One step electrosynthesis of conjugated polymers thin film for Fe³⁺ detection and its potential application

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ABSTRACT

Poly(9-fluorene-carboxylic acid) (PFCA) thin film was successfully fabricated onto the surface of indium tin oxide glass by one-step electropolymerization of commercially available 9-fluorene-carboxylic acid in boron trifluoride diethyl etherate. Meanwhile, PFCA thin film could specifically recognize Fe^{3+} . Fe^{3+} was generated by the oxidation of Fe^{2+} in the presence of H_2O_2 that was produced by biochemical reactions and resulted in fluorescence quenching of PFCA thin film. Furthermore, a sensing platform was designed for highly selective fluorescent detection of glucose. The qualitative and quantitative analysis of Fe^{3+} , H_2O_2 and glucose with detection limits of 1 μM , 2.9 μM and 3.4 μM respectively could be implemented by a simple, cost-effective, reusable, practical and multi-functional PFCA thin film. The fabricated PFCA-based chemo/bio sensing film for fluorescent detection of Fe^{3+} in rice sample or glucose in serum sample was assessed. Satisfactory results indicate that this work will provide not only a new strategy for fluorescent detection of important substances in agricultural science, but also significant theoretical guidance and important reference value for the practical application and development of multi-functional sensing device.

Keywords: Conjugated polymers, fluorescence sensors, Fe^{3+} detection, one step electrosynthesis, thin films

1. Introduction

The important species in animals and plants do not exist in isolation, and they can transform mutually with the help of biochemical reaction chains. In particular, biological toxic or hazardous substances like hyperoxides which can be generated by biochemical reactions in body can be eliminated by biochemical reactions in organisms. Thus, diverse biochemical reaction chains in organisms can realize the mutual transformation of different important substances and maintain a dynamic equilibrium of the body. This equilibrium can be controlled by the key steps of irreversible biochemical reactions such as enzyme catalyzed reactions or oxidation-reduction reactions. For example, iron ions are easily oxidized or reduced by biological oxidants or reductants, which can be realized by a series of biochemical reactions with the help of different biochemical reaction chains in organisms.

Among many biologically significant metal ions, Fe^{3+} is one of the most abundant and essential metal ions in the human body because it is involved in many biochemical processes, acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain [1–5], participates in electron transfer [6, 7] and oxidation reactions [8, 9]. However, both its deficiency and excess can cause a variety of diseases in the human body, such as dysfunction of liver [10, 11] and kidney [12, 13], anemia [14], cancer [15–17], Alzheimer's disease [18–20], hemochromatosis [21] and Parkinson's disease [22, 23]. Therefore, various efficient chemosensors for Fe^{3+} detection have gained extensive attentions of scientists. Conventional qualitative and quantitative strategies for Fe^{3+} detection, such as atomic absorption spectrometry [24], spectrophotometry [25], voltammetry [26] and inductively coupled plasma mass spectrometry [27], required

complex instrumentation and tedious sample preparation procedures, which limited their prosperous applications [28]. Very recently, use of fluorescence sensors for detection of iron has attracted significant attention because of their simplicity, rapid response and high sensitivity. In addition, fluorescence sensors usually do not require expensive or sophisticated instrumentation, complicated operation and detection procedures.

Considerable effort has been made to develop fluorescent bio/chemo sensors based on different materials in the past few decades [29–33]. Especially, conjugated polymers (CPs) have received much attention owing to their signal amplification properties and the versatility of their molecular design [34–37]. Although there are lots of reports on fluorescent sensors based on liquid phases (solution phases) [38–43], so far few efficient fluorescent sensors have been reported solid-phases (thin-films) [44–48]. In our previous work, we fabricated fluorescent sensors based on solution phases and thin films [42, 43, 48, 49]. In terms of practicality, thin-film sensors are more applicable than solution-phase sensors in recyclability, environment-friendly, and ease to make sensing device. CPs are more easily for the formation of thin films via chemical or electrochemical methods due to their insolubilization and infusibility, and thin-film sensing devices can be used in water and its mixed systems. Solution-phase sensors are limited to the solubility of sensing materials. In addition, We have also studied poly(9-fluorene-carboxylic acid) (PFCA) fluorescent sensors based on solution phases, which displayed ultrahigh-selectivity towards Fe^{3+} over different substances existing in agricultural crops and their products, such as common metal ions, anions, natural amino acids, carbohydrates, and organic acids, even organs of crops [49].

In this paper, firstly, we facilely electrosynthesized PFCA film onto the surface of indium tin oxide (ITO) glass as high-performance sensing devices for high-selective detection of Fe^{3+} . Then, a platform was designed for high-selective fluorescent detection of important substances like H_2O_2 and glucose using PFCA thin film as multi-functional sensing devices. Finally, the fabricated PFCA-based sensing device for fluorescent detection of Fe^{3+} or glucose in real samples was assessed.

2. Experimental

2.1 Chemicals and materials

9-Fluorencarboxylic acid (FCA) was bought from Acros Organics and used directly. BEFF (Aladdin Reagent Co., Ltd.) was distilled before use. H_2O_2 (30% w/v) was calibrated with KMnO_4 before use. glucose and glucose oxidase (GOD) were purchased from Beijing J&K Scientific Ltd. Bovine blood samples were obtained from Pingrui Biotechnology Company. Other chemicals were of analytical grade and used as received without further purification. Deionized water was used throughout the experiments.

Electrochemical measurements were performed in a one-compartment cell with the use of Model 263A potentiostat–galvanostat (EG&G Princeton Applied Research) under computer control. Absorption spectra were measured using PerkinElmer Lambda 900 Ultraviolet-Visible Near-Infrared spectrophotometer. All fluorescence experiments were carried out using a Hitachi F-4500 spectrophotometer with excitation slit set at 5 nm and emission slit at 5 nm. All pH measurements were made with a PHB-5 pH meter.

2.2 Preparation of PFCA thin film

The cyclic voltammetry (CV) was used to prepare the CPs films using a standard one-compartment, three-electrode electrochemical cell. Platinum (Pt) wire with a diameter of

0.5 mm was used as the counter electrode and ITO was used as the working electrode. The reference electrode was an Ag/AgCl electrode. The typical electrolytic solution was BFEE and 0.02 mol L⁻¹ FCA. All solutions were deaerated by a dry argon stream and maintained at a slight argon overpressure during experiments. The FCA was electropolymerized directly onto the ITO electrode through an oxidation coupling reaction. Finally, the prepared films were dried in a vacuum oven at 60 °C for 3 h.

2.3 Preparation of real samples

All bovine blood samples were centrifuged at 10000 rpm for 10 min after being stored for 2 h at room temperature. Then 0.4 mL serum sample was mixed with 0.6 mL acetonitrile. After vigorous shaking for 2 min, the mixture was centrifuged at 10 000 rpm for 10 min. The obtained supernatant was diluted at a 1:10 ratio with deionized water, which was used in real application.

Black rice sample was obtained from a local supermarket. Double-distilled deionized water was added into the above material, and homogenized for 10 min using a blender. Then homogenates were filtered to remove residues. The prepared sample was kept at 4 °C when not in use. The as-fabricated sensor was employed to detect the real samples with Fe³⁺ or without Fe³⁺. The real samples with Fe³⁺ were obtained by adding different contents of Fe³⁺.

2.4 General procedure for fluorescence measurements

2.4.1 Detection of Fe³⁺

A stock standard solution of 0.1 M Fe³⁺ was prepared by dissolving an appropriate amount of Fe(NO₃)₃·9H₂O in deionized water. This was further diluted to the corresponding concentrations. Different concentrations of Fe³⁺ ions were added in

MeOH-H₂O (v/v, 9:1). And the PFCA film was stuck to the bottom of the quartz cell, which made sure the same point was measured. The corresponding fluorescence spectra were recorded.

2.4.2 Detection of H₂O₂

Different concentrations of H₂O₂ were added to the MeOH-H₂O testing system containing redundant Fe²⁺ and the PFCA film. Then the fluorescence spectra were obtained.

2.4.3 Detection of glucose

PBS (pH = 7.0) was used to dissolve a glucose sample, which is the physiological pH value 7.0 of GOD. Different volumes of glucose solution and 1 mg mL⁻¹ GOD were added into a PBS in a 5 mL sample tube and reacted for 20 min under 37 °C water bath. Then the above reaction solution was added to the MeOH-H₂O solution of a constant concentration of redundant Fe²⁺ and PFCA thin film before fluorescent determination.

3. Results and Discussion

3.1 Preparation of PFCA thin film devices

3.1.1 Electrosynthesis of PFCA thin film

To better realize the practical application of the PFCA-based thin film sensor, a PFCA solid thin film was facilely prepared by one-step electrochemical polymerization, and its photophysical properties were characterized.

The electroactive monomer was coupled efficiently under anodic oxidation to form the thin film on the surface of ITO electrode by the CV method. Fig. 1 is the multicycle CV of FCA recorded for eight cycles at a scan rate of 200 mV s⁻¹. As can be seen, the peak

currents increase with the number of cycles, indicating that the PFCA thin film is gradually formed on the surface of ITO electrode.

Photophysical properties of PFCA thin film were characterized by UV-vis absorption and fluorescence emission spectra in MeOH-H₂O (Fig. 2). The absorption and emission maxima of PFCA thin film peaked at 330 and 455 nm, respectively. Its optical properties were very similar to those of the same polymer in solution states, which confirmed that the immobilization did not change its optical behaviors of PFCA. Notably, the PFCA thin film displayed excellent fluorescence stability in the PBS. There was nearly no fluorescence quenching when the film was placed in the PBS for 24 h. The poor solubility of the film may be the reason for this extraordinary stability. Such high stability of the film is very necessary for sensor application.

3.1.2 Optimization of PFCA thin film

To prepare a highly sensitive PFCA thin film sensor for Fe³⁺ detection, we optimized scan cycles and scan rates of the electropolymerization process, respectively. The fluorescent response of the PFCA thin film to Fe³⁺ sensing could be influenced by the thickness of the film, and the film thickness was controlled by scan cycles during the repetitive cyclic voltammetric polymerization of the PFCA thin film. Firstly, PFCA thin films were prepared by CV for 200 mV s⁻¹ at different scan cycles. The corresponding fluorescent quenching of thin film in the presence of Fe³⁺ was given in Fig. 3a. With the increase of scan cycles, the fluorescence quenching efficiency of thin film decreased. This was attributed to the permeability of the analyte in the polymer due to the increase of diffusion distance of Fe³⁺ with the increase of the film thickness. On the contrary, the fluorescence quenching efficiency of the thin film at six scan cycles was lower than that

at eight scan cycles. This might be because the thickness of the film was too thin and there was much doping in the film. Secondly, the PFCA thin film was prepared by CV for eight cycles at different scan rates. Fig. 3b showed the fluorescence quenching of thin film in the presence of Fe^{3+} at different scan rates. The fluorescence of the film prepared at 100 mV s^{-1} and 150 mV s^{-1} was quenched by 61% and 68%, respectively. Compared with the above mentioned film, the film fabricated at 200 mV s^{-1} showed higher sensitivity towards Fe^{3+} , and could be quenched by 73%. This might be because the increase of electrochemical cross-linking degree with the decrease of scan rates makes the film denser to hinder the diffusion of Fe^{3+} into the film. However, when the scan rate was 250 mV s^{-1} , the sensitivity to Fe^{3+} was lower than that at 200 mV s^{-1} . This might be because the scan rate was so high that the redox process was incomplete. The results indicated that the PFCA thin film prepared by CV for eight cycles at 200 mV s^{-1} was highly sensitive towards Fe^{3+} sensing.

3.2 High-performance PFCA devices towards Fe^{3+} sensing

3.2.1 High-selective detection of Fe^{3+}

The selectivity of the proposed thin film sensor was evaluated before its application in a real sample. The influence of various biological species was investigated. The thin film was immersed in MeOH- H_2O medium containing different biological species, then the corresponding fluorescence spectra were recorded. Remarkably, the addition of most of biological species to the PFCA thin film only brought about negligible changes in the fluorescence intensity. Fig. 4 depicts the fluorescence response of the PFCA thin film sensor to partial biological species. The anti-interference ability of the proposed PFCA-based thin film sensor was in accordance with that PFCA-based fluorescent sensor in

solution phase. Meanwhile, very recently we have reported PFCA sensing application in ethanol and the relevant sensing mechanism has been addressed and discussed in detail [49]. This result reflected the fact that the PFCA thin film sensor could selectively sense Fe^{3+} over the other biological species, which provide the prerequisite for the development and architecture of multi-functional sensing device.

The responsive properties of the PFCA thin film toward Fe^{3+} detection also were investigated. The PFCA thin film was immersed in MeOH- H_2O solution of iron nitrate. Then, the corresponding fluorescence spectra were recorded. Fluorescence emission spectra of the PFCA thin film in the presence of different concentrations of iron nitrate were shown in Fig. 5. The addition of Fe^{3+} led to a significant quenching of the PFCA thin film emission, and a 73% decrease in intensity was observed. In addition, good linear correlation was found over the concentration range from 3 μM to 1 mM (Fig. 5 inset). The detection limit of Fe^{3+} was determined to be 1 μM , which demonstrated that the use of the PFCA thin film sensor provided an approach to detect Fe^{3+} with satisfactory sensitivity.

3.2.2 Reversibility and reproducibility

One advantage of the film chemosensors was their reversible response. To evaluate the reversible sensing ability of PFCA thin film for Fe^{3+} , we monitored the corresponding changes of its fluorescence intensity when alternately immersing the film into MeOH- H_2O of 0.5 mM Fe^{3+} ion and the MeOH- H_2O solution containing dilute ammonia. Unsurprisingly, the emission of PFCA thin film quenched by Fe^{3+} was almost fully restored and the process could be repeated for at least three times (Fig. 6a).

Given the importance of the reproducibility of the thin film sensor in real application, the corresponding experiments were done. Fig. 6b depicts fluorescent response of four PFCA thin film fabricated on different days towards 0.5 mM Fe^{3+} under the same conditions. In these experiments, this film was immersed in the solution containing Fe^{3+} , respectively. And the average fluorescence-quenching efficiency of this film by Fe^{3+} was 52% with a relative standard deviation (RSD) of 4.1%. The result indicated that the PFCA thin film sensor presented excellent reproducibility for Fe^{3+} sensing, which made the practical application of the film sensor feasible.

3.3 Potential application of multi-functional Fe^{3+} sensing devices

To develop the potential application of Fe^{3+} sensor based on PFCA thin film, a platform was designed for highly selective qualitative and quantitative analysis of H_2O_2 , glucose using PFCA thin film as the high-performance and multi-functional sensing device (Scheme 1). Meanwhile, the quantification of different important substances via the stoichiometric relationship among oxidation of these important substances in this designed biochemical reaction chain could be also realized.

3.3.1 Detection of H_2O_2 or Fe^{2+}

To demonstrate the potential application of Fe^{3+} sensor system based on PFCA thin film in biochemical fields, a sensitive assay of H_2O_2 has been designed. As shown in Scheme 1, Fe^{3+} was generated by the oxidization of Fe^{2+} in the presence of H_2O_2 and resulted in fluorescence quenching of PFCA thin film emission, which can be directly detected of H_2O_2 or indirectly correlated to the quantification of Fe^{2+} (Fe^{2+} can also be indirectly detected by the stoichiometric relationship between H_2O_2 and Fe^{2+} or the addition of different concentrations of Fe^{2+} under the existence of a large number of

H₂O₂). Fig. 7 gives a comparison of fluorescence intensity changes when adding Fe²⁺, H₂O₂, Fe³⁺ and “Fe²⁺ + H₂O₂” to the MeOH-H₂O, respectively. As illustrated in Fig. 7, Fe²⁺ or H₂O₂ could not quench the fluorescence of PFCA thin film. However, after addition of 1 mM H₂O₂ to the solution containing PFCA thin film and 1 mM Fe²⁺, the fluorescence intensity was dramatically decreased to a state comparable to that caused by Fe³⁺, which to a large extent corroborated our sensing scheme.

Then, sensitive determination of H₂O₂ was performed upon titration of the MeOH-H₂O containing PFCA and 2 mM Fe²⁺ with different concentrations of H₂O₂. Fig. 8 illustrates the fluorescence quenching of the above mentioned titration system, indicating that titration with different concentrations of H₂O₂ resulted in a gradual decrease in fluorescence intensity of PFCA thin film. The fluorescence quenching of PFCA versus H₂O₂ concentrations in the range of 9 μM – 1.5 mM exhibited good linearity (Fig. 8, insert), providing a detection limit of 2.9 μM.

3.3.2 Determination of glucose

A sensitive assay of glucose has also been designed to demonstrate the potential application of Fe³⁺ sensing system based on PFCA thin film in biochemical fields. As shown in Scheme 1, under the existence of a certain amount of GOD, glucose is oxidized to gluconolactone and generates an equivalent H₂O₂. Immediately, the produced H₂O₂ oxidizes Fe²⁺ to Fe³⁺ and causes fluorescent quenching of PFCA thin film, which can be directly detected of glucose. The fluorescence of PFCA thin film with different concentrations of glucose or GOD has been detected, and the results were plotted in Fig. 9a, we could find that glucose or GOD could not quench the fluorescence of PFCA thin

film from Fig. 9a. Hence, there is no background interference in glucose determination with PFCA thin film.

PBS (pH = 7.0) containing GOD and glucose were put into a 37 °C water bath for 20 min. Then the as-prepared solution was added to the MeOH-H₂O of PFCA thin film and Fe²⁺ before recording fluorescence spectra. As shown in Fig. 9b, the fluorescence intensity decreased gradually as glucose concentration increased. A good linear relationship of I_0/I versus [glucose] was obtained in a range of 10 µM – 0.8 mM (Fig. 9b, insert), providing a detection limit of 3.4 µM. This demonstrated the sensor system could be used for glucose detection with satisfactory sensitivity. Moreover, a comparison between the proposed method and other reported methods for glucose determination was summed up in Table 1. Although the sensitivity of the method was not best among the reported methods, the advantages of the method were recyclability and ease to make sensing device.

3.4 Practical application of multi-functional PFCA sensing devices

3.4.1 Qualitative application in biological samples

To validate the practical application of the assay in biological samples, qualitative application in rice sample was firstly investigated by adding Fe³⁺, Fe²⁺ and H₂O₂ successively. As shown in Fig. 10a, when PFCA thin film was immersed in the rice samples, the fluorescence intensity decreased slightly. Then, Fe³⁺, Fe²⁺ and H₂O₂ were added to the PBS, respectively. The fluorescence intensity firstly decreased then remained unchanged and finally decreased. Similarly, this phenomenon could be observed in bovine serum samples. (Fig. 10b) Thus the proposed method was feasible and applicable for the analysis of qualitative application in rice samples or bovine serum samples.

3.4.2 Quantitative application in rice sample

To explore the applicability and feasibility of PFCA thin film in real sample analysis, the experiment where we took quantitative analysis of Fe^{3+} in rice samples by the standard addition method for example was made. From Table 2, it can be seen that the results obtained by the proposed method agree well with those in standard sample solutions. Furthermore, the recoveries for different concentration levels were observed in range from 97.6% to 104.0%. Thus the proposed method showed satisfactory results for the quantitative analysis of Fe^{3+} in rice samples.

3.4.3 Quantitative applications in bovine serum sample

Similarly, to further evaluate the applicability and validity of the present method for real samples, the bovine serum spiked with different concentrations of glucose was determined. The results were listed in Table 3 and the values measured by the developed biosensor were in good agreement with the results from the standard addition method. The recoveries of the samples were found to be in the range of 93.7% to 105.6% with the RSD within 5.0%. The recoveries of the samples and the RSD values were satisfactory compared with some reports [57-61], which proved the reliability of the proposed sensing platform for Glu determination in real samples.

4. Conclusions

In conclusion, a high-performance multi-functional PFCA thin film sensor has been successfully developed by one-step electrochemical method and used as multi-functional sensing device for detecting these substances (Fe^{3+} , H_2O_2 and glucose) in a designed sensing platform. Sensitive assays of Fe^{3+} , H_2O_2 and glucose were realized with detection limits of 1 μM , 2.9 μM and 3.4 μM , respectively. Meanwhile, PFCA thin film showed

also high selectivity, good reversibility, reproducibility and practicability. In addition, we also demonstrated the potential application of the present approach in rice or bovine serum samples. It is also noteworthy that H_2O_2 can be released in almost all oxidations catalyzed by oxidases, which suggests that this newly proposed strategy can be readily extended to sense other oxidases and their specific substrates.

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References

- [1] T. Ganz, Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation, *Blood* 102 (2003) 783–788.
- [2] T. Walczyk, F. von Blanckenburg, Natural iron isotope variations in human blood, *Science* 295 (2002) 2065–2066.
- [3] R.U. Onyenwoke, J. Wiegel, Iron (III) reduction: A novel activity of the human NAD (P) H: oxidoreductase, *Biochem. Biophys. Res. Commun.* 353 (2007) 389–393.
- [4] E.C. Theil, Ferritin protein nanocages use ion channels, catalytic sites, and nucleation channels to manage iron/oxygen chemistry, *Curr. Opin. Chem. Biol.* 15 (2011) 304–311.
- [5] T.A. Rouault, The role of iron regulatory proteins in mammalian iron homeostasis and disease, *Nat. Chem. Biol.* 2 (2006) 406–414.
- [6] G. Papanikolaou, K. Pantopoulos, Iron metabolism and toxicity, *Toxicol. Appl. Pharmacol.* 202 (2005) 199–211.
- [7] T.A. Rouault, W.H. Tong, Iron–sulfur cluster biogenesis and human disease, *Trends Genet.* 24 (2008) 398–407.
- [8] M. Andjelkovic, J. Van Camp, B. De Meulenaer, G. Depaemelaere, C. Socaciu, M. Verloo, R. Verhe, Iron-chelation properties of phenolic acids bearing catechol and galloyl groups, *Food Chem.* 98 (2006) 23–31.
- [9] K.D. Welch, T.Z. Davis, S.D. Aust, Iron autoxidation and free radical generation: effects of buffers, ligands, and chelators, *Arch. Biochem. Biophys.* 397 (2002) 360–369.
- [10] G.T. Sucak, Z.A. Yegin, Z.N. Oezkurt, S.Z. Aki, T. Karakan, G. Akyol, The role of

liver biopsy in the workup of liver dysfunction late after SCT: is the role of iron overload underestimated&quest, *Bone Marrow Transplant.* 42 (2008) 461–467.

- [11] J.C. Duvigneau, C. Piskernik, S. Haindl, B. Kloesch, R.T. Hartl, M. Huettemann, I. Lee, T. Ebel, R. Moldzio, M. Gemeiner, H. Redl, A.V. Kozlov, A novel endotoxin-induced pathway: upregulation of heme oxygenase 1, accumulation of free iron, and free iron-mediated mitochondrial dysfunction, *Lab. Invest.* 88 (2008) 70–77.
- [12] W.H. Hoerl, Clinical aspects of iron use in the anemia of kidney disease, *J. Am. Soc. Nephrol.* 18 (2007) 382–393.
- [13] R. Agarwal, N. Vasavada, N.G. Sachs, S. Chase, Oxidative stress and renal injury with intravenous iron in patients with chronic kidneydisease, *Kidney Int.* 65 (2004) 2279–2289.
- [14] D.A. Weinstein, C.N. Roy, M.D. Fleming, M.F. Loda, J.I. Wolfsdorf, N.C. Andrews, Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease, *Blood* 100 (2002) 3776–3781.
- [15] T.J. Wu, C.T. Sempos, J.L. Freudenheim, P. Muti, E. Smith, Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults, *Ann. Epidemiol.* 14 (2004) 195–201.
- [16] X. Huang, Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal, *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 533 (2003) 153–171.
- [17] R.L. Nelson, F.G. Davis, V. Persky, E. Becker, Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis, *Cancer* 76 (1995) 875–879.

- [18] J.A. Duce, A. Tsatsanis, M.A. Cater, S.A. James, E. Robb, K. Wikhe, S.L. Leong, K. Perez, T. Johanssen, M.A. Greenough, H.H. Cho, D. Galatis, R.D. Moir, C.L. Masters, C. McLean, R.E. Tanzi, R. Cappai, K.J. Barnham, G.D. Ciccotosto, J.T. Rogers, A.I. Bush, Iron-export ferroxidase activity of β -amyloid precursor protein is inhibited by zinc in Alzheimer's disease, *Cell* 142 (2010) 857–867.
- [19] S. Altamura, M.U. Muckenthaler, Iron toxicity in diseases of aging: Alzheimer's disease, Parkinson's disease and atherosclerosis, *J. Alzheimer's Dis.* 16 (2009) 879–895.
- [20] L. Silvestri, C. Camaschella, A potential pathogenetic role of iron in Alzheimer's Disease, *J. Cell. Mol. Med.* 12 (2008) 1548–1550.
- [21] X.F. Bao, X.W. Cao, X.M. Nie, Y. Xu, W.H. Guo, B.J. Zhou, L.Y. Zhang, H. Liao, T. Pang, A new selective fluorescent chemical sensor for Fe^{3+} based on rhodamine B and a 1,4,7,10-tetraoxa-13-azacyclopentadecane conjugate and its imaging in living cells, *Sens. Actuators, B* 208 (2015) 54–66.
- [22] D. Kaur, F. Yantiri, S. Rajagopalan, J. Kumar, J.O. Mo, R. Boonplueang, V. Viswanath, R. Jacobs, L. Yang, M.F. Beal, D. DiMonte, I. Volitaskis, L. Ellerby, R.A. Cherny, A.I. Bush, J.K. Andersen, Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease, *Neuron* 37 (2003) 899–909.
- [23] D. Berg, M. Gerlach, M.B.H. Youdim, K.L. Double, L. Zecca, P. Riederer, G. Becker, Brain iron pathways and their relevance to Parkinson's disease, *J. Neurochem.* 79 (2001) 225–236.
- [24] J.E.T. Andersen, A novel method for the filterless preconcentration of iron, *Analyst*

130 (2005) 385–390.

- [25] S. Lunvongsa, M. Oshima, S. Motomizu, Determination of total and dissolved amount of iron in water samples using catalytic spectrophotometric flow injection analysis, *Talanta* 68 (2006) 969–973.
- [26] C.M.G. Van den Berg, Chemical speciation of iron in seawater by cathodic stripping voltammetry with dihydroxynaphthalene, *Anal. Chem.* 78 (2006) 156–163.
- [27] A. Matusch, C. Depboylu, C. Palm, B. Wu, G.U. Hoeglinger, M.K.H. Schaefer, J.S. Becker, Cerebral bioimaging of Cu, Fe, Zn, and Mn in the MPTP mouse model of Parkinson's disease using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), *J. Am. Soc. Mass. Spectrom.* 21 (2010) 161–171.
- [28] S.H. Li, Y.C. Li, J. Cao, J. Zhu, L.Z. Fan, X.H. Li, Sulfur-doped graphene quantum dots as a novel fluorescent probe for highly selective and sensitive detection of Fe^{3+} , *Anal. Chem.* 86 (2014) 10201–10207.
- [29] J.B. Long, Y.X. Liu, Q.F. Cao, Q.P. Guo, S.Y. Yan, X.X. Meng, Sensitive and enzyme-free detection for single nucleotide polymorphism using microbead-assisted toehold-mediated strand displacement reaction, *Chin. Chem. Lett.* 26 (2015) 1031–1035.
- [30] G. Aragay, J. Pons, A. Merkoci, Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection, *Chem. Rev.* 111 (2011) 3433–3458.
- [31] D.T. Quang, J.S. Kim, Fluoro- and chromogenic chemodosimeters for heavy metal ion detection in solution and biospecimens, *Chem. Rev.* 110 (2010) 6280–6301.

- [32] C. Bazzicalupi, C. Caltagirone, Z.F. Cao, V. Lippolis, R. Paolesse, N. Zaccheroni, Multimodal use of new coumarin-based fluorescent chemosensors: towards highly selective optical sensors for Hg^{2+} probing, *Chem. Eur. J.* 19 (2013) 14639–14653.
- [33] D.T. McQuade, A.E. Pullen, T.M. Swager, Conjugated polymer-based chemical sensors, *Chem. Rev.* 100 (2000) 2537–2574.
- [34] L.J. Fan, Y. Zhang, C.B. Murphy, Fluorescent conjugated polymer molecular wire chemosensors for transition metal ion recognition and signaling, *Coord. Chem. Rev.* 253 (2009) 410–422.
- [35] J.S. Wu, W.M. Liu, J.C. Ge, New sensing mechanisms for design of fluorescent chemosensors emerging in recent years, *Chem. Soc. Rev.* 40 (2011) 3483–3495.
- [36] L.J. Fan, Y. Zhang, W.E. Jones, Design and synthesis of fluorescence “turn-on” chemosensors based on photoinduced electron transfer in conjugated polymers, *Macromolecules* 38 (2005) 2844–2849.
- [37] X.G. Li, Y.Z. Liao, M.R. Huang, V. Strong, R.B. Kaner, Ultra-sensitive chemosensors for Fe (III) and explosives based on highly fluorescent oligofluoranthene, *Chem. Sci.* 4 (2013) 1970–1978.
- [38] C.M. Yu, Y.L. Guo, H.T. Liu, N. Yan, Z.Y. Xu, G. Yu, Y. Fang, Y.Q. Liu, Ultrasensitive and selective sensing of heavy metal ions with modified grapheme, *Chem. Commun.* 49 (2013) 6492–6494.
- [39] J.L. Bricks, A. Kovalchuk, C. Trieflinger, M. Nofz, M. Büschel, A.I. Tolmachev, J. Daub, K. Rurack, On the development of sensor molecules that display Fe^{III} -amplified fluorescence, *J. Am. Chem. Soc.* 127 (2005) 13522–13529.

- [40] L.P. Ding, S.H. Wang, Y. Liu, J. Cao, Y. Fang, Bispirene/surfactant assemblies as fluorescent sensor platform: detection and identification of Cu^{2+} and Co^{2+} in aqueous solution, *J. Mater. Chem.* 23 (2013) 8866–8875.
- [41] K. Li, X.Y. Wang, A.J. Tong, A “turn-on” fluorescent chemosensor for zinc ion with facile synthesis and application in live cell imaging, *Anal. Chim. Acta* 776 (2013) 69–73.
- [42] G. Zhang, B.Y. Lu, Y.P. Wen, L.M. Lu, J.K. Xu, Facile fabrication of a cost-effective, water-soluble, and electrosynthesized poly(9-aminofluorene) fluorescent sensor for the selective and sensitive detection of Fe (III) and inorganic phosphates, *Sens. Actuators B* 171–172 (2012) 786–794.
- [43] G. Zhang, Y.P. Wen, C.Q. Guo, J.K. Xu, B.Y. Lu, X.M. Duan, H.H. He, J. Yang, A cost-effective and practical polybenzanthrone-based fluorescent sensor for efficient determination of palladium (II) ion and its application in agricultural crops and environment, *Anal. Chim. Acta* 805 (2013) 87–94.
- [44] P. Li, C.Y. Ji, H.W. Ma, M. Zhang, Y.F. Cheng, Development of fluorescent film sensors based on electropolymerization for iron(III) ion detection, *Chem. Eur. J.* 20 (2014) 5741–5745.
- [45] F.T. Lv, X.L. Feng, H.W. Tang, L.B. Liu, Q. Yang, S. Wang, Development of film sensors based on conjugated polymers for copper (II) ion detection, *Adv. Funct. Mater.* 21 (2011) 845–850.
- [46] M.H. Lee, T.V. Giap, S.H. Kim, Y.H. Lee, C. Kang, J.S. Kim, A novel strategy to selectively detect Fe(III) in aqueous media driven by hydrolysis of rhodamine 6G Schiff base, *Chem. Commun.* 46 (2010) 1407–1409.

- [47] X.F. Wu, B.W. Xu, H. Tong, L.X. Wang, Phosphonate-functionalized polyfluorene film sensors for sensitive detection of iron(III) in both organic and aqueous media, *Macromolecules* 43 (2010) 8917–8923.
- [48] W.C. Ding, J.K. Xu, Y.P. Wen, H. Zhang, J. Zhang, Facile fabrication of fluorescent poly (5-cyanoindole) thin film sensor via electropolymerization for detection of Fe^{3+} in aqueous solution, *J. Photochem. Photobiol. A* 314 (2016) 22–28.
- [49] H. Zhang, G. Zhang, J.K. Xu, Y.P. Wen, B.Y. Lu, J. Zhang, W.C. Ding, Novel highly selective fluorescent sensor based on electrosynthesized poly(9-fluorencarboxylic acid) for efficient and practical detection of iron(III) and its agricultural application, *Sens. Actuators B* 230 (2016) 123–129.
- [50] K. Wannajuk, M. Jamkatoke, T. Tuntulani, B. Tomapatanaget, Highly specific-glucose fluorescence sensing based on boronic anthraquinone derivatives via the GOx enzymatic reaction, *Tetrahedron* 68 (2012) 8899–8904.
- [51] M.U.A. Prathap, B. Kaur, R. Srivastava, Hydrothermal synthesis of CuO micro-/nanostructures and their applications in the oxidative degradation of methylene blue and non-enzymatic sensing of glucose/ H_2O_2 , *J. Colloid Interface Sci.* 370 (2012) 144–154.
- [52] X. Jia, G. Hu, F. Nitze, H.R. Barzegar, T. Sharifi, C.-W. Tai, T. Wagberg, Synthesis of palladium/helical carbon nanofiber hybrid nanostructures and their application for hydrogen peroxide and glucose detection, *ACS Appl. Mater. Inter.* 5 (2013) 12017–12022.

- [53] T. Zhang, H.L. Fan, G.L. Liu, J. Jiang, J.G. Zhou and Q.H. Jin, Different effects of Fe^{2+} and Fe^{3+} on conjugated polymer PPESO₃: a novel platform for sensitive assays of hydrogen peroxide and glucose, *Chem. Commun.* (2008) 5414–5416.
- [54] Y.H. Yi, J.H. Deng, Y.Y. Zhang, H.T. Li, S.Z. Yao, Label-free Si quantum dots as photoluminescence probes for glucose detection, *Chem. Commun.* 49 (2013) 612–614.
- [55] L.Z. Hu, Y.L. Yuan, L. Zhang, J.M. Zhao, S. Majeed, G.B. Xu, Copper nanoclusters as peroxidase mimetics and their applications to H_2O_2 and glucose detection, *Anal. Chim. Acta* 762 (2013) 83–86.
- [56] Y.F. Huan, Q. Fei, H.Y. Shan, B.J. Wang, H. Xua, G.D. Feng, A novel water-soluble sulfonated porphyrin fluorescence sensor for sensitive assays of H_2O_2 and glucose, *Analyst* 140 (2015) 1655–1661.
- [57] Y.Z. He, X.X. Wang, J. Sun, S.F. Jiao, H.Q. Chen, F. Gao, L. Wang, Fluorescent blood glucose monitor by hemin-functionalized graphene quantum dots based sensing system, *Anal. Chim. Acta* 810 (2014) 71–78.
- [58] X.Y. Huang, J.J. Wang, H. Liu, T. Lan, J.C. Ren, Quantum dot-based FRET for sensitive determination of hydrogen peroxide and glucose using tyramide reaction, *Talanta* 106 (2013) 79–84.
- [59] T. Wen, F. Qu, N.B. Li, H.Q. Luo, Polyethyleneimine-capped silver nanoclusters as a fluorescence probe for sensitive detection of hydrogen peroxide and glucose, *Anal. Chim. Acta.* 749 (2012) 56–62.
- [60] J. Yuan, Y. Cen, X.J. Kong, S. Wu, C.L. Liu, R.Q. Yu, X. Chu, MnO_2 -nanosheet-modified upconversion nanosystem for sensitive turn-on fluorescence detection of

H₂O₂ and glucose in blood, ACS Appl. Mater. Inter. 7 (2015) 10548–10555.

- [61] A.L. Hu, Y.H. Liu, H.H. Deng, G.L. Hong, A.L. Liu, X.H. Lin, X.H. Xia, W. Chen, Fluorescent hydrogen peroxide sensor based on cupric oxide nanoparticles and its application for glucose and L-lactate detection, Biosens. Bioelectron. 61 (2014) 374–378.

Figure captions

Scheme 1 A novel platform for selective fluorescent sensing of glucose using electrosynthesized PFCA thin film.

Fig. 1. Multicycle CV of FCA recorded for eight cycles at a scan rate of 200 mV s⁻¹. Inset: electrosynthesis of FCA.

Fig. 2. Normalized UV-vis absorption and emission spectra of PFCA thin film in MeOH-H₂O medium (v/v, 9:1).

Fig. 3. Fluorescence quenching efficiency of the PFCA thin films prepared by different (a) scan cycles (6, 8, 10 and 12) and (b) scan rates (100, 150, 200, 250) in the presence of 1 mM Fe³⁺ ions.

Fig. 4. Fluorescent response of PFCA thin film to some biological species at a concentration of 1 mM.

Fig. 5. Fluorescence emission spectra of the PFCA thin film in the presence of different concentrations of Fe³⁺ (3 μM-1 mM). Inset : I_0/I vs. [Fe³⁺].

Fig. 6. (a) Reversibility of the PFCA thin film in the presence/absence of 0.5 mM Fe³⁺ ions. (b) Fluorescent response of four PFCA thin films fabricated on different days to 0.5 mM Fe³⁺ ions under the same conditions.

Fig. 7. Fluorescence intensity changes of PFCA before (1) and after addition of Fe²⁺ (2), H₂O₂ (3), Fe³⁺ (4) and “Fe²⁺ + H₂O₂” (5), respectively. [Fe²⁺] = [H₂O₂] = [Fe³⁺] = 1 mM.

Fig. 8. Fluorescence emission spectra of a solution of the PFCA thin film and Fe²⁺ upon addition of different concentrations of H₂O₂ (9 μM-1.5 mM). Inset: I_0/I vs. [H₂O₂].

Fig. 9. (a) Fluorescence response of PFCA thin film towards glucose and GOD. (b) Fluorescence emission spectra for the determination of glucose (10 μ M- 0.8 mM). Inset: I_0/I vs. [Glucose].

Fig. 10. Fluorescence response of PFCA thin film in (a) black rice samples successively addition of Fe^{3+} , Fe^{2+} and H_2O_2 and (b) bovine serum samples with successively addition of Fe^{3+} , Fe^{2+} and glucose.

Table 1 A comparison of performance of a variety of glucose sensors based on different materials and analytical methods.

Method	System	LOD (μM)	Range (mM)	Ref.
Fluorometry	HAQB/GOx	11	0.08–0.42	[50]
Electrochemistry	CuO nano/methylene blue	20	0.9–16	[51]
Electrochemistry	Nafion/Pd–HCNFs/GCE	30	0.06–6	[52]
Fluorometry	PPESO ₃	0.43	0–0.005	[53]
Fluorometry	Si QDs	0.68	0.005–0.65	[54]
Colorimetry	Cu nano/GOx/TMB	100	0.1–2	[55]
Fluorimetry	H ₂ TEHPPS	0.32	0–0.005	[56]
Fluorimetry	Hemin-functionalized GQDs/GOx	0.1	0.009–0.3	[57]
Fluorimetry	PFCA thin film	3.4	0.01–0.8	This work

Table 2 Quantitative analysis of Fe³⁺ in rice sample using proposed sensors.

Sample	Fe ³⁺ added (μM)	Fe ³⁺ found (μM)*	Recovery (%)	RSD (%)
Rice	0	2	0	2.1
	50	54	104.0	3.5
	100	103	101.0	2.6
	500	490	97.6	1.8

*, mean of three determination.

Table 3 Quantitative analysis of glucose in serum sample using proposed sensors.

Sample	Glucose added (μM)	Glucose found (μM) [*]	Recovery (%)	RSD (%)
Bovine serum	0	0	–	–
	50	52.8	105.6	2.3
	200	187.4	93.7	3.4
	500	515.7	103.3	4.6

^{*}, mean of three determination.

Figure(s)

Scheme 1

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Biographies

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