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Determination of butyrylcholinesterase activity based on thiamine luminescence modulated by MnO_2 nanosheets

Zhengyi Qu [a], Tian Yu [b], Yuzhong Liu [c], Lihua Bi [a,*]

[a] College of Chemistry, Jilin University, Changchun, 130012, PR China
[b] College of Electrical and Electronic Engineering, Changchun University of Technology, Changchun, 130012, PR China
[c] Jilin University First Hospital, Changchun, 130021, PR China

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ABSTRACT

In this paper, a novel strategy for biosensing butyrylcholinesterase (BChE) activity is developed based on manganese dioxide (MnO_2) nanosheets to modulate the photoluminescence of thiamine (TH). The oxidase-like activity of MnO_2 nanosheets enables them to catalyze the oxidation of non-fluorescent substrate TH to generate strong fluorescent thiochrome (TC). When the target BChE is introduced to form thiocholine in the presence of S-butyrylthiocholine iodide (BTCh), MnO_2 nanosheets are reduced by thiocholine to Mn^{2+}, resulting in the loss of their oxidase-like activity and the reduction of TC fluorescence. Based on this, a BChE activity fluorescence biosensor is constructed utilizing the luminescence behavior variation of TH and the oxidase-like activity of MnO_2 nanosheets. The fluorescence biosensor shows a sensitive response to BChE, and the detection limit reaches 0.036 U L^{-1}. In addition, the feasibility of the biosensor in real samples analysis is studied with satisfactory results.

1. Introduction

Enzymes, as biocatalysts, play extremely important role in most metabolic processes due to high catalytic efficiency and precise specificity for related reactions [1]. Cholinesterase can be divided into acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [2]. Unlike AChE, which exists at the junction of muscle nerves, BChE is widely found in glial cells, plasma, liver and kidneys [3,4]. Enzyme synthesis is reduced when the liver undergoes substantial damage, so BChE activity is a sensitive indicator of hepatocyte protein synthesis, chronic liver disease, hepatocellular carcinoma and organophosphate poisoning, which can be reliably diagnosed by measuring BChE activity changes in serum [5,6]. As a consequence, it is of great significance to develop a method for the detection of BChE activity. Compared with AChE, there are few methods to detect BChE activity. To accurately determine BChE activity, some assay methods have been developed, including enzyme-linked immunosorbent assay [7,8], electrochemical analysis [9,10] and chemiluminescence detection [11]. However, most existing methods are based on nanomaterials that require complex synthesis, complicated labeling and purification procedures, which result in sophisticated procedures, consumption of time and labor, high costs and low sensitivity for the detection of BChE activity. And the optical methods for determining BChE activity still remain some disadvantages. Traditional fluorescent labeling methods are based on the fluorescent dyes, which are generally susceptible to environment and change specific enzymes, limiting their practical application in diagnosis [12,13].

Therefore, we propose a novel strategy based on thiamine (TH)-manganese dioxide (MnO_2) nanosheets to detect BChE activity with higher stability and lower biomolecular interference. Non-fluorescent TH is an inexpensive and easily available fluorescent substrate that is very attractive in bioassays [14,15]. Here, TH is selected for the detection of BChE activity, since it is easily converted into strong fluorescent thiochrome (TC) through oxidation reaction [16]. The change in fluorescence intensity of TC can be used as an indicator signal for detecting BChE activity. In addition, TH as indicator has the advantages of simple operation and time saving, so the TH-based assay method is environmentally friendly and cost effective [17,18]. Nanoenzyme as a kind of mimic enzyme with catalytic function, has been applied in the field of biosensing due to its unique physicochemical properties [19]. MnO_2 nanosheets, as a nanoenzyme, can induce the color reaction of the substrate based on its redox response characteristics for biological detection [20,21]. And MnO_2 nanosheets have good biocompatibility and low toxicity, which gives them advantageous in biological monitoring [22]. Therefore, MnO_2 nanosheets are used to catalyze the

* Corresponding author.
E-mail addresses: blh@jlu.edu.cn, blhytyyz@163.com (L. Bi).

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oxidation of non-fluorescent substrate TH and can be destroyed by target analyte, which allows them to be used in sensing for BChE activity detection. The detection mechanism utilizes the fluorescent signal of the luminescent substance generated by the reaction without adding fluorescent nanomaterials, so the advantage of this assay method is simple operation and easy implementation.

Scheme 1 illustrates the sensing strategy for BChE activity detection. The oxidase-like activity of MnO_2 nanosheets allows them to catalyze the oxidation of TH to blue fluorescent TC. When BChE is added in the presence of S-butyrylthiocholine iodide (BTCh) to produce thiocholine, MnO_2 nanosheets are reduced to Mn^{2+} by thiocholine and lose their oxidase-like activity, resulting in a decrease in the amount of fluorescent TC produced. The decrease of TC fluorescence intensity can be used as the signal to quantitatively detect BChE activity. Therefore, the novel strategy for the detection of BChE activity is established by utilizing the luminescence property of TH in oxidation state and the oxidase-like activity of MnO_2 nanosheets.

2. Experimental

2.1. Reagents and instruments

The reagents and instruments are shown in the Supplementary Material.

2.2. Synthesis of MnO_2 nanosheets

MnO_2 nanosheets were prepared as in the previous study [23]. Briefly, 2 mL of H_2O_2 (30 wt%), 12 mL of tetramethylammonium (TMA, 1 M) and 6 mL of deionized water were uniformly mixed and added to 10 mL of $MnCl_2{\cdot}4H_2O$ (3 mM) aqueous solution within 15 s. After the prepared mixture was stirred at room temperature overnight, the obtained MnO_2 nanosheets were washed by alternating washing with deionized water and methanol for purity. Then, the MnO_2 nanosheets were dissolved in water and ultrasonically dispersed for later use.

2.3. Fluorescence enhancement of TH by MnO_2 nanosheets

MnO_2 nanosheets with different concentrations were mixed with TH (10 mM) and PBS buffer solution (10 mM, pH 6.6), and then the mixture was diluted to 2.0 mL with distilled water. After 25 min, fluorescence spectra were recorded to investigate the effect of MnO_2 nanosheets concentration on the fluorescence intensity of TH (the excitation wavelength was 350 nm).

2.4. Fluorescence detection of BChE activity

For the BChE activity detection, different concentrations of BChE and BTCh solution (2 mM) reacted at 37 °C for 30 min. Subsequently, MnO_2 nanosheets (50 μg mL^{-1}), TH (10 mM) and pH 6.6 PBS buffer solution (10 mM) were added in the above solutions and diluted with distilled water to 2.0 mL. After 25 min, fluorescence spectra were recorded.

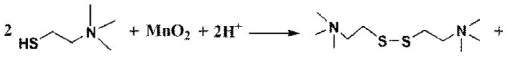
2.5. Processing and detection of real samples

For real samples detection, blood samples were acquired through venipuncture and allowed to stand for 2 h, and then centrifuged at room temperature for 10 min. The supernatant was taken, acetonitrile (CH_3CN) (CH_3CN: serum = 1:1) was added and shaken vigorously for 2 min for deproteinization. Subsequently, the mixture was centrifuged at 4 °C for 10 min. The resulting supernatant was filtered twice and the pH was adjusted to neutral to obtain the treated serum. Different concentrations of BChE solution were added to 5000-fold diluted serum sample and real sample analysis was performed in accordance with the same procedure as described in Section 2.4.

3. Results and discussion

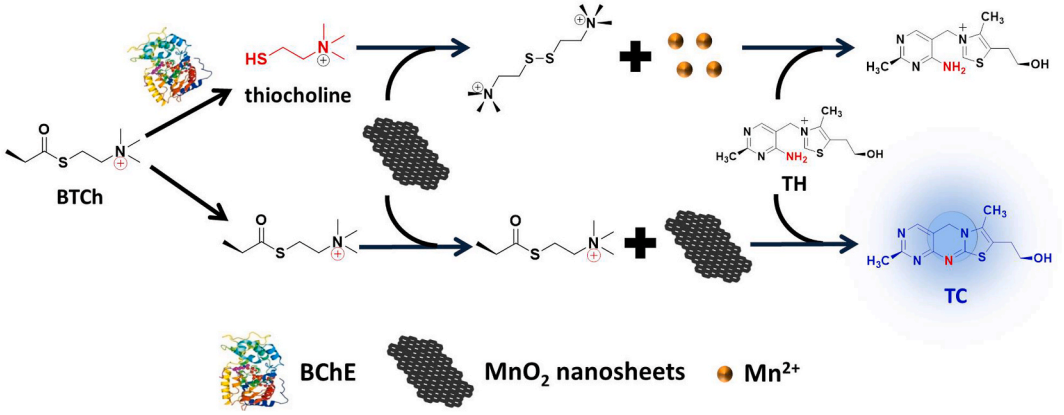
3.1. Characterization of MnO_2 nanosheets

In this work, the BChE activity detection was performed by modulating the luminescence of TH with MnO_2 nanosheets. The structure and optical property of MnO_2 nanosheets were characterized by XPS, TEM and UV–vis absorption, which are shown in Fig. 1. The XPS spectrum of MnO_2 nanosheets exhibits two peaks at 529.8 eV and 642.2 eV, belonging to O_{1s} and Mn_{2p}, respectively. The $Mn_{2p3/2}$ peak as shown is centered at 654 eV, whereas the $Mn_{2p1/2}$ peak is observed at 642.3 eV in Fig. S1. As shown in Fig. 1B and C, MnO_2 nanosheets dispersed in solution show large pieces with large surface area, which can provide multiple active sites to catalyze the oxidation of TH. And after the addition of BChE and BTCh, the structures of MnO_2 nanosheets are destroyed by thiocholine via –SH hydrolysis reaction on MnO_2 nanosheets. In addition, the characteristic absorption peak of MnO_2 nanosheets in UV–vis spectrum is described in Fig. 1D (black curve), which is consistent with the reported literature [23]. It can be seen that the absorbance of MnO_2 nanosheets decreases after adding BChE and BTCh (red curve in Fig. 1D), which also illustrates that the structure of MnO_2 nanosheets are destroyed. As shown in equation (1), MnO_2 nanosheets were reduced to produce Mn^{2+} by thiocholine.



(1)

Further, it is obvious that the color of MnO_2 nanosheets solution fades (see the inset of Fig. 1D), which is consistent with the absorption spectra recorded. And as the concentration of BChE increases, the color shows continuous changes (see Fig. S2). Such observation further



Scheme 1. Schematic illustration of the novel biosensor for the detection of BChE activity.

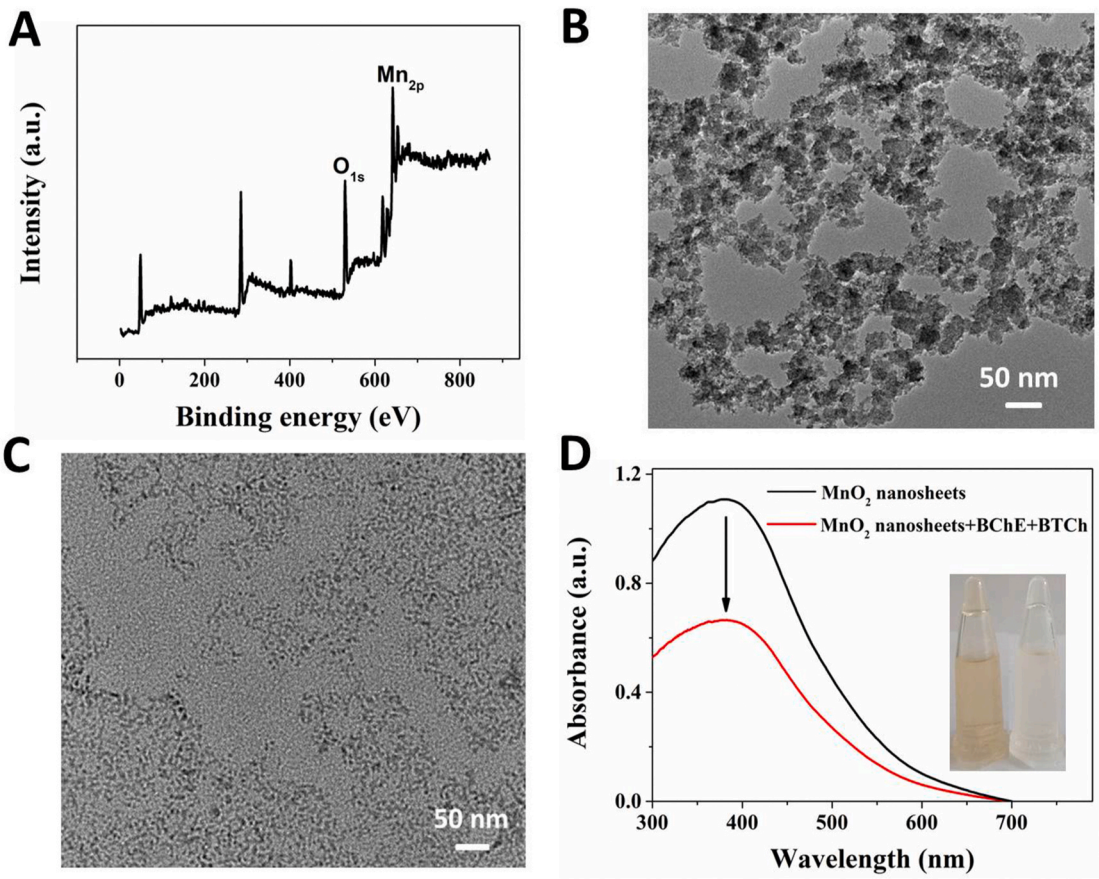


Fig. 1. (A) XPS spectra of MnO_2 nanosheets; (B) TEM image of MnO_2 nanosheets ; (C) TEM image of MnO_2 nanosheets after adding BChE and BTCh; (D) UV–vis absorption spectra of MnO_2 nanosheets in absence (black line) and presence (red line) of BChE and BTCh; The inset shows the corresponding images of MnO_2 nanosheets solution. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

indicates that MnO_2 nanosheets are decomposed. In short, the combination of XPS, TEM sheet structures and UV–vis spectrum can prove that MnO_2 nanosheets are successfully obtained and the MnO_2 nanosheets can be decomposed by BChE and BTCh reaction. Thereby the MnO_2 nanosheets-based biosensor for BChE activity detection can be established.

3.2. The design strategy for BChE activity detection

To validate the feasibility of the biosensor to detect BChE activity, we used 350 nm as the excitation wavelength to investigate the fluorescence variation of TH after the addition of MnO_2 nanosheets, BChE and BTCh. As shown in Fig. 2, it can be observed that TH itself does not exhibit the fluorescence behavior, while TC produced by catalytic oxidation of MnO_2 nanosheets exhibits blue fluorescence behavior with a fluorescence peak appearing obviously at 451 nm. And the fluorescence intensity decreases after the addition of BChE and BTCh. In addition, we found the introduction of single BTCh or BChE does not cause significant change in fluorescence intensity (as shown in Fig. S3). Combining with the reaction equation (1) of MnO_2 nanosheets and thiocholine, these results show that only thiocholine produced by the enzymatic reaction can reduce MnO_2 nanosheets to Mn^{2+}. These results above further demonstrate the feasibility of the proposed scheme. Moreover, we systematically measured fluorescence response of TH to different concentrations of MnO_2 nanosheets, 50 μg mL^{-1} MnO_2 nanosheets are therefore selected for the subsequent detection because they can produce maximum fluorescence intensity for TH (see Fig. S4).

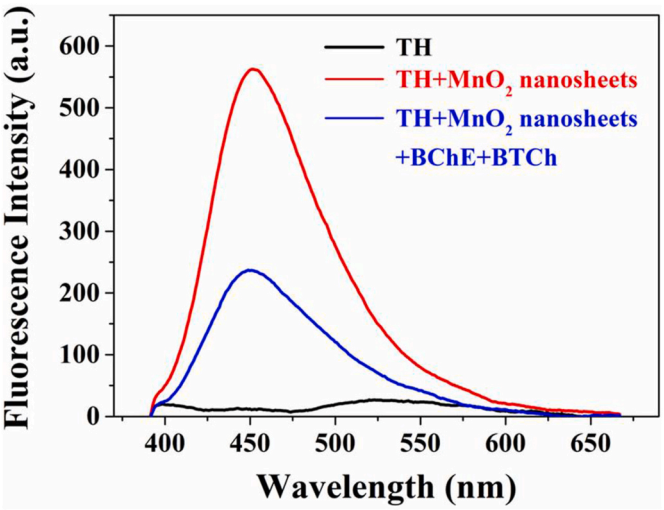


Fig. 2. Fluorescence emission spectra of TH, TH/MnO_2 nanosheets and TH/MnO_2 nanosheets/BChE/BTCh system.

3.3. Optimization of detection conditions

We further optimized the experimental conditions in order to obtain the fluorescence biosensor with good sensing properties. We optimized the time curve as shown in Fig. 3. It can be observed that the reaction of MnO_2 nanosheets with TH reaches equilibrium at 25 min and enzymatic reaction reaches equilibrium at 30 min, indicating that two reactions are

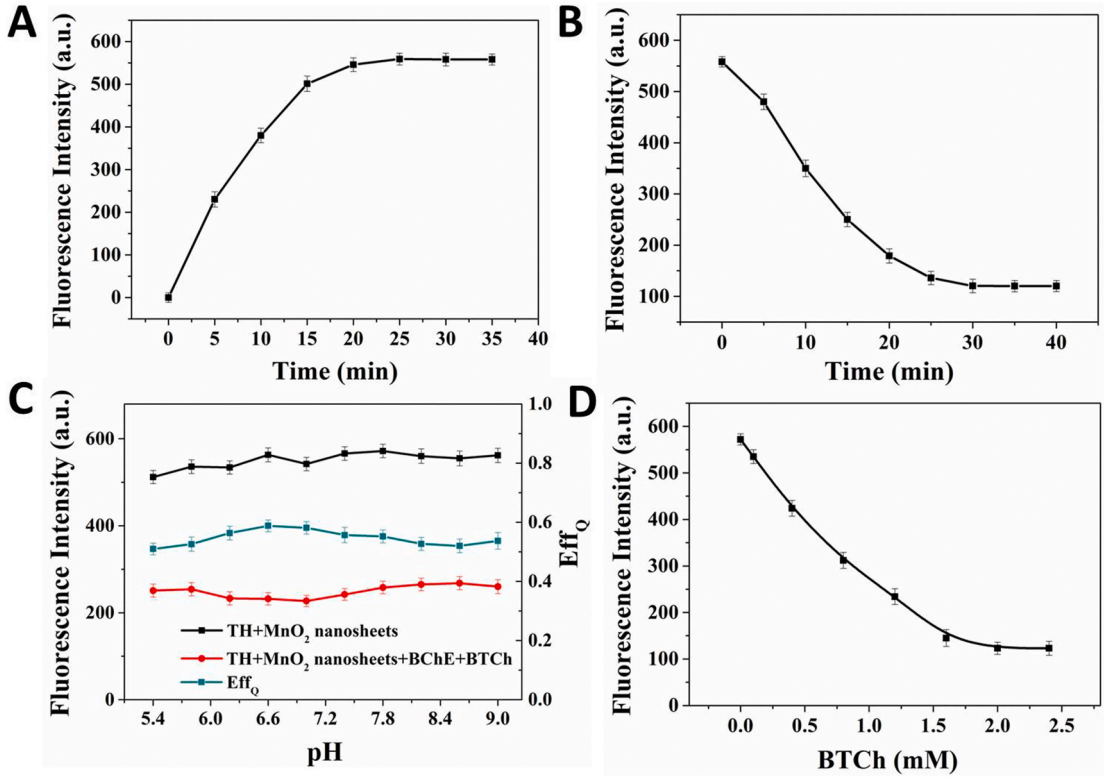


Fig. 3. (A) Effect of incubation time on the fluorescence intensity of TH and MnO_2 nanosheets; (B) Effect of incubation time on the fluorescence intensity of BChE and BTCh; (C) Effect of pH on the fluorescence intensity of TH/MnO_2 nanosheets (black squares) and TH/MnO_2 nanosheets/BChE/BTCh (red dots); (D) Effect of BTCh concentration on the fluorescence intensity of TH/MnO_2 nanosheets/BChE. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

completed (see Fig. 3A and B). Therefore, the reaction time of MnO_2 nanosheets-TH and BChE-BTCh were set to 25 min and 30 min, respectively. Additionally, the effect of pH on the reactions were investigated and calculated the quenching efficiency Eff_Q ($Eff_Q = (F_0\text{-}F_{BChE})/F_0 \times 100\%$), where F_{BChE} represents the fluorescence intensity of TH/MnO_2 nanosheets/BChE/BTCh, F_0 stands the fluorescence intensity of TH/MnO_2 nanosheets). It can be seen from Fig. 3C that the maximum quenching efficiency is at pHa 6.6, so pH 6.6 is selected for the subsequent detection process. The substrate concentration of the BChE enzyme reaction was optimized by adding a series of concentrations of BTCh to system. Fig. 3D displays that a BTCh concentration of 2 mM is sufficient for the reaction to be completed.

3.4. Fluorescence detection of BChE activity

The fluorescence curves for detecting BChE activity are shown in Fig. 4A. It can be seen that with the increasing level of BChE, the fluorescence intensity of the sensing system gradually decreases. Fig. 4 shows that there is a good linear relationship (correlation coefficient $R^2 = 0.997$) between fluorescence intensity ratio I/I_0 and BChE concentration and the linear regression equation $I/I_0 = 0.989–0.051$ [BChE] (U L^{-1}) is obtained in the range of 0.125–15 U L^{-1}. In order to show more intuitively, the inset further enlarges the graph of the low concentration range. And the detection limit (LOD) reaches 0.036 U L^{-1} (The LOD is defined by the equation LOD = 3σ/s, where σ is the standard deviation

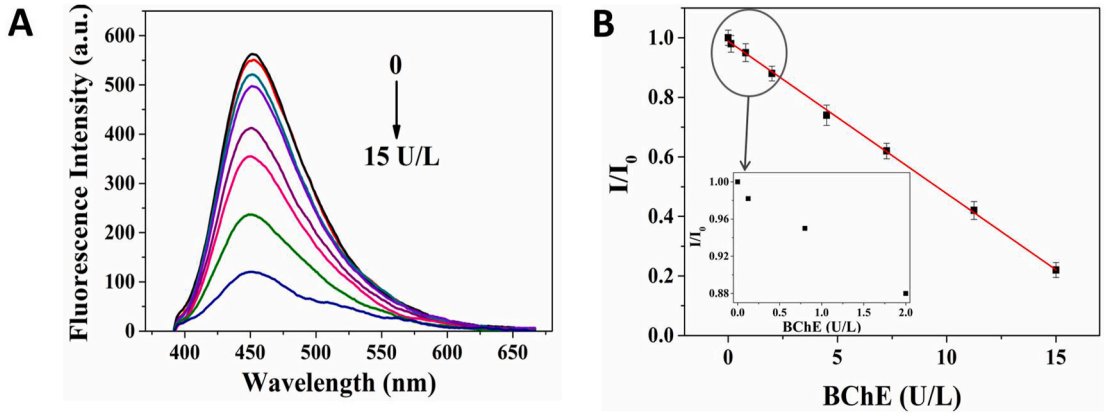


Fig. 4. (A) Fluorescence spectra of the TH (10 mM)/MnO_2 nanosheets (50 μg mL^{-1})/BTCh (2 mM) system with different concentrations of BChE (0, 0.125, 0.8, 2, 4.5, 7.25, 11.25 and 15 U L^{-1}) in pH 6.6 buffer solution; (B) Plot of the relative fluorescence intensity (I/I_0) of the TH/MnO_2 nanosheets/BTCh system against the BChE concentration, where I_0 and I stand for the fluorescence intensity of the TH/MnO_2 nanosheets/BTCh system in the absence and presence of BChE. The illustration shows a dot plot in the 0–2 U L^{-1} range.

of the corrected blank signals and s is the slope of the calibration curve). In addition, we compared our detection methods with other BChE activity assays reported in the literature (Table S1). In comparison with previous methods, the assay method possesses high sensitivity and comparable detection range. On the other hand, in previous studies, synthetic luminescent materials are required. Whereas the method is based on the change of TH luminescence behavior, which illustrates that the assay method is simple to operate, time-saving and cost-effective. Therefore, the sensing strategy can be better applied to sensitive detection of BChE activity.

3.5. The selectivity and interference study

The selectivity for BChE detection was further investigated by using potential ions, biological small molecules, proteins and enzymes. It can be seen from Fig. 5 that they do not cause a significant response to the TMB/MnO_2 nanosheets system like BChE except GSH, cysteine and Hcy. However, the interference can be eliminated by adding N-ethylmaleimide (NEM), a kind of masking agent that can specially react with the thiol group [24]. And because BChE can specifically hydrolyze BTCh, AChE cannot hydrolyze BTCh [25]. Therefore, when taking BTCh as the substrate, only BChE can cause the fluorescence intensity change, indicating that this sensing platform can effectively resist the interference of AChE on BChE activity detection. To further verify the specificity of BChE detection, paraoxon as a specific inhibitor of BChE, was introduced into the system [26]. The addition of paraoxon effectively inhibits the enzymatic reaction, leading to the restoration of fluorescence intensity, which further illustrates the specificity of the method for detecting BChE. These results clearly show that the system has high selectivity to BChE.

We further studied the ability of the probe to resist interference. It can be clearly observed from Fig. 6 that even in the presence of interfering substances, the platform can still work the same for BChE detection, which indicates that the proposed strategy can provide good resistance to interference.

3.6. Detection of BChE activity in real samples

To further validate the detection method in this work, this BChE assay was applied in the measurement of BChE activity in human serum samples. First of all, the standard addition method was carried out. As shown in Table S2, the recoveries of BChE in serum samples ranged from 98.5% to 108% with the relative standard deviations (RSDs) lower than 4.26%, which proves that the method possesses high reliability for BChE assay in actual samples. In order to further verify the accuracy of BChE activity detection, the traditional Ellman colorimetric method by detecting the absorbance of 5-thio-2-nitroben zoic acid produced by thiocholine and 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) was used as a standard method for comparison [27]. Table S3 lists the measured values of BChE level in three human serum samples based on our method and Ellman method. It is found that the average value of the measured BChE is consistent with that of Ellman method and is in agreement with normal BChE level (9010 ± 2041 U L^{-1}) [28]. The good agreement convincingly indicates that our assay is capable of accurately measuring BChE activity in human serum.

4. Conclusion

In this paper, we constructed a novel biosensor for the detection of BChE activity. This strategy is based on the change in the fluorescence signal of TC produced by catalytic oxidation of non-fluorescent substrate TH. There is no need to synthesize fluorescent materials, so this assay method has great advantages in simple operation, high cost-effectiveness, and environmental friendliness. Therefore, the sensing platform can be better applicable to actual detection. In addition, this assay method has been successfully used to detect BChE activity in actual samples. These results indicate that this assay method has practical value for the detection of BChE activity.

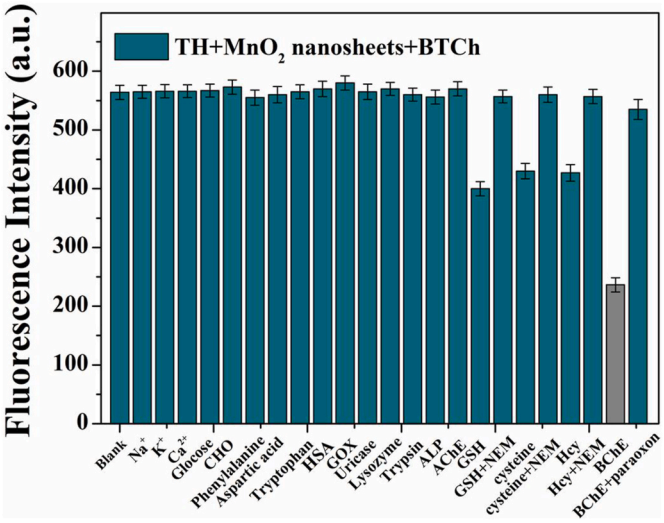


Fig. 5. The selectivity of TH/MnO_2 nanosheets/BTCh probe for the detection of BChE (800 μmol L^{-1} of Na^+, K^+, Ca^{2+}, 50 μmol L^{-1} of glucose, CHO, phenylalanine, aspartic acid, tryptophan, GSH, GSH/NEM, cysteine, cysteine/NEM, Hcy and Hcy/NEM, 5 μmol L^{-1} of HSA, GOX, uricase, lysozyme, trypsin, ALP and AChE, 100 ng mL^{-1} paraoxon).

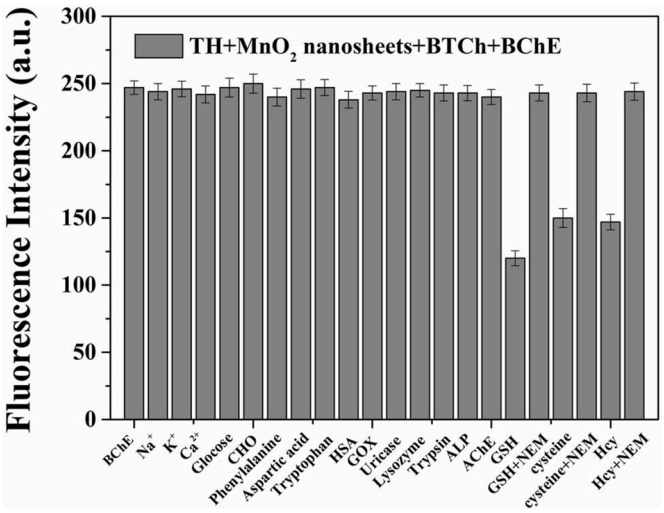


Fig. 6. The fluorescence intensity of the TH/MnO_2 nanosheets/BTCh system with interfering substance (800 μmol L^{-1} of Na^+, K^+, Ca^{2+}, 50 μmol L^{-1} of glucose, CHO, phenylalanine, aspartic acid, tryptophan, GSH, GSH/NEM, cysteine, cysteine/NEM, Hcy and Hcy/NEM, 5 μmol L^{-1} of HSA, GOX, uricase, lysozyme, trypsin, ALP and AChE).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121831.

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