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Anionic polythiophene derivative as peroxidase mimetics and their application for detection of hydrogen peroxide and glucose

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ABSTRACT

In this paper, we discovered that the anionic polythiophenes derivative, poly[2-(3-thienyl)ethyloxy-4-butylsulfonate] (PTEBS), possesses intrinsic peroxidase-like activity that can catalyze the reaction of peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a blue color reaction, which provides colorimetric detection of H_2O_2. PTEBS exhibits several advantages such as high catalytic efficiency, good stability, and rapid response over horseradish peroxidase (HRP). By coupling the oxidation of glucose catalyzed by glucose oxidase, a simple, inexpensive, highly sensitive and selective colorimetric method for glucose detection has been developed. The absorbance was proportional to the concentration of glucose in the range from 0.01 to 0.5 mM with a detection limit of 0.004 mM. This work is not only of importance for a better understanding of the unique properties of polythiophenes derivative but also of great potential for medical diagnostics and biotechnology.

1. Introduction

Enzyme-catalyzed reactions are of widespread interest due to their high efficiency, high specificity, and mild conditions needed. Artificial enzyme mimetics are a current research interest because natural enzymes bear some serious disadvantages, such as their catalytic activity can be easily inhibited due to denaturation with environmental changes and they can be digested by proteases [1]. As a result, a lot of effort has been made to extend the natural enzymes to enzyme mimetics [2]. Peroxidase enzymes activate H_2O_2 to perform a myriad of oxidations in nature and have been targeted by biomimetic chemists. Many peroxidase mimics including hemin [3,4], molecularly imprinted hydrogels [5], DNAzyme [6] and porphyrin [7], have been applied in different fields. Since Yan and co-workers first reported that Fe_3O_4 magnetic nanoparticles possess intrinsic peroxidase-like activity similar to horseradish peroxidase (HRP) [8], opening the door for developing nanomaterials for biochemical applications, a large of nanomaterials as HRP mimetics have emerged such as $CoFe_2O_4$ nanoparticles [9], $ZnFe_2O_4$ nanoparticles [10], gold nanoparticles [11], CuO nanoparticles [12], polymer-coating CeO_2 nanoparticles [13], Co_3O_4 nanoparticles [14], single-wall carbon nanotube [15], carbon nanodots [16] and graphene oxide [17]. Smaller nano-particles means higher activities because of the larger specific area. However, as well known, nanoparticles easily aggregate into microscale or precipitate in the aqueous solution due to their high specific surface energy, which results in the degradation of catalytic performance [18]. Thus, it is obvious that further efforts to find or develop new peroxidase mimetics with more sensitivity, reusability and stability are still required to underway and hold significant incentives in the field of biochemistry analysis.

Conjugated polyelectrolytes (CPEs) are water-soluble polymers that contain a π-delocalized backbone bearing pendant ionic functionalities. Owing to their extensively delocalized π-network and conformational restrictions, CPEs have gained enormous attention as novel functional materials [19]. Over the past several years, the use of CPEs as sensory materials, with emphasis on biosensing, has been the subject of considerable research interest. Relevant targets include polynucleic acids [20], proteins [21], enzymes [22], and small molecules [23], and metal ions [24]. The presence of biomolecules or biological events can be detected through fluorescence resonance energy transfer between the CPEs and an acceptor molecule, or through their impact on the conformational alteration of the conjugated backbone. Although the optical amplification of CPEs makes them useful as fluorescence probes, some defects would lower the fluorescence quantum yields of CPEs. For example, the highly hydrophobic backbone of CPEs leads to close proximity of the optically active units, and then CPEs could aggregate in water, resulting in intractable self-quenching of fluorescence. The noticeable photobleaching still exists in CPEs-based fluorescence bioassays. These defects would limit the application of CPEs-based fluorescence bioassays.

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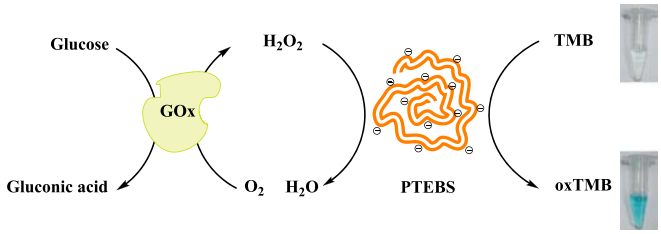

Scheme 1. Schematic illustration of colorimetric detection of glucose by using glucose oxidase (GOx) and PTEBS-catalyzed reactions. oxTMB is the oxidation product of TMB.

In this study, we made the interesting discovery that poly[2-(3-thienyl)ethyloxy-4-butylsulfonate] sodium salt (PTEBS), one of the anionic polythiophene derivative, has intrinsic peroxidase-like activity that can catalyze the reaction of peroxidase substrate TMB in the presence of hydrogen peroxide (H_2O_2) to produce a blue color reaction. Kinetic studies indicate that PTEBS has even higher catalytic activity to TMB than the natural HRP. Peroxidase has a wide range of practical applications and can be used as a diagnostic kit for H_2O_2 and glucose. Detection of glucose has been paid attention to more and more in biomedical fields and plays an increasingly important role in the improvement of life quality [25]. Up to now, a number of glucose detection methods have been reported using mimic peroxidase [10,15,16,26,27]. As a mimic peroxidase, PTEBS exhibited good catalytic properties, stability, and less vulnerable to denaturation compared to other mimic peroxidases and HRP, and was also successfully used as peroxidase mimetics for colorimetric detection of glucose (Scheme 1).

2. Experimental

2.1. Chemical reagents and materials

Poly[2-(3-thienyl)ethyloxy-4-butylsulfonate] sodium salt (PTEBS) was purchased from American Dye Source Inc. The weight of the polymer was afforded by the suppliers (MW=80,000–1,000,000, GPC vs polystyrene). The polymer's concentration was given using its monomer. Glucose, fructose, lactose, and maltose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich, horseradish peroxidase (HRP, EC1.11.1.17, 300 U mg^{-1}) and glucose oxidase (GOx, EC 1.1.3.4. 47, 200 U mg^{-1}) were purchased from Sigma-Aldrich and stored in a refrigerator at −18 °C. All other chemicals were of analytical reagent grade and used without further purification. Millipore Milli-Q water (18 MΩ cm^{-1}) was used in all experiments.

2.2. Instrumentation

UV–visible adsorption spectra were recorded on a U-3900H UV–vis Spectrophotometer (Hitachi, Japan) at room temperature using a 500 μL black-body quartz curette with 1 cm path length. The photographs were taken with a Canon 500 digital camera. The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing). SEM images were taken using a TM3000 environmental scanning electron microscopy operated at the accelerating voltage of 15 KV (Hitachi, Japan).

2.3. Detection of H_2O_2 using PTEBS as peroxidase mimetics

A typical colorimetric analysis was realized as follows. Firstly, 100 μL of 0.5 mM TMB, 50 μL of 1.0×10^{-7} M PTEBS, and 100 μL of H_2O_2 with different concentrations were added into 250 μL of 0.2 M acetate buffer (pH 4.0). Secondly, the mixed solution was incubated in a 45 °C water bath for 10 min and cooled to the room temperature. Finally, the resulting solution was used for adsorption spectroscopy measurement.

2.4. Detection of glucose using PTEBS and GOx

Glucose detection was performed as follows: (1) 20 μL of 5 mg mL^{-1} GOx and 200 μL of glucose of different concentrations in 10 mM PBS buffer (pH 7.4) were incubated at 37 °C for 15 min; (2) 100 μL of 0.5 mM TMB, 50 μL of the PTEBS solution (1.0×10^{-7} M, on a monomeric unit basis of polymer) and 630 μL of 0.2 M acetate buffer (pH 4.0) were added to the above glucose reaction solution; and (3) the mixed solution was incubated at 45 °C for 10 min and then for standard curve measurement. In control experiments, 5 mM maltose, 5 mM lactose, and 5 mM fructose were used instead of glucose in a similar way. For glucose determination in serum, the samples from local hospital were first treated by centrifugation at 10,000 rpm for 30 min. After that, the supernatant solution was diluted 100 times using PBS (10 mM, pH 7.4) for the following work. This diluted serum was then used with GOx for glucose catalyzed reaction as stated above instead of glucose aqueous solution and the corresponding absorbance was measured at a wavelength of 652 nm.

3. Results and discussion

3.1. Peroxidase-like activity of PTEBS

In this work the PTEBS was used to catalyze the oxidation of a peroxidase substrate TMB by H_2O_2 to the oxidized colored product. To investigate the peroxidase-like activity of the polyelectrolyte PTEBS, the catalytic oxidation of peroxidase substrate TMB in the presence of H_2O_2 was tested. The results (Fig. 1) showed that PTEBS could catalyze the oxidation of the substrate by H_2O_2 in acetate buffer, and produced the typical color reaction.

The inset in Fig. 1 shows the photographs of TMB solutions under different conditions. As you can see that it is only TMB and H_2O_2 coexist with PTEBS, the blue color can be observed. The typical absorbance peak of oxidation product of TMB (oxTMB) is at 652 nm. The absorbance of the PTEBS–TMB–H_2O_2 system at 652 nm was much higher than that of the TMB–H_2O_2 systems.

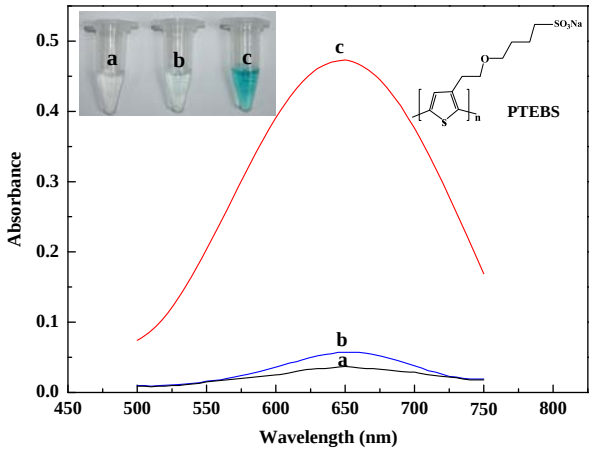

Fig. 1. Typical absorption spectra of TMB solution catalytically oxidized by PTEBS in the presence of H_2O_2 incubated at 45 °C in 200 μL of 0.2 M pH 4.0 acetate buffer (from a to c: 0 mM H_2O_2 with PTEBS, 1 mM H_2O_2 without PTEBS, 1 mM H_2O_2 with PTEBS). Inset: typical photographs of three samples to the corresponding lines and the chemical structure of PTEBS.

Furthermore, in order to check whether the structure of PTEBS changed before and after the oxidation reaction with H_2O_2, we measured the absorption spectra and the fluorescence spectra of PTEBS before and after the oxidation reaction in the same medium. The optical character of conjugated polyelectrolytes is closely related to their structure. There was no obvious change on the absorption spectra and the fluorescence spectra of PTEBS (Fig. S1), which indicated that PTEBS itself did not change during the oxidation reaction with H_2O_2. Therefore, all these observations further confirm that PTEBS has peroxidase-like catalytic ability and thus can catalyze oxidation of TMB in the presence of H_2O_2.

In order to further verify the above inference, the morphology of polymer PTEBS was studied. Fig. S2 shows SEM images for PTEBS before and after reacting with H_2O_2. It can be observed that the PTEBS before and after reacting with H_2O_2 have very similar morphology. All samples show an agglomerated grain structure and the grains have diameters of around 3 μm. It was reported that the morphology of films prepared by polyelectrolyte is not controlled via the physical conformation of the polyelectrolyte chains in solution but via their chemical structure [28]. During the whole reaction, the morphology of PTEBS was not changed, so it was confirmed that the structure of PTEBS was not changed. When PTEBS was dispersed in homogeneous solution and reacted with H_2O_2, the electron transfer to hydrogen peroxide is an important pathway to accelerate the reaction.

3.2. Optimization of experimental conditions

According to the above discussion, PTEBS can catalyze the oxidation of TMB by H_2O_2 to obtain the oxTMB form with blue color, which shows that PTEBS has intrinsic peroxidase-like catalytic activity. Similar to peroxidase, the catalytic activity of PTEBS was dependent on pH, temperature, and H_2O_2 concentration (Fig. S3). The pH-dependent response curve showed that the catalytic activity of PTEBS was highest at pH 4.0. The possible reason is that PTEBS is stable in weak acidic media [29]. At the same time, the oxidation of TMB occurs easily under weakly acidic conditions [30]. Thus, the 0.2 M pH 4.0 acetate buffer solution was taken as the reaction media. The catalytic activity of PTEBS increased with increasing reaction temperature in the range of 20–45 °C, and above 45 °C the activity decreased due to the decomposition of H_2O_2 itself under high temperature. So, the reaction temperature was fixed at 45 °C. The optimal PTEBS concentration was evaluated to 0.1 μM. Previous literature has reported that the excess 1 mM H_2O_2 can inhibit the catalytic activity of HRP through conversion of HRP to inactive forms [31]. However, inhibition was found for the PTEBS-catalyzed reaction beyond 20 mM H_2O_2 concentration, which shows that the catalytic activity of the PTEBS is more stable at high H_2O_2 concentration than that of HRP.

3.3. Mechanism of peroxidase-like activity of PTEBS

The study of peroxidase-like kinetics is fundamental and necessary to understand the enzymatic characteristics and the catalytic mechanism of PTEBS. Michaelis – Menten constant (K_m) and maximum initial velocity (V_{max}) were obtained using Lineweaver – Burk plot [32,33] and are shown in Table S1. K_m represents the affinity of a given enzyme towards the substrate. Smaller K_m values thus indicate a stronger affinity between the enzyme and the substrate. Whether TMB or H_2O_2 as the substrate, the apparent K_m value for the PTEBS was lower than that for HRP and other peroxidase nanomimetics (Table S1), suggesting that the PTEBS had higher affinity for both H_2O_2 and TMB than HRP and other peroxidase nanomimetics. The catalytic effect is related to PTEBS electronic structures and its interactions with H_2O_2 and TMB. Polythiophene derivative exhibited the good electrocatalytic activity to reduction of oxygen, which was attributed to their ability to promote electron transfer between electronic acceptor and electronic donator [34]. In this system, PTEBS with negative charge in side chain can adsorb TMB with positive charge in acid condition by electrostatic interaction, consulted the previous relative research, which is propitious to the catalytic activity of PTEBS in TMB–H_2O_2 system [15–17]. The peroxidase-like activities of PTEBS may originate from an increase in the electron density and mobility in the PTEBS because of electron transfer from lone-pair electrons in TMB amino groups to the electronically delocalized backbone of PTEBS. This would result in acceleration of electron transfer from PTEBS to H_2O_2 and the reaction rate of TMB oxidation by H_2O_2 would increase.

At the same time, another nature of peroxidase-like activities of the PTEBS may originate from their catalytic ability to decompose H_2O_2 into •OH radicals, being confirmed with the ESR spin-trapping technique (Fig. S4). The ESR spectra in the presence of PTEBS displayed a 4-fold characteristic peak of the typical DMPO–•OH adduct with an intensity ratio of 1:2:2:1. It suggested that •OH radicals have produced in the PTEBS–H_2O_2 system. In order to further demonstrate that the mechanism of peroxidase-like activity of the PTEBS is from •OH formation, terephthalic acid was adopted as a fluorescence probe to evaluate the effects of the PTEBS on •OH signal intensity, in which terephthalic acid easily reacted with •OH to form highly fluorescent 2-hydroxy terephthalic acid [35]. It was clearly shown that gradual increase of the fluorescence intensity was observed as the concentration of the PTEBS increased (Fig. S5), suggesting that the amount of generated •OH was increased by the increase in PTEBS. However, there was no fluorescence intensity in

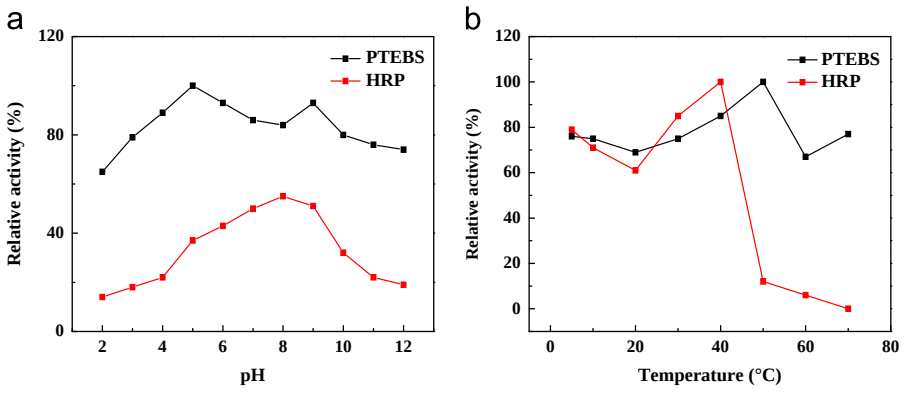

Fig. 2. Comparison of the stability of PTE-BS and HRP. (a) PTE-BS and HRP were first incubated at pH 2–12 for 2 h and then their peroxidase activities were measured under standard conditions. (b) The catalytic activities of PTE-BS and HRP were measured under different temperatures form 5 to 70 °C.

the absence of H_2O_2. These results indicated that PTEBS could decompose H_2O_2 to generate the •OH radical.

According to the above results, the catalytic mechanism of PTEBS on TMB–H_2O_2 reaction can be summarized. PTEBS as electron donors reacts with H_2O_2, and the O–O bond of H_2O_2 might be broken up into double •OH radicals; the reactive •OH radicals can oxidize TMB to produce color. For anionic PTEBS, there is also another way to catalyze the TMB–H_2O_2 reaction: the electrostatic binding of TMB toward the polymer results in the local concentration effect, which would also accelerate the reaction of TMB and H_2O_2.

3.4. Robustness of peroxidase activity of PTEBS

The stability of the PTEBS in wide pH and temperature ranges is crucial to extend their applications. As a novel material with peroxidase-like activity, the PTEBS is expected to be more stable than the enzyme HRP. To confirm this, both PTEBS an HRP were incubated at a range of values of pH and a range of temperatures for 2 h, and then their activities were measured under standard conditions. The catalytic activity of HRP was largely inhibited after incubation at lower and higher pH or higher than 50 °C (Fig. 2). In contrast the catalytic activity of the PTEBS remained stable over wide range of pH (2–12) and temperature (5–70 °C). The robustness of the PTEBS makes it potentially applicable under harsh conditions. In addition, the stability of the polymer with the change of time was investigated. The catalytic activity of the polymer PTEBS which was preserved for one month, three months, six months and one year was compared. The result indicated that the catalytic activity of the polymer PTEBS had no significant difference with the change of time. It was shown that the stability during this time was good.

3.5. Detection of glucose

Because the catalytic activity of the PTEBS was H_2O_2 concentration dependent, this can be used to detect H_2O_2. The absorbance at 652 nm is proportional to H_2O_2 concentration from 0.001 to 0.01 mM with a detection limit of 0.3 μM (Fig. 3b). H_2O_2 is the main product of glucose oxidase-catalyzed reaction. When combined with glucose oxidase, the proposed colorimetric method could be used for the determination of glucose. The linear range for glucose is from 0.01 to 0.5 mM, and the detection limit (taken to be 3 times the standard deviation in the blank solution) is as low as 4 μM (Fig. 3a). The color variation for glucose response was also obvious by visual observation to as low as 0.01 mM (Fig. 3a inset). In comparison with the reported colorimetric glucose measure (Table 1), our assay for glucose detection is relatively simple, cost-effective, and sensitive. In addition, the stability of the low-cost PTEBS is much better than that of nanoparticles. The enzyme mimics property of PTEBS holds a great potential for practical applications. To test the selectivity of detection of glucose, the control experiments were taken using fructose, lactose, and maltose. The selectivity of the colorimetric method

Table 2
Results of determination of glucose in serum.

Sample	Added (mM)	Total found (mM) ± SD [a]	Recovery (%)	RSD	Glucosemeter method (mM) [b]
Serum 1	–	0.047 ± 0.002	–	3.2	4.74
	0.05	0.094 ± 0.003	96.7	4.2	
	0.1	0.138 ± 0.005	93.9	3.7	
Serum 2	–	0.053 ± 0.004	–	3.9	5.24
	0.05	0.091 ± 0.003	93.8	3.3	
	0.1	0.157 ± 0.006	102.6	4.1	
Serum 3	–	0.062 ± 0.002	–	2.8	6.22
	0.05	0.116 ± 0.006	103.6	3.6	
	0.1	0.155 ± 0.007	95.7	4.4	

[a] The serum samples were diluted 100-fold for glucose determination by the proposed method.
[b] The glucose determination was performed directly without dilution for clinical analysis in the laboratory of the hospital of Shaanxi Normal University.

Table 1
Comparison of colorimetric glucose analytical performance for PTEBS with other glucose biosensors.

Catalyst	Linear range (μM)	Detection limit (μM)	Usage of catalyst (mg)	Detection time (min)	Reference
PTEBS	10–500	4	0.0015	25	This work
Fe_3O_4 MNPs	50–1000	30	0.037	40	[8]
Co_3O_4 nanoparticles	10–1000	5	0.045	60	[14]
Gold nanoparticles	18–1100	4	–	25	[16]
CuO nanoparticles	100–8000	–	0.01	40	[12]
Fe (III)-based coordinationpolymer nanoparticles	2–20	1	0.52	80	[27]

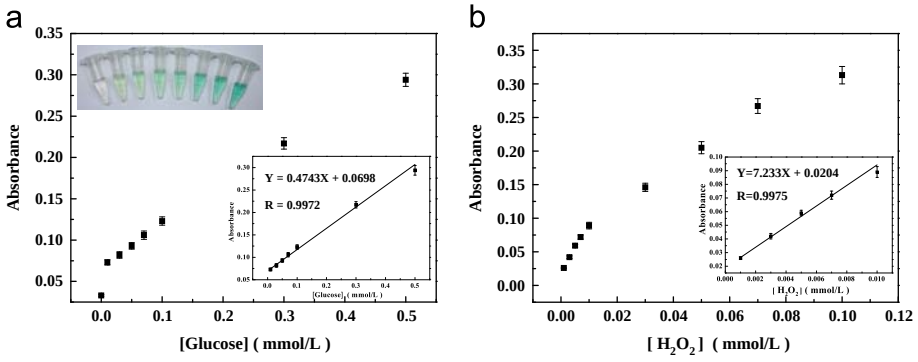

Fig. 3. Response curves of glucose (a) and H_2O_2 (b). Error bars represent the standard deviation for three measurements. Inset: images of production of colored products for different concentrations of glucose.

was shown in Fig. S6. Even when the concentration of control samples was 5 times larger than glucose, there are no detectable signals observed. Thus, the colorimetric method developed here showed high selectivity toward glucose detection.

The present novel PTEBS-based enzyme mimic colorimetric system was applied to glucose determination in blood samples with recoveries in the spiked samples from 93 to 104% and the results agree well with those obtained by the conventional electrochemical method (Table 2). The general range of blood glucose concentration in healthy and diabetic persons is about 4.4–6.6 mM and 9–40 mM, respectively [36]. Therefore, the proposed method is suitable and satisfactory for glucose analysis of real samples.

4. Conclusions

In summary, we provide the first report that the PTEBS possesses intrinsic peroxidase-like activity. As a novel mimic peroxidase, the PTEBS shows several advantages over HRP, such as stability, long lifespan and high catalytic efficiency. On the basis, we provide a simple, inexpensive, highly sensitive, and selective method for colorimetric detection of glucose. The assay is homogenous and occurs in the liquid phase, which makes it easy to automate by standard robotic manipulation of microwell plates. Taking into account the advantages of PTEBS, we confidently expected that PTEBS would have potential applications in biotechnology and clinical diagnosis as enzymatic mimics.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.063.

References

[1] D.L. Nelson, M.M. Cox, Lehninger Principles of Biochemistry, fourth ed., W.H. Freeman & Co. Ltd., New York, 2005.
[2] G. Wulff, Chem. Rev. 102 (2002) 1–27.
[3] H. Aissaoui, R. Bachmann, A. Schweiger, W.D. Woggon, Angew. Chem. Int. Ed. 37 (1998) 2998–3002.
[4] L. Fruk, C.M. Niemeyer, Angew. Chem. Int. Ed. 44 (2005) 2603–2606.
[5] Z. Chen, L. Xu, Y. Liang, M. Zhao, Adv. Mater. 22 (2010) 1488–1492.
[6] G. Pelossof, R. Tel-Vered, J. Elbaz, I. Willner, Anal. Chem. 82 (2010) 4396–4402.
[7] R.P. Bonar-Law, J.K.M. Sanders, J. Am. Chem. Soc. 117 (1995) 259–271.
[8] L.Z. Gao, J. Zhuang, L. Nie, J.B. Zhang, Y. Zhang, N. Gu, T.H. Wang, J. Feng, D.L. Yang, S. Perrett, X. Yan, Nat. Nanotechnol. 2 (2007) 577–583.
[9] W. Shi, X. Zhang, S. He, Y. Huang, Chem. Commun. 47 (2011) 10785–10787.
[10] L. Su, J. Feng, X. Zhou, C. Ren, H. Li, X. Chen, Anal. Chem. 84 (2012) 5753–5758.
[11] Y. Jv, B. Li, R. Cao, Chem. Commun. 46 (2010) 8017–8019.
[12] W. Chen, J. Chen, A.L. Liu, L.M. Wang, G.W. Li, X.H. Lin, ChemCatChem 3 (2011) 1151–1154.
[13] A. Asati, S. Santra, C. Kaittanis, S. Nath, J.M. Perez, Angew. Chem. Int. Ed. 48 (2009) 2308–2312.
[14] J. Mu, Y. Wang, M. Zhao, L. Zhang, Chem. Commun. 48 (2012) 2540–2542.
[15] Y. Song, X. Wang, C. Zhao, K. Qu, J. Ren, X. Qu, Chem. Eur. J. 16 (2010) 3617–3621.
[16] W. Shi, Q. Wang, Y. Long, Z. Cheng, S. Chen, H. Zheng, Y. Huang, Chem. Commun. 47 (2011) 6695–6697.
[17] Y. Song, K. Qu, C. Zhao, J. Ren, X. Qu, Adv. Mater. 22 (2010) 2206–2210.
[18] Y. Zhao, L. Jiang, Adv. Mater. 21 (2009) 3621–3638.
[19] H. Jiang, P. Taranekar, J.R. Reynolds, K.S. Schanze, Angew. Chem. Int. Ed. 48 (2009) 4300–4316.
[20] J.W. Hong, W.L. Hemme, G.E. Keller, M.T. Rinke, G.C. Bazan, Adv. Mater. 18 (2006) 878–882.
[21] K. Li, B. Liu, Polym. Chem. 1 (2010) 252–259.
[22] F. Feng, Y. Tang, S. Wang, Y. Li, D. Zhu, Angew. Chem. Int. Ed. 46 (2007) 7882–7886.
[23] Q. Fan, Y. Zhou, X. Lu, X. Hou, W. Huang, Macromolecules 38 (2005) 2927–2936.
[24] C. Qin, W.-Y. Wong, L. Wang, Macromolecules 44 (2011) 483–489.
[25] J. Castillo, S. Gaspar, S. Leth, M. Niculescu, A. Mortari, I. Bontidean, V. Soukharev, S.A. Dorneanu, A.D. Ryabov, E. Csoregi, Sens. Actuators B 102 (2004) 179–194.
[26] J. Wang, D. Han, X. Wang, B. Qi, M. Zhao, Biosens. Bioelectron. 36 (2012) 18–21.
[27] J. Tian, S. Liu, Y. Luo, X. Sun, Catal. Sci. Technol. 2 (2012) 432–436.
[28] R. Kniprath, S. Duhm, H. Glowatzki, N. Koch, S. Rogaschewski, J.P. Rabe, S. Kirstein, Langmuir 23 (2007) 9860–9865.
[29] G. Tourillon, F. Garnier, J. Electrochem. Soc. 130 (1983) 2042–2044.
[30] P.D. Josephy, T. Eling, R.P. Mason, J. Biol. Chem. 257 (1982) 3669–3675.
[31] J.A. Nicell, H.A Wright, Enzyme Microb. Technol. 21 (1997) 302–310.
[32] M. Chayer, K. Faïd, M. Leclerc, Chem. Mater. 9 (1997) 2902–2905.
[33] Y. Peng, X. Li, J. Ren, X. Qu, Chem. Commun. 0 (2007) 5176–5178.
[34] V.G. Khomenko1, V.Z. Barsukov, A.S. Katashinskii, Electrochim. Acta 50 (2005) 1675–1683.
[35] K. Ishibashi, A. Fujishima, T. Watanabe, K.J. Hashimoto, Photochem. Photobiol. A 134 (2000) 139–142.
[36] Y. Xu, P.E. Pehrsson, L.W. Chen, R. Zhang, W. Zhao, J. Phys. Chem. C 111 (2007) 8638–8643.