



A simple and reusable fluorescent sensor for heme proteins based on a conjugated polymer-doped electrospun nanofibrous membrane

Huaming Wang, Zhou Peng, Yuanyuan Long, Haibo Chen, Yufei Yang, Na Li, Feng Liu*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry, Peking University, Beijing 100871, China

ARTICLE INFO

Article history:

Received 5 March 2012

Accepted 17 March 2012

Available online 22 March 2012

Keywords:

Fluorescent sensor

Electrospun nanofibrous membrane

Conjugated polymer

Heme proteins

Blood sample

ABSTRACT

We reported a simple and reusable fluorescent sensor for heme proteins based on the electrospun nanofibrous membrane doped with a fluorescent conjugated polymer **P**. The sensor showed favorable fluorescence sensing performance towards the heme proteins, including hemoglobin (Hb), myoglobin (Mb) and cytochrome *c* (Cyt *c*). The surface wettability and sensing performance of the electrospun nanofibrous membrane were investigated in detail using Hb as the model. The nanofibrous sensor showed satisfactory reversibility with less than 10% signal loss after nine quenching–regeneration cycles, and good batch-to-batch reproducibility with a relative standard deviation of 3.4% ($n = 3$). The linear range of the sensor for Hb determination was 2.0×10^{-8} to 3.0×10^{-6} M with a detection limit of 1.2×10^{-8} M. The quenching process is mainly based on the fluorescence resonance energy transfer mechanism between the fluorescent conjugated polymer **P** and the heme prosthetic groups, therefore the sensor was selective against most of the common interferents. As an example to evaluate the feasibility of the sensor in practical application, Hb in human blood samples was determined and the results were in good agreement with the data provided by the hospital. To the best of our knowledge, this is the first work using fluorescent electrospun nanofibrous sensor for protein analysis in real biological sample.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Novel approaches to sensitive, reusable and practical sensors have attracted considerable research interests due to the growing demands of simple and low-cost analysis. In order to improve the sensitivity of the sensor, much effort has focused on increasing the specific surface area of the sensing materials. The electrospun nanofibrous membranes possess a series of unique properties including controllable morphology, large surface area-to-volume ratio, and easy preparation procedure, thus are considered as a kind of promising sensing materials [1,2]. Apart from the well studied electrospun sensors for volatile solvents [3,4], nitroaromatic explosives [5–7] and electroactive species [8–10], only a few works about electrospun nanofibrous sensors for biomacromolecules have been reported. Wang et al. combined electrospinning and layer-by-layer adsorption to fabricate fluorescent sensor for cytochrome *c* [11]. Davis et al. reported fluorescent dendrimers-based electrospun nanofibers sensors for protein [12,13]. Luo et al. designed electrospun biosensor for *Escherichia coli* O157:H7 and bovine viral diarrhoea virus [14]. However, these works mainly focus on the

fabrication of the electrospun nanofibrous sensors and the evaluation of their sensing performance, no analysis of real biological sample has been studied. It is still challenging to fabricate electrospun nanofibrous sensor which is highly sensitive and selective for protein determination directly in complex biological matrix.

Heme proteins including hemoglobin, myoglobin, cytochrome *c*, etc. are a class of the most widely distributed metallic proteins containing heme prosthetic groups which are responsible for oxygen delivery, electron transfer, catalysis and many other biological functions in the living organism. The determination of heme proteins is of great importance in the diagnosis of clinical diseases and the monitoring of metabolic pathways. The direct detection of heme proteins using its absorption spectra around 405 nm is low in sensitivity and easily influenced by scattered light and coexisting interferences. The cyanmethemoglobin (HiCN) method provides improved sensitivity for the UV–vis determination of Hb in blood, and is used as the current standard method in hospital. However, it requires the use of toxic cyanide reagent. In recent years, some methods were developed based on electrochemistry [15,16], absorption spectrometry [17,18], fluorimetry [19], chemiluminescence [20], electrophoresis [21] or high-performance liquid chromatography [22]. However, the methods developed still suffer from complexity, matrix interference or reagent instability. In the current efforts, we aimed to develop a novel method for the

* Corresponding author. Tel.: +86 10 62761187; fax: +86 10 62751708.
E-mail address: liufeng@pku.edu.cn (F. Liu).

Table 1
Determination and recoveries of Hb in human blood samples.

Sample	Proposed method ^a (10^{-3} M)	Reference method ^b (10^{-3} M)	Added (10^{-3} M)	Found ^a (10^{-3} M)	Recovery ^a (%)
1	2.53 (4.5%)	2.53	1.72	4.31 (2.9%)	104 (6.7%)
2	2.36 (3.2%)	2.29	1.72	3.98 (3.1%)	95 (7.9%)
3	2.37 (4.0%)	2.34	1.72	4.14 (2.2%)	103 (5.1%)

^a Average of three determination with respective RSD in the parentheses.

^b The data were provided from Peking University Hospital (Beijing, China) using HiCN method.

determination of heme proteins based on a simple and reusable fluorescent electrospun nanofibrous sensor.

Conjugated polymers are high performance fluorophores widely studied in various chemical and biochemical sensing systems due to their large extinction coefficient, high fluorescence quantum yields and the signal amplification “molecular wire effect” [23,24]. In this work, a simple, sensitive and reusable fluorescent sensor for heme proteins was fabricated using the conjugated polymer **P** [6] as the fluorescent probe and electrospun nanofibrous membrane as the porous substrate. The morphology and wettability of the electrospun nanofibrous membrane were characterized. And the fluorescence sensing performance of the sensor for heme proteins were investigated. Hb was taken as a model to evaluate practical application of the heme protein sensor. The present strategy extended the application of electrospinning technique in sensor construction for protein determination in real biological samples.

2. Experimental

2.1. Reagents

Conjugated polymer **P** ($M_n = 18,000$) was synthesized according to the Ref. [6]. Polystyrene (PS, $M_n = 250,000$) was purchased from Acros Organics (NJ, USA). Trimethylchlorosilane (TMCS) was obtained from Shanghai Chemical Reagent Plant (Shanghai, China). Dimethylformamide (DMF) was purchased from Beijing Chemical Reagent Plant (Beijing, China) and distilled before used. Tetrahydrofuran (THF) was obtained from Fluka (Switzerland). Phosphate buffer solution (PBS) was prepared by adjusting 0.02 M NaH_2PO_4 with 1.0 M NaOH to pH 7.4. Hemoglobin, myoglobin, cytochrome c, and fibrinogen (Fib) were offered by Sigma (MO, USA). Lysozyme (LZM) was purchased from Fluka (Switzerland). Bovine serum albumin (BSA) was obtained from Amresco (Solon, USA). All protein stock solutions (1.0×10^{-5} M) were prepared in PBS (pH 7.4). Other chemicals used for interference study were purchased from Beijing Chemical Reagent Plant (Beijing, China). Human blood samples from three different healthy persons were provided by Peking University Hospital (Beijing, China) after the addition of anticoagulant. All the reagents were of analytical grade unless specially mentioned. Deionized water was used for aqueous solutions preparation.

2.2. Apparatus

The **P**-PS electrospun nanofibrous membranes were fabricated using a set of homemade electrospinning setup, which contained a high voltage supply (Beigao, Beijing, China), a syringe pump (Lion, Zhejiang, China) and a grounded rotary collector. The drying of the membranes was carried out in a DZF-6020 vacuum drying oven (Yiheng, Shanghai, China). The morphology of the fibers was observed with a S-4800 scanning electron microscope (Hitachi, Tokyo, Japan). Surface wettabilities of the membranes were obtained using an OCA20 contact angle measuring system (Dataphysics, Stuttgart, Germany). Fluorescence spectra were measured using a F-4500 fluorescence spectrophotometer (Hitachi,

Tokyo, Japan). UV–vis absorption spectra measurements were performed on a U-3010 spectrophotometer (Hitachi, Tokyo, Japan). Fluorescence decays were recorded using a FLS920 time-resolved and steady-state fluorescence spectrometer (Edinburgh Instruments, UK). pH values were measured using a FE20 pH meter (Mettler Toledo, Switzerland). Centrifugation was conducted on a Legend Micro 17 centrifuge (Thermo, Miami, US).

2.3. Methods

2.3.1. Preparation of the **P**-PS electrospun nanofibrous membranes

Stock solution of conjugated polymer **P** was prepared by dissolving 3.6 mg **P** into 10 mL DMF/THF (1:1) mixed solvent. 0.13 g PS was added into 1.0 mL **P**-DMF/THF solution and stirred to obtain homogeneous solution. The solution was loaded into the electrospinning setup and electrospun using typical parameters as follow: the voltage was 15.0 kV, the collecting distance was 15 cm, and the solution feeding rate was 0.40 mL/h. Glass slides with the size of 1.4 cm \times 2.5 cm were treated with TMCS for 24 h to be hydrophobic coated. The **P**-PS electrospun nanofibrous membranes were deposited on the glass slides mounted on the rotary collector with an electrospinning time of 10 min. The membranes were then dried in the vacuum oven at 40 °C for 12 h to remove residual organic solvent. For comparison, **P**-PS dense film was fabricated by spin-coating using the same electrospinning solution.

2.3.2. Fluorescence measurement

All standard protein solutions were prepared by diluting appropriate volumes of stock solution with PBS (pH 7.4). The **P**-PS electrospun nanofibrous sensor was fixed in a 1 cm quartz cell along the diagonal. About 2 mL of the sample solution was then introduced into the cell. The cell was kept at room temperature for 20 min to allow adsorption equilibration. Fluorescence spectra of the membranes were recorded by the fluorescence spectrophotometer in the wavelength range of 350–550 nm with an excitation wavelength of 327 nm.

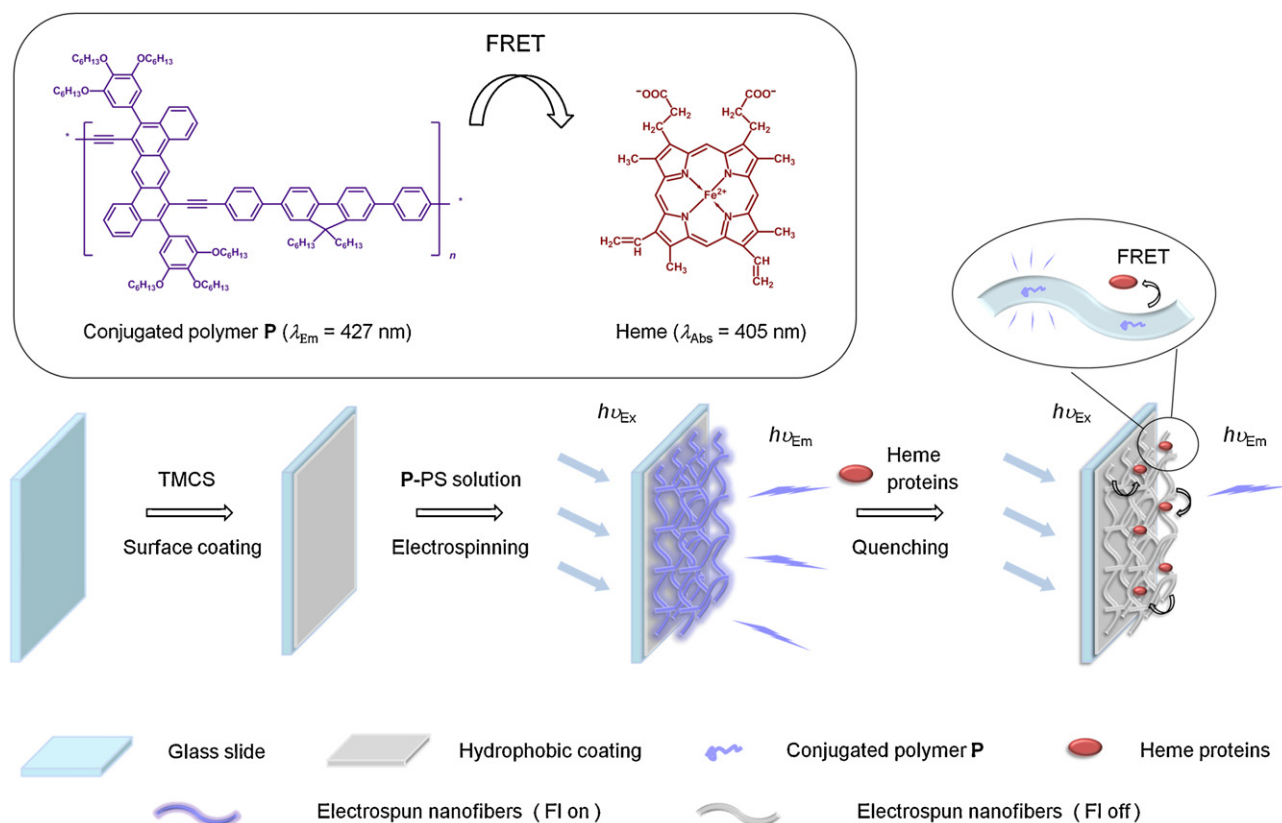
2.3.3. Preparation of human blood sample

The human blood samples were handled as the following procedure. 100 μL blood sample was first diluted to 10 mL with deionized water and shaken vigorously to break the cells. The diluted sample was then centrifuged at 5000 rpm for 30 min. The supernatant was transferred and stored at 4 °C as sample stock solution. The test solution was prepared by further diluting the stock solution 100 times with PBS (pH 7.4).

3. Results and discussion

3.1. Fabrication and sensing rationale of the **P**-PS electrospun nanofibrous sensor

The rationale of the **P**-PS electrospun nanofibrous sensor for heme proteins is shown in Scheme 1. Blank glass slide was first treated with TMCS to generate a hydrophobic surface coating. The **P**-PS nanofibrous membrane was then deposited on the slide via



Scheme 1. Schematic illustration of the fabrication and heme protein sensing procedure of the P-PS electrospun nanofibrous sensor. The insert shows the FRET process between the conjugated polymer **P** and the heme prosthetic groups in heme proteins.

an electrospinning approach. The conjugated polymer **P** doped nanofibers had a strong emission maximum at 427 nm, while the maximum absorption spectrum of heme prosthetic groups located around 405 nm, which provided the spectral overlap to facilitate the fluorescence resonance energy transfer (FRET). In the sensing procedure, the protein molecules were adsorbed on the surface of the P-PS nanofibrous membrane via hydrophobic interaction, thus FRET [13,25] would be expected to occur from the conjugated polymer **P** to the heme prosthetic groups, leading to the fluorescence quenching of the sensor.

3.2. Preparation and characterization of the P-PS electrospun nanofibrous membranes

Polystyrene (PS) was chosen as the host matrix for electrospinning due to its controllable molecule weight, good chemical stability, and suitable compatibility with the conjugated polymer. The electrospinning process was conducted with a typical condition described in Section 2.3.1. Fig. 1a–c shows the scanning electron microscope (SEM) images of nanofibers obtained from 0.13 g/mL PS in mixed solution of various DMF/THF ratio (v/v) from 1:5 to 1:1. As can be seen, the increase DMF in the mixed solvent led to less bead formation, which was attributed to the elevation of the solvent boiling point. Further addition of DMF lowered the solubility of **P** in the mixed solvent, thus weaken the fluorescence intensity of the sensing membrane. Therefore, the final solvent composition was chosen as DMF/THF = 1:1. The optimal P-PS electrospun nanofibers exhibited uniform and smooth morphology with average diameters of 400–500 nm, no bead was observed along the nanofibers (Fig. 1c). The random arrangement of the one-dimensional nanofibers built up three-dimensional nanoarchitecture with large amount of

porous structures within the membrane, which provided large surface area for protein adsorption and led to great enhancement in speed and sensitivity for sensing application.

Wettability is an important property revealing the chemical composition and charge density of a solid surface. The wetting properties of various surfaces were investigated (Fig. 2). The water contact angles (WCAs) of the glass slides before and after TMCS hydrophobic surface coating were $23.1 \pm 1.6^\circ$ (Fig. 2a) and $79.9 \pm 4.2^\circ$ (Fig. 2b), respectively. The electrospinning deposition of P-PS nanofibrous membrane further raised the WCA to $136.5 \pm 2.0^\circ$ (Fig. 2c). The hydrophobicity of the nanofibrous membrane was attributed to both the nonpolar PS matrix and the rough network structure. During the sensing procedure, the WCA of P-PS nanofibrous membrane after dipped in Hb solution reduced significantly to $36.4 \pm 9.9^\circ$ (Fig. 2d), while the membrane in blank PBS solution remained $134.7 \pm 3.4^\circ$ (Fig. 2e). The dramatically decrease in WCA indicated the adsorption of heme proteins on the nanofibers, which contributed to the sensitivity of the P-PS electrospun nanofibrous sensor.

3.3. Fluorescence properties of the P-PS electrospun nanofibrous sensor for heme proteins

3.3.1. Fluorescence quenching response of the sensor

The spectral and quenching responses of the sensing system are shown in Fig. 3. The P-PS electrospun nanofibrous sensor had a sharp emission maximum at 427 nm with the excitation of 327 nm (Fig. 3a). Comparing with the emission spectrum of **P** in CHCl_3 solution (Fig. 3b), no significant red-shift was observed, indicating that the fluorescence property of the conjugated polymer **P** in the nanofibrous membrane was well maintained. The absorption

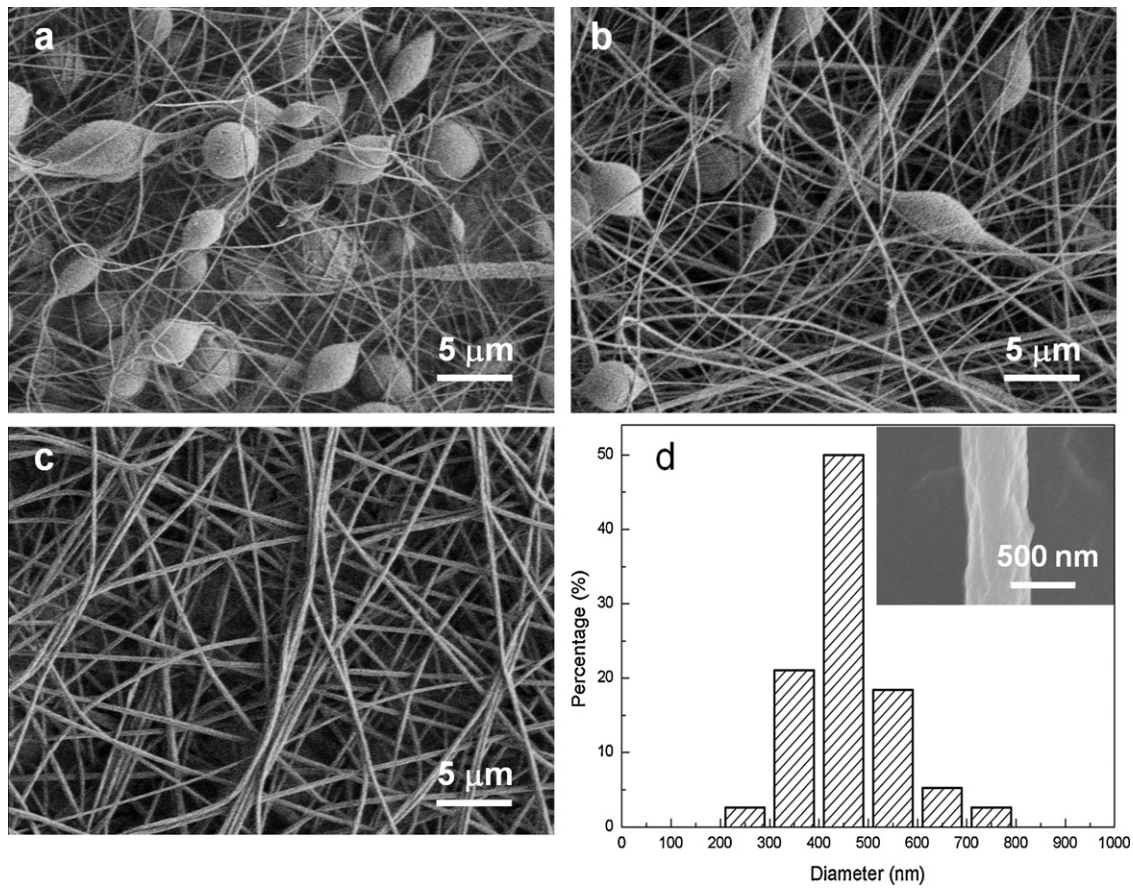


Fig. 1. SEM images of P-PS electrospun nanofibrous membranes from different solvents of DMF/THF ratio: 1:5 (a), 1:3 (b), 1:1 (c), and diameter distribution of 50 independent optimal nanofibers (d). Insert: the zoomed-in image of a single nanofiber.

peaks of heme proteins were around 405 nm (Fig. 3c–e), which partially overlapped with the emission of P. Therefore, FRET process occurred from P to the heme prosthetic groups when the heme proteins were adsorbed on the nanofibers, resulting fluorescence quenching of the sensor [13,25]. Among three heme proteins, Hb induced highest quenching signal of the sensor, thus was chosen

as the model for further investigation of the performance of the sensor.

3.3.2. Optimization of the sensing conditions

Sensing conditions including membrane thickness and solution pH were optimized. The effect of membrane thickness on the

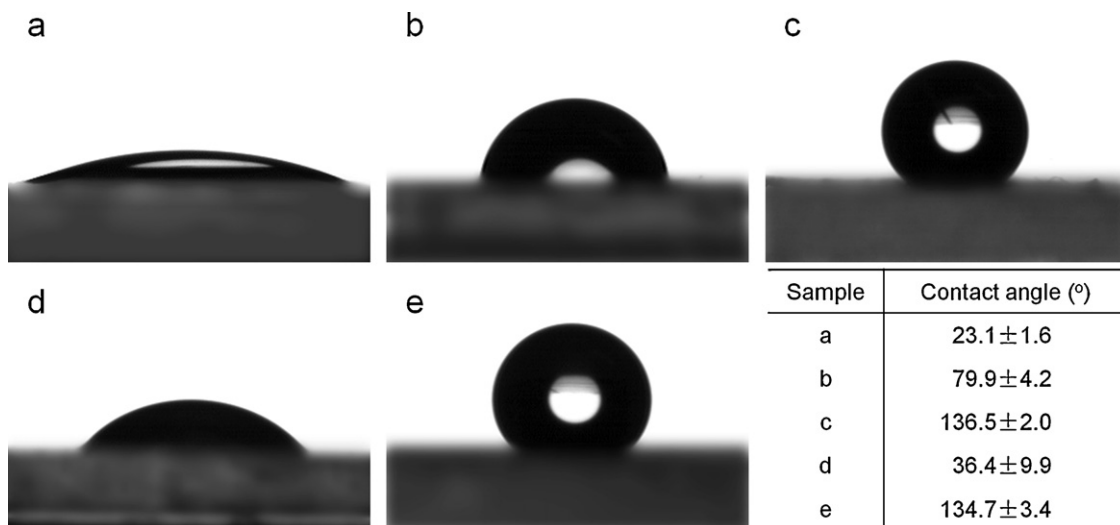


Fig. 2. Photographs of water contact angles (WCAs) on original glass slide (a), TMCS surface coated glass slide (b), the as-spun P-PS nanofibrous membrane (c), the P-PS nanofibrous membranes after dipped in PBS with (d) and without (e) 5×10^{-7} M Hb.

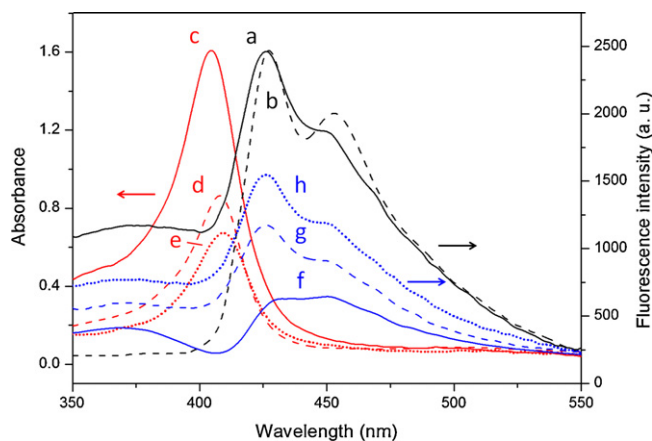


Fig. 3. Spectral properties of the sensing system: the emission spectra of the **P**-PS electrospun nanofibrous sensor (a) and **P** in CHCl_3 solution (2.0×10^{-8} M) (b), the absorption spectra of Hb (c), Mb (d) and Cyt c (e) solutions (5.0×10^{-6} M), and the fluorescence emission of the sensor after quenched in Hb (f), Mb (g) and Cyt c (h) solutions (5.0×10^{-6} M).

properties of the fluorescent sensor was investigated by varying the electrospinning time from 5 to 20 min (Fig. 4). As can be seen, the sensitivity of the sensor represented as equilibrated F_0/F increased along with the reduction of the membrane thickness (Fig. 4a, c, and d). However, further thinning led to instability and dissociation of the sensing membrane from the glass slide after 15 min immersing in Hb solution (Fig. 4b), while the others remained stable during the experiment. The membrane by 10 min electrospinning was chosen as the sensing membrane which had the highest sensitivity as well as good stability. Comparing with the spin-coated film (Fig. 4e), the electrospun nanofibrous membranes showed much higher sensitivity for Hb, which again demonstrated the advantage of the enlarged surface area-to-volume ratio of the electrospun nanofibrous membranes. It is appealing that the easy fabrication of the proposed sensor requires only 10 min of electrospinning, which provides extensible potential for mass production and routine analysis.

The influence of solution pH in the range of 2.0–12.0 on the sensitivity of the sensor was also investigated. The fluorescence quenching response reached a maximum around pH 7.0 for Hb

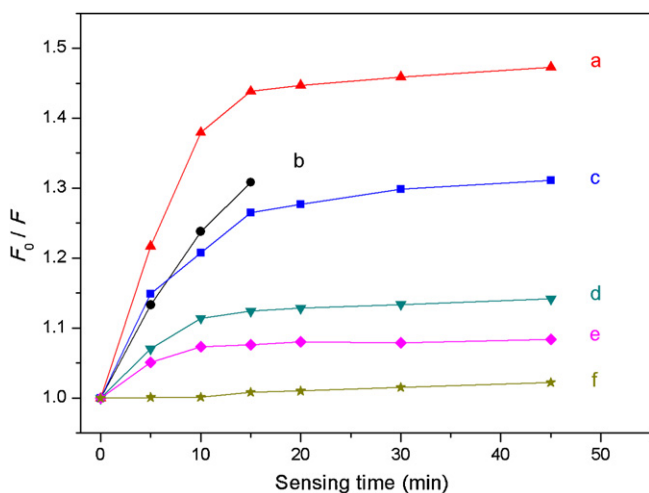


Fig. 4. Effect of membrane thickness on sensitivity and stability of the **P**-PS electrospun nanofibrous sensor for 5.0×10^{-7} M Hb solution. The electrospinning times: 10 min (a), 5 min (b), 15 min (c), and 20 min (d). For comparison: spin-coated film in 5.0×10^{-7} M Hb solution (e), the **P**-PS electrospun nanofibrous membrane (10 min) in blank PBS (f).

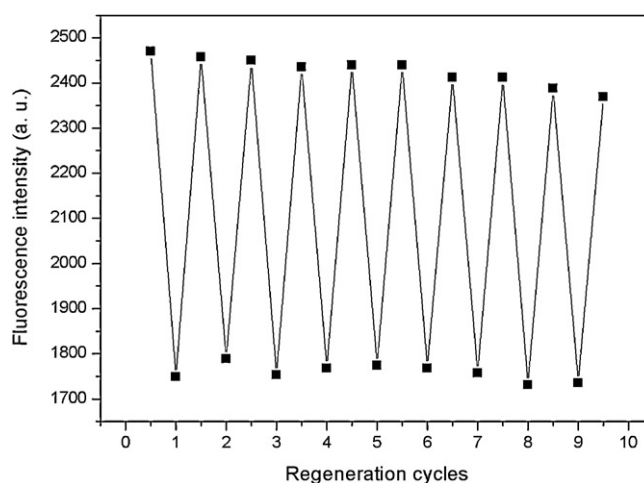


Fig. 5. Fluorescence intensity of the sensing membrane at 427 nm during nine times of regeneration cycles. Quenching condition: 1.0×10^{-6} M Hb solution for 5 min. Regeneration condition: immersed in 50 mL blank PBS for 20 min, rinsed with deionized water, and dried in air.

sensing. The proper explanation is that Hb has an isoelectric point of 6.8, thus was charge-free and more apt to be adsorbed on the surface of the **P**-PS nanofibers in neutral pH. Hence, all the sensing experiments below were performed under pH 7.4 to mimic the physiological ionic conditions.

3.3.3. Sensing performances of the sensor

As important parameters to evaluate a practical sensor, the stability, reproducibility, reusability and selectivity of the **P**-PS electrospun nanofibrous sensor were investigated. The sensing membrane dipped in PBS lost less than 5% fluorescence intensity under continuous excitation for 2 h, indicating satisfactory photostability of the sensor. Three sensing membranes produced from three batches showed a good reproducibility in the average $F_0/F = 1.46$ for 5.0×10^{-7} M Hb solution with a relative standard deviation (RSD) of 3.4%. Since Hb was adsorbed on the nanofibers via non-covalent hydrophobic interaction, the sensor could be recovered by simply immersing in 50 mL PBS for 20 min. As shown in Fig. 5, more than 90% signal intensity of the sensing membrane remained after nine quenching–regeneration cycles, indicating excellent reusability of the sensor. The selectivity of the sensor was also investigated and shown in Fig. 6. Heme proteins including Hb, Mb, Cyt c lead to obvious fluorescence quenching of the sensor, while no significant quenching response was observed for other proteins including BSA, LZM, Fib. All the results demonstrated remarkable sensing performances of the **P**-PS electrospun nanofibrous sensor.

3.3.4. Linear calibration range and detection limit of the sensor

Under the optimized conditions, the fluorescence spectral responses of the **P**-PS electrospun nanofibrous sensor to Hb of different concentrations are illustrated in Fig. 7. F_0/F of the sensor showed a linear relationship with the concentration of Hb in the range of 2.0×10^{-8} to 3.0×10^{-6} M. The linear regression equation was $F_0/F = 8.49 \times 10^5 c(\text{Hb}) + 1.051$ ($r = 0.9967$). The limit of detection (LOD) of the sensor to Hb was 1.2×10^{-8} M (signal/noise ratio = 3). The RSD of the sensor for nine determinations of Hb (5.0×10^{-7} M) was 4.3%. These results indicated that the sensitivity and precision of the present method were good enough for the determination of heme proteins in blood or other biological samples.

Table 2
Comparison of analytical performance of various methods for determination of Hb.

Method/technique	Linear range/ detection limit (M)	Reusability	Sample analysis	Special requirement	Ref.
Hydroxylamine induced replacement reaction, polarography	1.5×10^{-10} to 2.9×10^{-7}	–	Hb in blood sample	Dropping mercury electrode	[15]
Hb modified pencil lead electrode, cyclic voltammetry	1.5×10^{-7} to 2.0×10^{-6} , 1.1×10^{-7}	4 cycles	–	–	[16]
Catalytic oxidation of o-phenylenediamine, UV–vis	6.5×10^{-11} to 8.5×10^{-5} , 3.2×10^{-12}	–	Hb in spiked urine sample	Strict temperature control	[17]
HiCN and HbO ₂ method, ^a microfluidic absorption photometry	4.7×10^{-4} to 3.1×10^{-3}	–	Hb in blood sample	Toxic cyanide solution	[18]
Spiro form rhodamine B hydrazide in micellar medium, fluorescence	2.0×10^{-10} to 1.2×10^{-8} , 8.6×10^{-11}	–	Hb in blood sample	–	[19]
β -Cyclodextrin sensitized chemiluminescence	3.1×10^{-11} to 1.6×10^{-7} , 1.9×10^{-11}	–	Hb in blood and serum samples	–	[20]
Capillary zone electrophoretic, photodiode-array detection	2.0×10^{-13} to 6.0×10^{-10} , 6.0×10^{-14}	–	Cyt c in mitochondrial extracts and cytosolic fractions	–	[21]
HPLC coupled to enzyme amplified biochemical detection	2.5×10^{-9} to 5.0×10^{-8} , 1.0×10^{-9}	–	Hb in spiked urine sample	Enzyme labeling	[22]
Fluorescent conjugated polymer P doped nanofibrous sensing membrane, fluorescence	2.0×10^{-8} to 3.0×10^{-6} , 1.2×10^{-8}	9 cycles	Hb in blood sample	–	This work

^a HiCN and HbO₂ are the abbreviation for cyanmethemoglobin and oxihemoglobin methods, respectively.

3.4. Human blood sample analysis

In order to evaluate the practicality of the P–PS electrospun nanofibrous sensor for heme proteins, we applied the sensor for the determination of hemoglobin in human blood samples. The tolerance of the sensor towards most of the possible interferences in a relative error of less than 5% was investigated. Most anions (CO_3^{2-} , $\text{C}_2\text{O}_4^{2-}$, SO_4^{2-} , NO_3^- , Br^- , and I^-), cations (Na^+ , K^+ , NH_4^+ , Mg^{2+} , and Ca^{2+}), neutral compounds (vitamin C, glucose, and urea) or amino acids (glycine, phenylalanine, and tyrosine) had minimal influence on Hb sensing even at 1000 times molar ratio. While the tolerance concentrations for proteins (γ -globulin, Fib, HSA, and LZM) were in

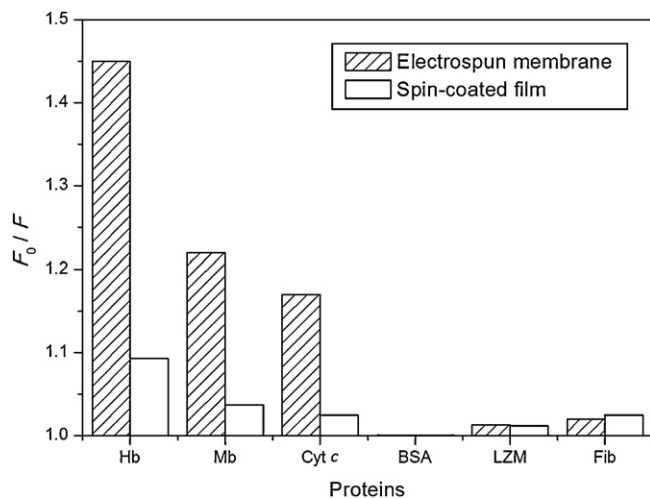


Fig. 6. The fluorescence quenching responses in F_0/F of the electrospun nanofibrous membrane and spin-coated film to heme proteins (Hb, Mb, and Cyt c) and other proteins (BSA, LZM, and Fib). All the protein concentrations were 5.0×10^{-7} M.

the same order of magnitude as the concentration of Hb. Considering Hb was the most-abundant protein in blood, the interference of other proteins could be minimized by sample dilution. Thus the proposed sensor can be applied to direct Hb determination in blood samples without complicated sample pretreatment.

According to the procedure described in Sections 2.3.2 and 2.3.3, Hb contents in three healthy human blood samples together with the recoveries of the method were determined. As listed in Table 1, the results of Hb using the proposed P–PS electrospun nanofibrous sensor were in good agreement with the values of the

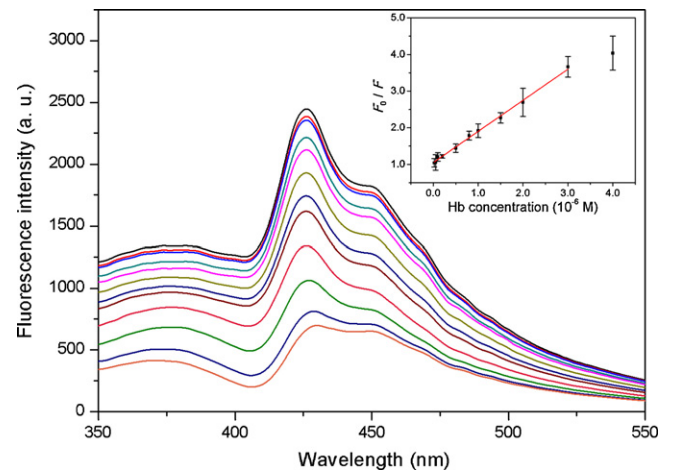


Fig. 7. Fluorescence spectral response of the P–PS electrospun nanofibrous sensor to Hb solutions of increasing concentrations from top to bottom: 0 , 2.0×10^{-8} , 5.0×10^{-8} , 1.0×10^{-7} , 2.0×10^{-7} , 5.0×10^{-7} , 8.0×10^{-7} , 1.0×10^{-6} , 1.5×10^{-6} , 2.0×10^{-6} , 3.0×10^{-6} , 4.0×10^{-6} M. Inset: the linear relationship between F_0/F and Hb concentration in the range of 2.0×10^{-8} to 3.0×10^{-6} M. The error bars represent the standard deviation (SD) of three measurements.

same samples provided by Peking University Hospital, while the recoveries of the method were 95–104% with the RSD of 5.1–7.9%. The results demonstrated that the sensor had a satisfactory reproducibility and accuracy for the determination of Hb in human blood samples. Table 2 listed the comparison of the analytical performances of various methods in the literatures (Refs. [15–22]). It is notable that all the methods are theoretically sensitive enough for the real sample analysis since the Hb concentration in blood sample is at 10^{-3} M level. Comparing with the pervious reported methods, the sensor proposed in this work was provided with simple fabrication procedure and favor reusability, which also avoided the use of toxic/instable reagents or strict condition control, proffering a promising strategy for the simple, cost-effective and environment-friendly sensing application in biological samples.

4. Conclusion

In summary, a sensitive and cost-effective fluorescent sensor for heme proteins was developed based on the P-PS electrospun nanofibrous membrane. The nanofibrous sensor showed sensitive fluorescence quenching response to hemoglobin, myoglobin, cytochrome *c*, and exhibited excellent stability, reproducibility, reusability and selectivity. The results of Hb determination in human blood samples were in good agreement with the data provided by hospital with satisfying recoveries of 95–104%, demonstrating the practicality of the heme protein sensor. Comparing with the current method, the proposed sensor was simple and straightforward, which avoided complex sample pretreatment or the use of instable reagents. The strategy herein extends the use of electrospinning technique for sensing application, which opens up new possibilities for the construction of simple and reusable sensors for other bioactive substances in real samples.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (20975006, 21035005 and 20675003).

The authors would like to thank Prof. J. Pei's group of Peking University for synthesizing the fluorescent conjugated polymer, and also thank Peking University Hospital for providing human blood samples with reference data.

References

- [1] D. Li, Y.N. Xia, *Adv. Mater.* 16 (2004) 1151–1170.
- [2] A. Greiner, J.H. Wendorff, *Angew. Chem. Int. Ed.* 46 (2007) 5670–5703.
- [3] J. Yoon, Y.S. Jung, J.M. Kim, *Adv. Funct. Mater.* 19 (2009) 209–214.
- [4] Z.Y. Li, Y. Dzenis, *Talanta* 85 (2011) 82–85.
- [5] S.Y. Tao, G.T. Li, J.X. Yin, *J. Mater. Chem.* 17 (2007) 2730–2736.
- [6] Y.Y. Long, H.B. Chen, Y. Yang, H.M. Wang, Y.F. Yang, N. Li, K.A. Li, J. Pei, F. Liu, *Macromolecules* 42 (2009) 6501–6509.
- [7] Y.F. Yang, H.M. Wang, K. Su, Y.Y. Long, Z. Peng, N. Li, F. Liu, *J. Mater. Chem.* 21 (2011) 11895–11900.
- [8] J.S. Huang, Y. Liu, H.Q. Hou, T.Y. You, *Biosens. Bioelectron.* 24 (2008) 632–637.
- [9] Y. Ding, Y. Wang, L. Su, M. Bellagamba, H. Zhang, Y. Lei, *Biosens. Bioelectron.* 26 (2010) 542–548.
- [10] F. Cao, S. Guo, H.Y. Ma, G.C. Yang, S.X. Yang, J. Gong, *Talanta* 86 (2011) 214–220.
- [11] X.Y. Wang, Y.G. Kim, C. Drew, B.C. Ku, J. Kumar, L.A. Samuelson, *Nano Lett.* 4 (2004) 331–334.
- [12] B.W. Davis, N. Niamnont, C.D. Hare, M. Sukwattanasinitt, Q. Cheng, *ACS Appl. Mater. Interface* 2 (2010) 1798–1803.
- [13] B.W. Davis, N. Niamnont, R. Dillon, C.J. Bardeen, M. Sukwattanasinitt, Q. Cheng, *Langmuir* 27 (2011) 6401–6408.
- [14] Y.L. Luo, S. Nartker, H. Miller, D. Hochhalter, M. Wiederoder, S. Wiederoder, E. Setterington, L.T. Drzal, E.C. Alocilja, *Biosens. Bioelectron.* 26 (2010) 1612–1617.
- [15] D.B. Luo, J.X. Huang, *Anal. Chem.* 81 (2009) 2032–2036.
- [16] M.R. Majidi, A. Saadatirad, E. Alipour, *Electroanalysis* 23 (2011) 1984–1990.
- [17] K. Zhang, R.X. Cai, D.H. Chen, L.Y. Mao, *Anal. Chim. Acta* 413 (2000) 109–113.
- [18] T. Noda, H. Takao, K. Yoshioka, N. Oku, M. Ashiki, K. Sawada, K. Matsumoto, M. Ishida, *Sens. Actuators B* 119 (2006) 245–250.
- [19] X.F. Yang, X.Q. Guo, H. Li, *Talanta* 61 (2003) 439–445.
- [20] S.C. Zhang, H.X. Ju, *Anal. Chim. Acta* 475 (2003) 163–170.
- [21] L. del Valle-Mondragon, M. Ramirez-Ortega, G. Zarco-Olvera, A. Sanchez-Mendoza, G. Pastelin-Hernandez, F.A. Tenorio-Lopez, *Talanta* 74 (2008) 478–488.
- [22] M.R. van Bommel, A.P.J.M. de Jong, U.R. Tjaden, H. Irth, J. van der Greef, *J. Chromatogr. A* 886 (2000) 19–29.
- [23] L.H. Chen, D.W. McBranch, H.L. Wang, R. Helgenson, F. Wudl, D.G. Whitten, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 12287–12292.
- [24] S.W. Thomas, G.D. Joly, T.M. Swager, *Chem. Rev.* 107 (2007) 1339–1386.
- [25] M. Liu, P. Kaur, D.H. Waldeck, *Langmuir* 21 (2005) 1687–1690.