Biosensors and Bioelectronics 52 (2014) 304–309

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An eco-friendly, simple, and sensitive fluorescence biosensor for the detection of choline and acetylcholine based on C-dots and the Fenton reaction

Jianfei Wei [a,b], Jun Ren [a], Jing Liu [a], Xianwei Meng [a,*], Xiangling Ren [a], Zhenzhen Chen [a], Fangqiong Tang [a,*]

[a] Laboratory of Controllable Preparation and Application of Nanomaterials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, No. 29, Zhongguancun East Road, Haidian District, Beijing 100190, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

ARTICLE INFO

Article history:
Received 31 July 2013
Accepted 4 September 2013
Available online 14 September 2013

Keywords:
Carbon dots
Fluorescence
Choline
Acetylcholine
Fenton reaction

ABSTRACT

A simple and novel method is proposed for the preparation of Carbon dots (C-dots) with excellent properties. We firstly demonstrated that the fluorescence of C-dots decreased apparently in the presence of H_2O_2 and Fe^{2+}. Based on the this finding, C-dots are successfully adopted as probes for the detection of H_2O_2. After the experimental conditions are optimized, the limit of detection (LOD) for H_2O_2 is found to be 0.1 μM. Furthermore, we established an eco-friendly, simple and sensitive biosensor for the detection of choline and acetylcholine (ACh) based on the detection of H_2O_2 using C-dots as probes. The detection limit for choline is 0.1 μM and the linear range is 0.1–40 μM. The detection limit for ACh is found to be 0.5 μM and the linear range is 0.5–60 μM. The excellent performance of the proposed biosensor shows that this method possesses the potential for practical application.

1. Introduction

As a kind of strong organic base, choline is the component of lecithin. Furthermore, it participates in the synthesis of ACh, which is one of the most significant neurotransmitters in both peripheral and central nervous systems of mammals. Choline and ACh play an essential role in many biological processes. They affect learning, memory and emotions and regulate the movement of skeletal muscle and cardiac muscle (Sattarahmady et al., 2010; Wessler et al., 1998). An abnormal amount of these metabolites causes some neuropsychiatric disorders such as Alzheimer's and Parkinson's diseases, progressive dementia and schizophrenia (Fintelmann and Lindner, 1972; Ikarashi et al., 2004; Newhouse et al., 2004; Pepeu and Giovannini, 2004; Savage et al., 2003; Sletten et al., 2005; White and Cummings, 1996). Thus, the development of a simple method to sensitively and selectively detect choline and ACh has received a lot of attention. Numerous kinds of methods have been applied in the detection of choline and ACh, such as high performance liquid chromatography (HPLC) detection (Dunphy and Burinsky, 2003; Kuribayashi et al., 2007), electrochemical detection (Çevik et al., 2012; Jia et al., 2012; Li et al., 2012; Pundir et al., 2012; Ren et al., 2009; Sattarahmady et al., 2010; Wu et al., 2011), colorimetric detection (Fiamegos et al., 2002; M et al., 1977), chemiluminescence detection (Buiculescu et al., 2010; Burmeister et al., 2008; Dai et al., 2010; Wei et al., 2011, 2009) and fluorescent detection (Chen et al., 2011). Of all these methods, fluorescent sensors have several advantages over the other methods, such as rapidity, specificity, sensitivity and real-time monitoring (Jung et al., 2009; Lai et al., 2003; Numata et al., 2005). As a new kind of harmless and eco-friendly fluorescent nanometer-sized particles, carbon dots (C-dots) are ideal materials used as a fluorescent probes, which is the core component of fluorescent sensors. Futhermore, the preparation of C-dots does not need stringent, tedious, and costly preparation steps. To the best of our knowledge, sensing of choline and ACh using C-dots has not been reported so far.

In this paper, we have constructed an eco-friendly, simple and sensitive biosensor for the detection of choline and ACh using C-dots. C-dots were prepared using the hydrothermal method and had been utilized as a fluorescence probe for the detection of H_2O_2 in the presence of Fe^{2+}. It was found that the fluorescence of C-dots was quenched linearly by H_2O_2 in a certain concentration range (0.1–40 μM). Based on the detection of H_2O_2, an eco-friendly, simple and sensitive fluorescence sensor for the determination of choline and ACh was constructed. The biosensor showed a wide linear detection range, good selectivity, and low detection limit.

* Corresponding authors. Tel.: +86 10 82543521; fax: +86 10 62554670.
E-mail addresses: mengxw@mail.ipc.ac.cn (X. Meng), tangfq@mail.ipc.ac.cn (F. Tang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.006

2. Material and methods

2.1. Materials

Polyvinylpyrrolidone (average molecular weight 360,000), choline, ACh, acetylcholinesterase (AChE, EC 3.1.1.7, from Electrophorus electricus (electric eel.), 425.94 Units/mg), choline oxidase (ChOx, EC 1.1.3.17, from Alcaligenes Sp. Lyophilized powder, 13 Units/mg) were purchased from Sigma-Aldrich. Sodium dihydrogen phosphate, disodium hydrogen phosphate, and ammonium iron (II) sulfate hexahydrate were purchased from Xilong Chemical Co., Ltd.

All reagents used are of analytical grade and used without further purification. All solutions are prepared using ultrapure water and the ultrapure water (0.22 μm) is produced using a Millipore-Q water system. Phosphate Buffer (PB) (0.05 M, pH 8.0): 8.4790 g $Na_2HPO_4 \cdot 12H_2O$, 0.2068 g $NaH_2PO_4 \cdot 2H_2O$, and 500 mL ultrapure water. The stock solutions of choline and ACh are freshly diluted with 0.05 M PB.

2.2. Apparatus

Fluorescence property is detected with a Cary Eclipse fluorescence spectrophotometer (from Varian, Inc.), and the UV–vis absorption spectra are measured by a UV–vis spectrometer (from Jasco V-570). The solution for the fluorescence measurement is put in a quartz fluorescence cuvette with 10 mm optical path length, while the emission slit and the exciting slit are 5 nm. The emission spectra excited at 330 nm are detected in the wavelength of 350–530 nm.

2.3. Synthesis of water-soluble C-dots using the hydrothermal method

Water-soluble C-dots are prepared using the hydrothermal method. Briefly, 0.24 g PVP is dissolved in 8 mL water and loaded into a 25 mL Teflon-lined stainless steel autoclave. After that, the autoclave is kept at 240 °C for 9 h. The product can be used after being filtered with cylinder filtration membrane filter (0.22 μm).

2.4. Fluorescence measurements

The procedure for H_2O_2 detection is described as follows: 16.5 μL C-dots is diluted into a volume of 1 mL with PB solution (pH 8.0) containing hydrogen peroxide with different concentrations. After that, the pH value of the solution is adjusted to 3.0 with 1 M HCl and the fluorescence intensity is recorded (F_0). After the

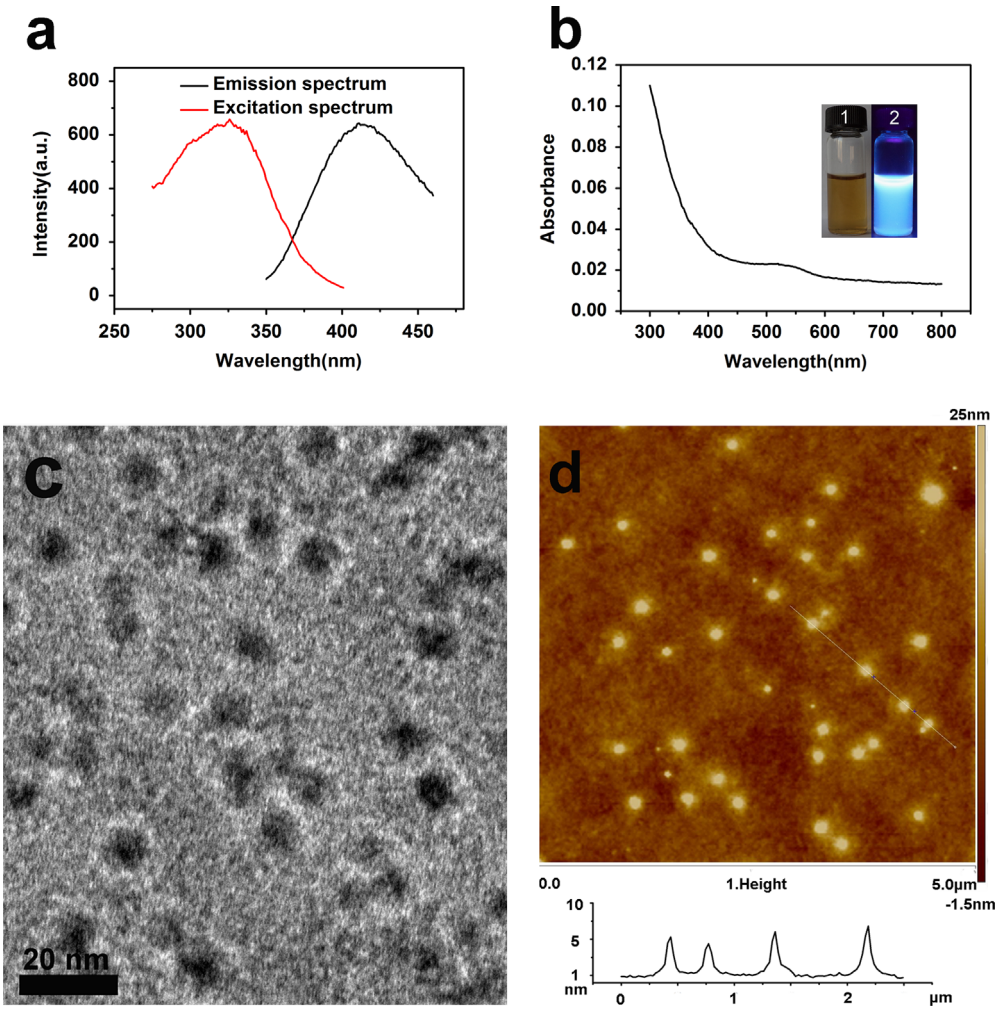

Fig. 1. (a) Photoluminescence spectrum of the C-dots, (b)UV–vis absorption spectrum of the C-dots, the insets in Fig. 1(b) are the photographs of the solution of C-dots under natural light (1) and UV light (254 nm) (2), (c) TEM image of the C-dots, and (d) AFM image of the C-dots. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

solution containing Fe^{2+} is added to the obtained mixture, the fluorescence intensity is recorded at different time (F_t).

The procedure for choline detection is described as follows: firstly, the choline solution is prepared by dissolving different amount of choline in 0.05 M PB solution (pH 8.0). Then, ChOx is added into the choline solution and is kept in room temperature for 30 min. 16.5 μL C-dots is added to the 1 mL above reaction solution. After pH value is adjusted to 3.0 with 1 M HCl, F_0 and F_t were measured the same as the operation in H_2O_2 detection.

The procedure for ACh detection is described as follows: firstly, the ACh solution with different concentrations is prepared by dissolving ACh in 0.05 M PB solution (pH 8.0). Then, AChE and ChOx are added to the choline solution, which is kept in room temperature for 30 min. 16.5 μL C-dots is added to the 1 mL above reaction solution. After the pH value is adjusted to 3.0 with 1 M HCl, F_0 and F_t were measured the same as the operation in H_2O_2 detection.

3. Results and discussion

3.1. Characterization of C-dots

C-dots were synthesized in aqueous solution by a novel method. The as-prepared C-dots were characterized by fluorescence spectroscopy, UV–vis absorption spectroscopy, transmission electron microscopy (TEM) and atomic force microscopy (AFM). To evaluate the optical properties of C-dots, the PL emission and excitation spectrum (Fig. 1(a)) and UV–vis absorption spectrum (Fig. 1(b)) were investigated. The PL spectrum of C-dots showed a 410 nm emission peak (black line) under the excitation of 330 nm, while the fluorescence excitation spectrum showed a peak centered at 330 nm (red line) upon emission at 410 nm. The inset in Fig. 1(b) shows that the as-prepared C-dots water solution was transparent brown in color under visible light (1), while it emitted a blue fluorescence under UV light (254 nm) (2). According to Fig. 1(c), the C-dots are well dispersed and the diameter is about 5–12 nm, which is supported by AFM results (Fig. 1(d)).

3.2. Fluorescence quenching of C-dots in the presence of H_2O_2 and Fe^{2+}

Firstly, the fluorescence change of C-dots in the presence of H_2O_2 is investigated. According to Fig. 2(a), the fluorescence intensity of C-dots solution containing 0.5 mM H_2O_2 at 410 nm changes inconspicuously. The result shows that C-dots are insensitive to H_2O_2. Secondly, the influence of Fe^{2+} on the fluorescence intensity of C-dots is studied. The result demonstrates that the fluorescence of C-dots changes slightly in the presence of Fe^{2+} (0.5 mM) (Fig. 2(a)). However, the fluorescence intensity of C-dots decreases apparently, when Fe^{2+} and H_2O_2 are simultaneously added into the C-dots solution. Fig. 2(b) shows the time-dependent fluorescence quenching of the C-dots in the presence of Fe^{2+} (0.338 mM) and H_2O_2 (25 μM). According to the above results, we propose that Fe^{2+} and H_2O_2 are needed for the quenching of C-dots. As is well known, H_2O_2 reacts with Fe^{2+} to yield the hydroxyl radical (OH) in sulfuric acid, which is called as the Fenton reaction. Hence, it is possible that the ·OH produced from the Fenton reaction cause the quenching of C-dots.

3.3. Detection of hydrogen peroxide on the basis of the Fenton reaction

We have optimized the experimental conditions for the detection of H_2O_2 (20 μM), including pH and the concentration of Fe^{2+}. We used ΔF as a signal for the detection of H_2O_2, where $\Delta F = F_t - F_0$. We first investigated the influence of pH on ΔF values

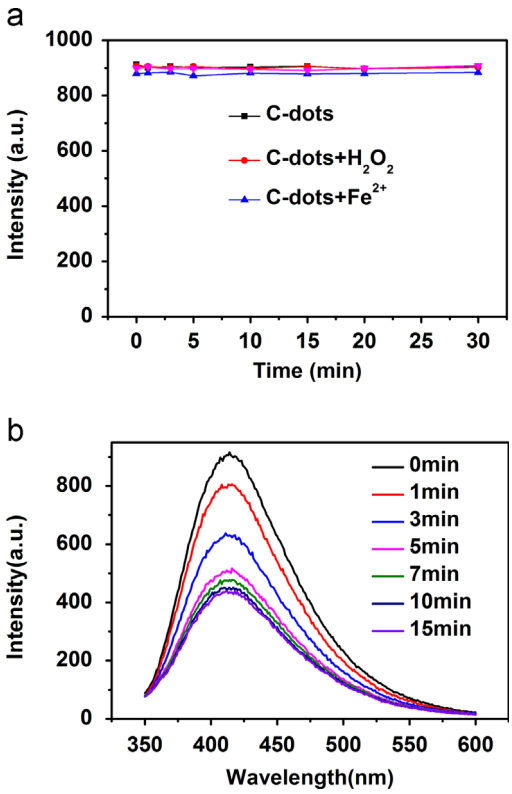

Fig. 2. (a) Stability of C-dots in the presence of H_2O_2 (0.5 mM) and Fe^{2+} (0.5 mM), separately, and (b) time-dependent fluorescence changes of C-dots in the presence of H_2O_2 (25 μM) and Fe^{2+} (0.338 mM).

(Fig. 3(a)). The pH values chosen in this study were 2.5, 3.0, 3.5, 4.0 and 4.5, while the Fe^{2+}concentration was constant (0.338 mM). The results clearly demonstrated that the optimum pH value was 3.0. Then, we investigated the influence of the concentration of Fe^{2+} on ΔF (Fig. 3(b)). In order to determine the optimum concentration of Fe^{2+}, different concentrations of Fe^{2+} (0.300, 0.325, 0.338, 0.350 mM) was selected. According to Fig. 3(b), the ΔF increased to the maximum value with the increasing of Fe^{2+} concentration to 0.338 mM. However, the ΔF decreased, when the concentration of Fe^{2+} was further increased. As a result, the concentration of Fe^{2+} was determined as 0.338 mM. Furthermore, we can see from Fig. 3(a) and (b) that the fluorescence intensity changed inconspicuously after 10 min, so the time was decided as 10 min.

After experimental conditions are optimized, calibration plots are established for H_2O_2 (Fig. 3(c)). The results exhibited a good linear relationship ($R^2 = 0.9956$) in the range of 0.1–40 μM between ΔF and the concentration of H_2O_2 with the following equation: $\Delta F = 18.76 + 13.06C$. The detection limit for H_2O_2 (at a S/N of 3) is as low as 0.05 μM, which can be compared favorably with those of previous reports of H_2O_2 detection (Han et al., 2012; Wen et al., 2012; Yang et al., 2013; Yuan et al., 2009; Zhang et al., 2013).

3.4. The principle for the detection of ACh and choline

The ACh decomposes into choline and acetric acid through the AChE catalyzed reaction, then the product choline can be decomposed into betaine and H_2O_2 by ChOx catalyzing. Thus, biosensors for the detection of choline and ACh could be constructed based on the detection of H_2O_2.

It is known that the optimum pH values for AChE and ChOx are pH 8.0–9.0 and 7.0–8.0, respectively (Gao et al., 2012) and the pH optimum for the Fenton reaction is usually reported in the acidic

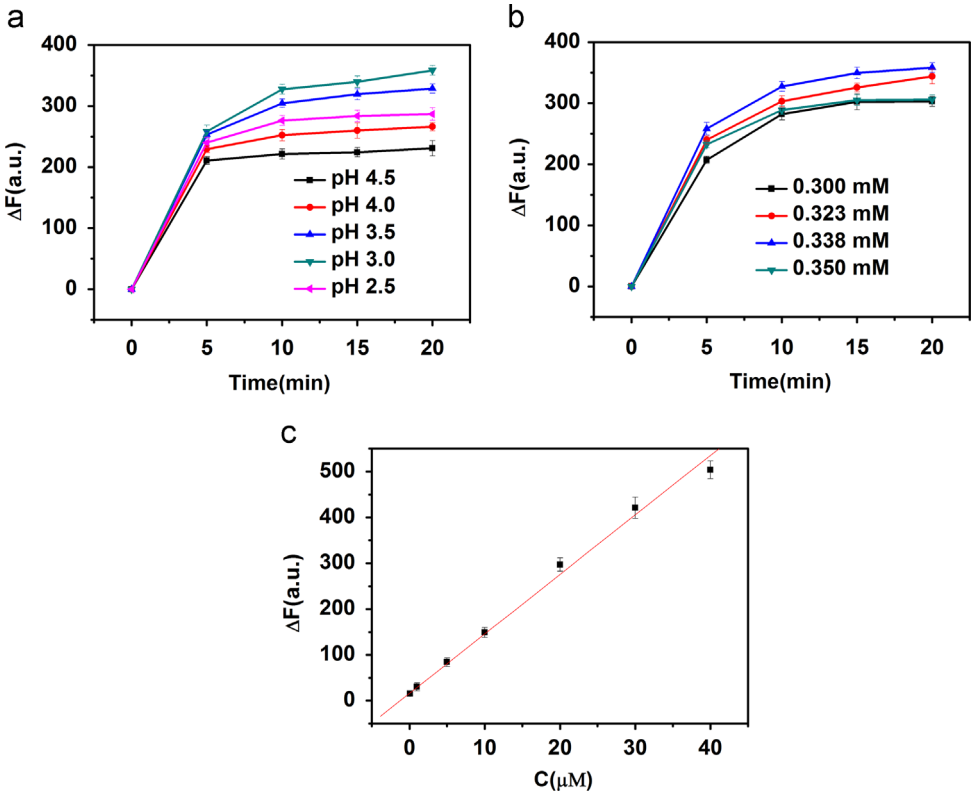

Fig. 3. Absolute changes of the fluorescence intensity of C-dots at 410 nm within 10 min as a function of (a) buffer pH and (b) the concentration of Fe^{2+}, and (c) change of fluorescence intensity of C-dots solution versus the concentration of H_2O_2. Error bars represent the standard deviations of five independent measurements.

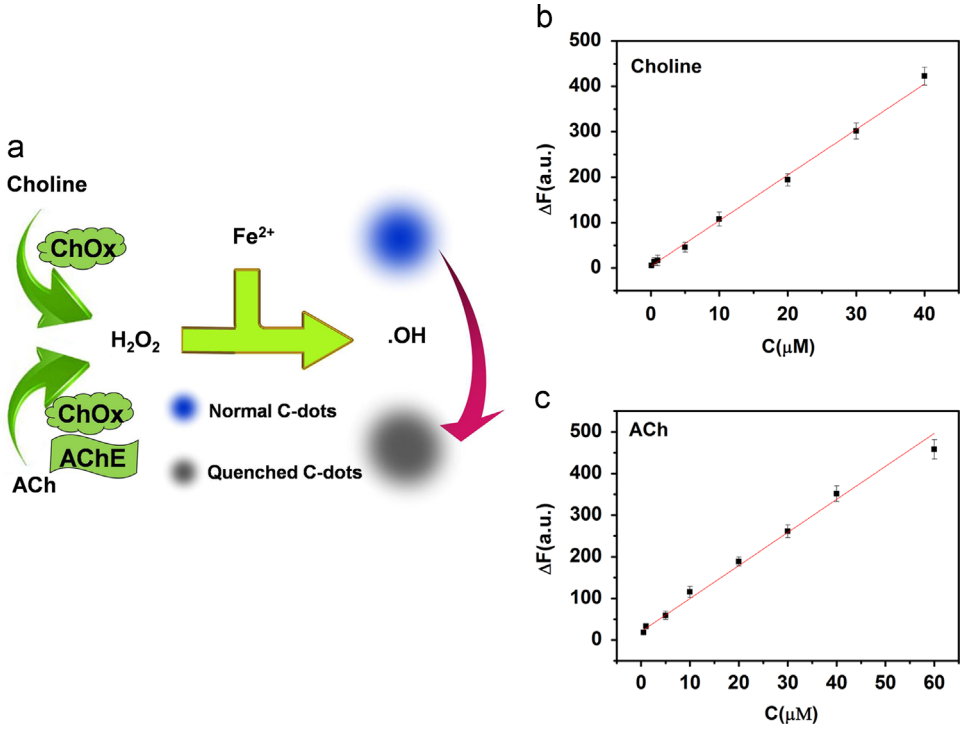

Fig. 4. (a) Scheme for the detection of choline and ACh based on C-dots, (b) change of fluorescence intensity of C-dots solution versus the concentration of choline, and (c) change of fluorescence intensity of C-dots solution versus the concentration of ACh. Error bars represent the standard deviations of five independent measurements.

range near pH 3 (Georgi et al., 2007; Lu et al., 2002; Pignatello et al., 2006). Thus, the detection of choline and ACh has to be achieved through two steps: enzyme reaction and the Fenton reaction (Fig. 4(a)). Firstly, hydrogen peroxide is generated from biocatalyzed reaction in solution (pH 8.0) and the pH of the solution was adjusted to 3.0. Then, C-dots and Fe^{2+} are introduced into the solution and the change of fluorescence intensity before and after the addition of Fe^{2+} is determined.

3.5. Detection of choline based on C-dots

In most living cells, choline is an essential nutrient required for the synthesis of phosphatidylcholine, which is an important constituent of lipid membranes in the cell (Babb et al., 2004; Guerrieri and Palmisano, 2001; Mitchell, 2004). Without choline, many fat-based nutrients and waste products cannot pass in and out of our cells (Bai et al., 2007). In addition, choline is the precursor of ACh, which is an important neurotransmitter in peripheral nervous systems, and is often adopted as a marker of cholinergic activity in the brain (Rahman et al., 2004). Choline is synthesized in the human body, but dietary supplementation is necessary to maintain proper function (Blusztajn, 1998). As an essential nutrient for humans, choline is widely added in food such as milk, fortified foods and dietary supplements (Chen et al., 2011; Phillips, 2012; Wei et al., 2011). As a result, the quantitative determination of choline in clinical analysis, food industry, feed additives and many other fields is of great importance.

Under the optimum experimental conditions established (Fig. S1), calibration plots were generated for choline (Fig.4(b)). There is a good linear correlation (R^2=0.9935) between ΔF and the concentration of choline over the range of 0.1–40 μM with the following equation: $\Delta F = 2.292 + 10.03C$. The LOD of choline (at a S/N of 3) was calculated to be 0.45 μM, which is lower than the average concentration of choline in the serum (10.8 μM) (Pundir et al., 2012). The LOD for the proposed biosensor is lower than that for the fluorescence biosensor adopting semiconductor quantum dots (QDs) or other materials as probes (Chen et al., 2011; Hu et al., 2013; Jin, 2010).

The control experiment of choline detection showed that there was no significant interference on the fluorescence of C-dots observed from the 60 μM choline, which is higher than the range of detection (0.1–40 μM) (Fig. S1(c)). Furthermore, it was also proved that no observable interference on the fluorescence of QDs was observed in the presence of ChOx (2 U/mL). All the above results prove that the quenching of C-dots is caused by the hydroxyl radical produced from the Fenton reaction.

3.6. Detection of ACh based on C-dots

ACh is a neurotransmitter appearing in both the peripheral and central nervous systems. ACh regulates muscle contraction by binding to its receptors at the neuromuscular junction. Thus, the concentration of ACh is related to behavioral activities, learning, sleeping, etc. (De Bundel et al., 2008). In addition, various neuropsychological and neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease, progressive dementia and schizophrenia may caused by the accumulation of ACh in nervous tissue when it is not metabolized (Sattarahmady et al., 2010). As a result, determining the concentration of ACh in biological samples such as blood is of paramount importance.

According to the procedure, the ΔF at different concentrations of ACh was recorded, and was used to plot working curve (Fig. 4(b)). The results exhibited a good linear relationship in the range of 0.5–60 μM. The correlation coefficient (R) was 0.9976. The LOD (0.1 μM) for the biosensor of ACh is lower than that for the fluorescence biosensor adopting QDs or other fluorescent materials as probes (Chen et al., 2011; Jin, 2010). The control experiment showed that there was no apparent change in the fluorescence intensity of C-dots observed from the 200 μM ACh, which was higher than the range of detection (0.5–60 μM) (Fig. S2). Furthermore, it was also proved that no obvious interference in the fluorescence of C-dots was observed in the presence of 200 μM ACh and 50 U/mL AChE. All the above results proved that the quenching of C-dots is caused by the hydroxyl radical produced from the Fenton reaction.

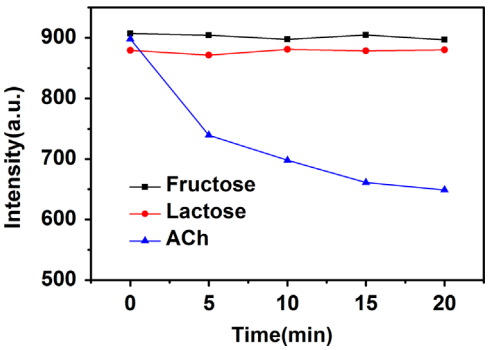

Fig. 5. The change of fluorescence intensity of C-dots solution in the presence of different substrates.

3.7. The anti-interference ability

The study on the anti-interference ability is of great importance for the biosensor. The study on the anti-interference ability was carried out using buffer solution with lactose and fructose in place of ACh with the same concentration (50 μM) under the same conditions. The results indicated that the ΔF which was caused by fructose and lactose was negligible compared with that of glucose (Fig. 5). The results proved that the proposed biosensor can selectively detect the concentration of ACh and can offer credible signal when the interfering species reach high concentrations.

3.8. The reproducibility

The study of reproducibility is another important evaluation for the choline ACh biosensor. The reproducibility was evaluated by comparing response of the determination of 15 μM ACh (see Fig. S3) at different days. The relative standard deviation (RSD) was 3.45% for 6 assays with an average response of 152.1 a.u.

4. Conclusions

In summary, a novel method was proposed for the preparation of C-dots in aqueous solution. The as-prepared C-dots possess excellent fluorescence property and high stability. A turn-off fluorescence method was proposed for the detection of H_2O_2 based on the fluorescence quenching of C-dots in the presence of Fe^{2+}. On the basis of the production of H_2O_2 coupled with fluorescence quenching of C-dots in the presence of H_2O_2 and Fe^{2+}, an eco-friendly, sensitive and simple biosensor for the detection of choline and ACh was constructed. It is the first report of sensing of choline and ACh using C-dots. The proposed method exhibits several advantages, including eco-friendly, sensitivity, wide linearity, and a low degree of sample matrix interference. Because numerous kinds of O_2-dependent oxidase generate H_2O_2, the method may also be utilized to detect other substrates by using their corresponding enzymes. What is more, the biosensor could be used for the detection of activity of enzymes, when the concentration of substrates was constant. As a result, this work presents a new design model to construct fluorescence biosensor for detection of numerous substrates and enzymes.

Acknowledgments

The authors acknowledge the financial support from the National Natural Science Foundation of China (Nos. 61171049, 81171454, and 61178035) and National Hi-Tech. Research and Development Program of China (No. 2012AA022701).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.09.006.

References

Babb, S.M., Ke, Y., Lange, N., Kaufman, M.J., Renshaw, P.F., Cohen, B.M., 2004. Psychiatry Research-Neuroimaging 130, 1–9.
Bai, Y.H., Du, Y., Xu, J.J., Chen, H.Y., 2007. Electrochemistry Communications 9, 2611–2616.
Blusztajn, J.K., 1998. Science 281, 794–795.
Buiculescu, R., Hatzimarinaki, M., Chaniotakis, N., 2010. Analytical and Bioanalytical Chemistry 398, 3015–3021.
Burmeister, J.J., Pomerleau, F., Huettl, P., Gash, C.R., Werner, C.E., Bruno, J.P., Gerhardt, G.A., 2008. Biosensors and Bioelectronics 23, 1382–1389.
Çevik, S., Timur, S., Anik, Ü., 2012. Microchimica Acta 179, 299–305.
Chen, Z., Ren, X., Meng, X., Chen, D., Yan, C., Ren, J., Yuan, Y., Tang, F., 2011. Biosensors and Bioelectronics 28, 50–55.
Dai, H., Chi, Y., Wu, X., Wang, Y., Wei, M., Chen, G., 2010. Biosensors and Bioelectronics 25, 1414–1419.
De Bundel, D., Sarre, S., Van Eeckhaut, A., Smolders, I., Michotte, Y., 2008. Sensors 8, 5171–5185.
Dunphy, R., Burinsky, D.J., 2003. Journal of Pharmaceutical and Biomedical Analysis 31, 905–915.
Fiamegos, Y., Stalikas, C., Pilidis, G., 2002. Analytica Chimica Acta 467, 105–114.
Fintelmann, V., Lindner, H., 1972. Minerva Medica 63, 4148–4149.
Gao, X., Tang, G., Su, X., 2012. Biosensors and Bioelectronics 36, 75–80.
Georgi, A., Schierz, A., Trommler, U., Horwitz, C.P., Collins, T.J., Kopinke, F.D., 2007. Applied Catalysis B: Environmental 72, 26–36.
Guerrieri, A., Palmisano, F., 2001. Analytical Chemistry 73, 2875–2882.
Han, K.N., Li, C.A., Bui, M.-P.N., Pham, X.H., Kim, B.S., Choa, Y.H., Seong, G.H., 2012. Sensors and Actuators B: Chemical 174, 406–413.
Hu, R., Liu, Y.R., Zhang, X.B., Tan, W., Shen, G.L., Yu, R.Q., 2013. Biosensors and Bioelectronics 41, 442–445.
Ikarashi, Y., Harigaya, Y., Tomidokoro, Y., Kanai, M., Ikeda, M., Matsubara, E., Kawarabayashi, T., Kuribara, H., Younkin, S.G., Maruyama, Y., Shoji, M., 2004. Neurobiology of Aging 25, 483–490.
Jia, X., Li, J., Wang, E., 2012. Nanoscale 4, 5572–5575.
Jin, T., 2010. Sensors 10, 2438–2449.
Jung, H.S., Kwon, P.S., Lee, J.W., Kim, J.I., Hong, C.S., Kim, J.W., Yan, S., Lee, J.Y., Lee, J. H., Joo, T., Kim, J.S., 2009. Journal of the American Chemical Society 131, 2008–2012.
Kuribayashi, M., Tsuzuki, M., Sato, K., Abo, M., Yoshimura, E., 2007. Chromatographia 67, 339–341.
Lai, C.-Y., Trewyn, B.G., Jeftinija, D.M., Jeftinija, K., Xu, S., Jeftinija, S., Lin, V.S.Y., 2003. Journal of the American Chemical Society 125, 4451–4459.
Li, H., Kang, Z., Liu, Y., Lee, S.T., 2012. Journal of Materials Chemistry 22, 24230–24478.
Lu, M.C., Chen, J.N., Huang, H.H., 2002. Chemosphere 46, 131–136.
Takayama, M., Itoh, S., Nagasaki, T., Tanimizu, I., 1977. Clinica Chimica Acta 79, 93–dummy98.
Mitchell, K.M., 2004. Analytical Chemistry 76, 1098–1106.
Newhouse, P., Singh, A., Potter, A., 2004. Current Topics in Medicinal Chemistry 4, 267–282.
Numata, M., Li, C., Bae, A.H., Kaneko, K., Sakurai, K., Shinkai, S., 2005. Chemical Communications 37, 4655–4657.
Pepeu, G., Giovannini, M.G., 2004. Learning and Memory 11, 21–27.
Phillips, M.M., 2012. Analytical and Bioanalytical Chemistry 403, 2103–2112.
Pignatello, J.J., Oliveros, E., MacKay, A., 2006. Critical Reviews in Environmental Science and Technology 36, 1–84.
Pundir, S., Chauhan, N., Narang, J., Pundir, C.S., 2012. Analytical Biochemistry 427, 26–32.
Rahman, M.A., Park, D.S., Shim, Y.B., 2004. Biosensors and Bioelectronics 19, 1565–1571.
Ren, X., Tang, F., Liao, R., Zhang, L., 2009. Electrochimica Acta 54, 7248–7253.
Sattarahmady, N., Heli, H., Moosavi-Movahedi, A.A., 2010. Biosensors and Bioelectronics 25, 2329–2335.
Savage, L.M., Chang, Q., Gold, P.E., 2003. Learning and Memory 10, 242–246.
Sletten, D.M., Nickander, K.K., Low, P.A., 2005. Journal of the Neurological Sciences 234, 1–3.
Wei, W., Kang, X., Deng, H., Lu, Z., Jie, Z., 2011. Analytical Letters 44, 1381–1391.
Wei, W., Wei, M., Kang, X., Deng, H., Lu, Z., 2009. Electrophoresis 30, 1949–1952.
Wen, T., Qu, F., Li, N.B., Luo, H.Q., 2012. Analytica Chimica Acta 749, 56–62.
Wessler, I., Kirkpatrick, C.J., Racké, K., 1998. Pharmacology and Therapeutics 77, 59–79.
White, K.E., Cummings, J.L., 1996. Comprehensive Psychiatry 37, 188–195.
Wu, B., Ou, Z., Ju, X., Hou, S., 2011. Journal of Nanomaterials 2011, 1–6.
Yang, H.W., Hua, M.Y., Chen, S.L., Tsai, R.Y., 2013. Biosensors and Bioelectronics 41, 172–179.
Yuan, J., Guo, W., Yin, J., Wang, E., 2009. Talanta 77, 1858–1863.
Zhang, X., Ji, R., Wang, L., Yu, L., Wang, J., Geng, B., Wang, G., 2013. Crystal Engineering Communications 15, 1173–1178.