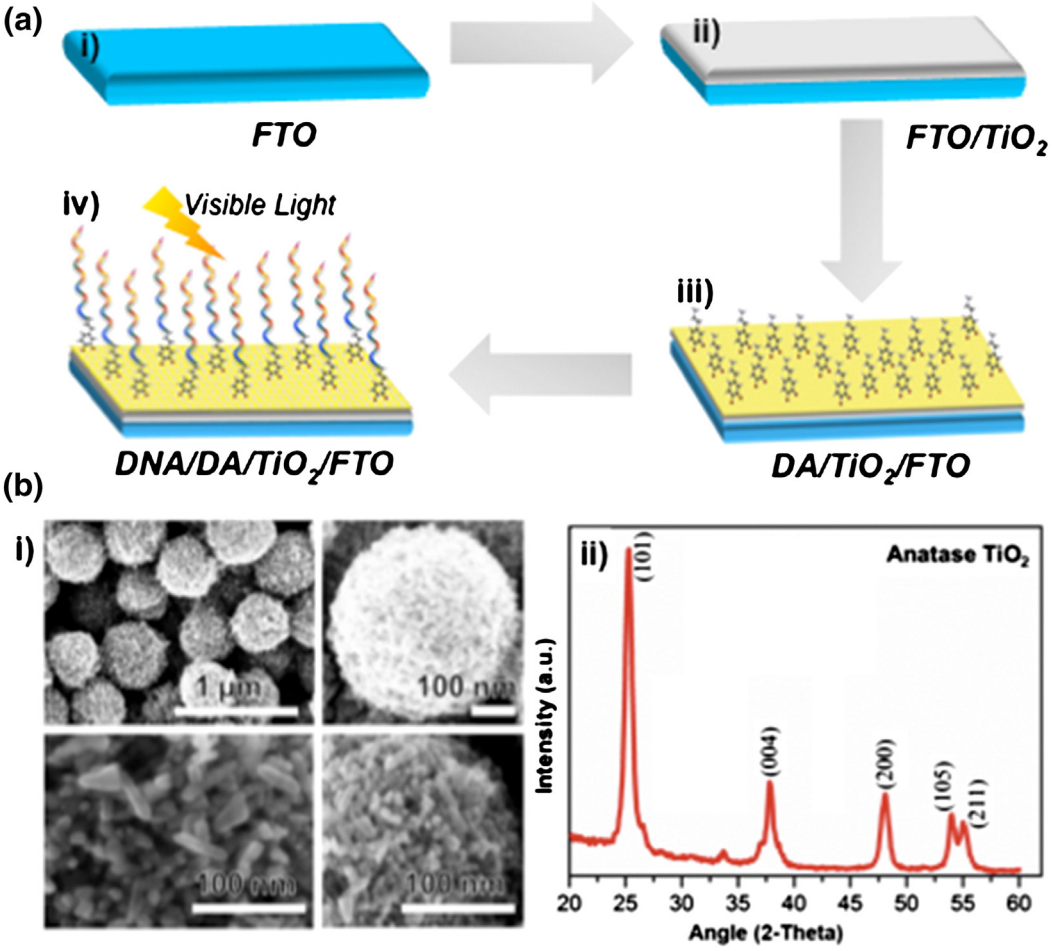

Fig. 1. (a) Overall steps (i–iv) involved in the fabrication of DNA/DA/TiO_2/FTO electrode and (b) SEM images (i) and anatase X-ray diffraction patterns of mesoporous TiO_2 microbeads after solvothermal treatment (ii).

XRD pattern of the mesoporous TiO_2 microbeads. All the peaks observed at $2\theta = 25.3$, 37.9, 48.1, 54 and 55.2 in the XRD pattern are consistent with anatase (101), (004), (200), (105), and (211) spacing. This confirmed that TiO_2 microbeads consisted of a crystalline anatase structure (JCPDS number: 01-073-1764). The crystallite size was calculated by the Debye–Scherrer formula as 14 nm.

3.2. Electrochemical characterisation of the modified electrode

Fig. 2a shows the CVs of the electrode achieved after each modification stage. A symmetric reversible voltammogram with peak-to-peak separation of ΔE_p ~0.32 V was obtained with a scan rate of 50 mV/s at the bare FTO electrode interface. For the TiO_2/FTO-modified electrode, peak currents were decreased and the peak-to-peak separation was larger than that for FTO. Electrostatic repulsion between $Fe(CN)_6^{3-/4-}$ and the highly negatively charged mesoporous TiO_2 microbead surface can explain the lower current of TiO_2 compared to that obtained with FTO. This is consistent with the electrochemical response of $Fe(CN)_6^{3-/4-}$ to the mesoporous TiO_2 microbead film and the bare FTO electrode. For the DA/TiO_2/FTO-modified electrode, the redox current was decreased due to the dopamine layer on the electrode surface. For this step, the narrower peak-to-peak separation could be due to the electrostatic attraction between $Fe(CN)_6^{3-/4-}$ and the positively charged dopamine. In the final step, after covalent bonding of DNA to the amine group of dopamine (via the carboxyl group at the 5′-end of DNA) on the surface of the modified electrode, a substantial decrease in the redox peak current and a major widening of the peak-to-peak separation (ΔE_p) were observed, which confirmed that ss-DNA had been successfully bound to the modified electrode. In this case, there is a strong electrostatic repulsion between $Fe(CN)_6^{3-/4-}$ and the

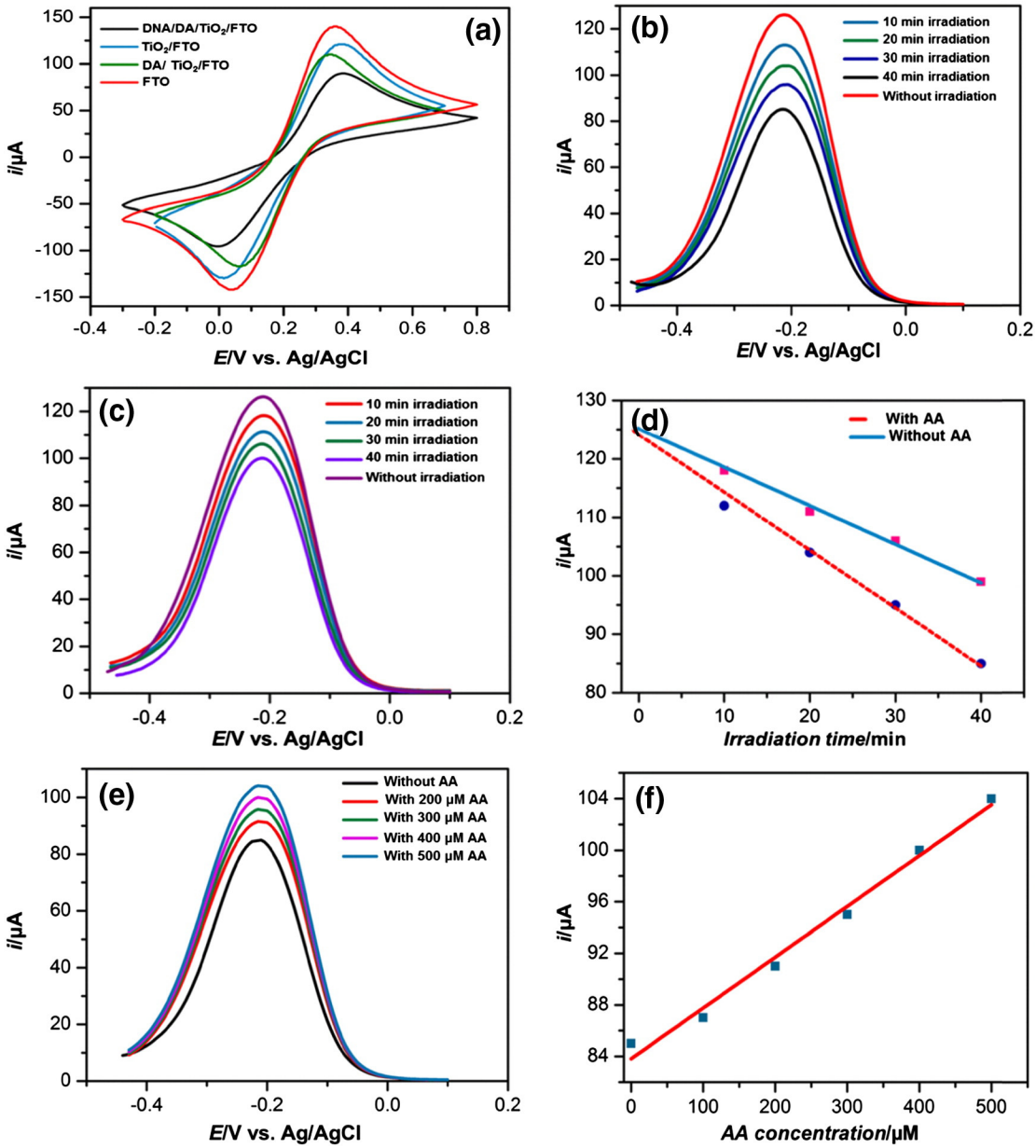

Fig. 2. (a) Cyclic voltammograms of modified electrodes recorded after each modification step in the phosphate buffer at pH 7.4 containing 1 mM $Fe(CN)_6^{3-/4-}$. Square-wave voltammetry of a DNA/DA/TiO_2/FTO-modified electrode in phosphate buffer (pH 7.4) containing 1 mM $[Ru(NH_3)_6]^{3+}$; (b) in the absence of AA; (c) in the presence of 200 μM AA after the photocatalytic reaction with different irradiation times; (d) comparison of the relationship between oxidation current and irradiation time of the photocatalytic reaction in the absence of AA and in the presence of 200 μM AA; (e) different concentrations of AA (irradiation time was 40 min); and (f) relationship between oxidation current and AA concentration.

highly negatively charged phosphate backbone of the insulating DNA probe [10]. Overall, the results generated from the CV measurements correlated well with each step of the electrode modifications.

3.3. DNA oxidation damage detection and the effect of antioxidant for DNA protection

A TiO_2/DA complex enables absorption of visible light and, additionally, the DA acts as a bridge to covalently bond DNA to TiO_2. The TiO_2/DA complex has an absorption with a shoulder of around 420 nm. Dopamine absorbs the incident photons, resulting in molecule-to-surface charge transfer. In the TiO_2/DA complex, there is a direct catechol-to-TiO_2 charge transfer (i.e., the electron is directly photoinjected from catechol into the conduction band of TiO_2 without the participation of excited states in dopamine). Dopamine introduces an occupied π-level at the lower end of the TiO_2 band gap; this π-level in the gap gives rise to a direct charge-transfer excitation from the π-level to the bottom of the conduction band, dominated by an excitation to a level with a significant contribution from a Ti(3d) atomic orbital close to the adsorbate. Localisation of hole on dopamine and electron in TiO_2 is well established [7], as $TiO_2/DA + h\nu \rightarrow e^- [(Ti^{3+})]/DA^+$.

In the photocatalytic tests under visible light, the same radicals produced by the UV light were also observed. Moreover, with the bare TiO_2, because of the electron–hole recombination processes, the amount of generated ROS during the photocatalytic experiment was lower than that with TiO_2/DA. TiO_2/DA promotes the spatial separation of photogenerated charges in TiO_2/DA, in which holes are placed on DA and electrons are injected to TiO_2 and charge recombination is suppressed [8].

The proposed DNA oxidation damage detection was tested with $[Ru(NH_3)_6]^{3+}$ as the electrochemical probe. Placing the DNA-modified electrode in an aqueous solution of a redox cation such as ruthenium hexaammine $[Ru(NH_3)_6]^{3+}$ leads to an ion exchange equilibrium between $[Ru(NH_3)_6]^{3+}$ and the native charge compensation ions (presumably Na^+) associated with the anionic DNA backbone. Upon the application of a negative potential to the hexaammineruthenium redox couple formal potential in a solution containing $[Ru(NH_3)_6]^{3+}$, $[Ru(NH_3)_6]^{2+}$ is electrogenerated on the electrode and diffuses to react with the adsorbed ss-DNA. The surface densities of single stranded oligonucleotides can be determined by the integration of the current for the reduction of $[Ru(NH_3)_6]^{3+}$ to $[Ru(NH_3)_6]^{2+}$ [11].

The oxidation peak decreased dramatically with increasing irradiation time (Fig. 2b), which is consistent with previous findings that DNA can be seriously damaged by ROS generated in photocatalytic reactions [12]. The $[Ru(NH_3)_6]^{3+}$ oxidation current for different illumination times was observed at −0.21 V. The peak current is directly proportional to the remaining ss-DNA present on the electrode and can be considered as a direct measurement of the ss-DNA damage; the stronger the damage, the smaller the peak current. The ability of AA to protect ss-DNA from oxidation at different time intervals was observed and compared to the DNA oxidation damage effect during the photocatalytic reaction (Fig. 2c). Fig. 2d shows the oxidation current changes as a function of the irradiation time for two different photocatalytic reactions, with and without AA, respectively. The results clearly show that the oxidation current recorded after the photocatalytic reaction in the presence of AA, decreased more slowly than the oxidation current recorded in the absence of AA. TiO_2/DA nanostructures are photoactive and under visible illumination they produce ROS, which damaged the DNA. In the absence of AA, the lowest DNA oxidation damage was determined to be 11% for 10 min of irradiation and the highest oxidation damage was observed to be about 33% for 40 min of irradiation. However, the lowest DNA oxidation damage was observed at 200 μM AA; i.e., only 7% for 10 min of irradiation and also the highest oxidation damage was measured about 22% for 40 min of irradiation. In the presence of antioxidant, the ROS were quenched by antioxidant prior to reaching the DNA backbone and higher current signals were observed. We repeated the photocatalytic experiment for different concentrations of AA (irradiation time was fixed at 40 min, Fig. 2e). Fig. 2f shows the electrochemical signals recorded for different concentrations of AA. The results show that with increasing concentration of antioxidant, there was less DNA oxidation damage and a greater oxidation current. In the absence of AA, DNA oxidation damage was at 33% while in the presence of 500 μM AA, DNA oxidation damage was decreased to 17%.

4. Conclusion

A new strategy for the rapid detection of DNA damage following photocatalytic generation of ROS was devised; this strategy can be highlighted by a relatively simple and inexpensive procedure for the fabrication of the electrode — no need of external ROS species, and use of in situ generated ROS under visible light for genotoxicity test. Dopamine had a dual role in this composite; it improved visible light harvesting of DA/TiO_2 in the complex, but additionally provided a bridge linking the DNA electronically to TiO_2. We also demonstrated that by applying AA as antioxidant during the photocatalytic reaction, we were able to significantly protect the DNA from oxidation damage. Thus, this provides a novel and sensitive approach to detecting DNA damage and investigating protection of DNA from oxidative damage and has promising applications in the screening of new bio-nanoconjugations and their genotoxicity. Further work would be targeted to include the interferences and influence of real media composition in the bioassay.

Acknowledgement

The authors wish to acknowledge the Swedish Research Council (VR-2011-6058357), R.I., A.I., and the Slovenian Research Agency (ARRS, grants J3-2120, J1-4109, J1-4136, J3-4108 & P2-0232) for their generous financial support in order to carry out this research. We also gratefully acknowledge Dr . Meysam Pazoki from Uppsala University for his valuable advice and help in the doctor-blade method and the material synthesis.

References

[1] N. Gehrke, C. Mertens, T. Zillinger, J. Wenzel, T. Bald, S. Zahn, T. Tüting, G. Hartmann, W. Barchet, Immunity 39 (2013) 482.
[2] S. Kumari, R.P. Rastogi, K.L. Singh, S.P. Singh, R.P. Sinha, EXCLI J. 7 (2008) 44.
[3] M. Boncheva, L. Scheibler, P. Lincoln, H. Vogel, B. Åkerman, Langmuir 15 (1999) 4317.
[4] A. Houlton, A.R. Pike, M. Angel Galindo, B.R. Horrocks, Chem. Commun. 14 (2009) 1797.
[5] T. Paunesku, T. Rajh, G. Wiederrecht, J. Maser, S. Vogt, N. Stojićević, M. Protić, B. Lai, J. Oryhon, M. Thurnauer, G. Woloschak, Nat. Mater. 2 (2003) 343.
[6] H. Kisch, Angew. Chem. 52 (2013) 812.
[7] K. Syres, A. Thomas, F. Bondino, M. Malvestuto, M. Grätzel, Langmuir 26 (2010) 14548.
[8] T. Rajh, Z. Saponjic, J. Liu, N.M. Dimitrijevic, N.F. Scherer, M. Vega-Arroyo, P. Zapol, L.A. Curtiss, M.C. Thurnauer, Nano Lett. 4 (2004) 1017.
[9] A. Mathew, M.R. Gowravaram, M. Nookala, Adv. Mater. Lett. 4 (2013) 737.
[10] S. Ito, P. Chen, P. Comte, M. Nazeeruddin, P. Liska, P. Pechy, M. Gratzel, Prog. Photovolt. Res. Appl. 15 (2007) 603.
[11] H.Z. Yu, C.Y. Luo, C.G. Sankar, D. Sen, Anal. Chem. 75 (2003) 3902.
[12] Q. Guo, Q. Yue, J. Zhao, L. Wang, H. Wang, X. Wei, J. Liu, J. Jia, Chem. Commun. 47 (2011) 11908.