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Functionalized photonic crystal for the sensing of Sarin agents

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ABSTRACT

The indiscriminate use of nerve agents by terrorist groups has attracted attention of the scientific communities toward the development of novel sensor technique for these deadly chemicals. A photonic crystal (PhC) hydrogel immobilized with butyrylcholinesterase (BuChE) was firstly prepared for the sensing of Sarin agents. Periodic polystyrene colloidal (240 nm) array was embedded inside an acrylamide hydrogel, and then BuChE was immobilized inside the hydrogel matrix via condensation with 3-(diethoxyphosphoryloxy)−1,2,3-benzotriazin-4(3 h)-one (DEPBT). It indicated that a total of 3.7 units of BuChE were immobilized onto the PhC hydrogel. The functionalized hydrogel recognized the Sarin agent and then shrunk, thus the diffraction of PhC hydrogel blue shifted significantly, and a limit of detection (LOD) of 10^{-15} mol L^{-1} was achieved.

1. Introduction

Nerve agents are a class of organophosphates (Fig. 1) that disrupt nerves transfer messages by blocking AChE, an enzyme that normally destroys acetylcholine, a neurotransmitter. Nerve agents are now classified as weapons of mass destruction by the United Nations according to UN Resolution 687, their production and stockpiling are outlawed by the Chemical Weapons Convention of 1993. However, the easy production of nerve agents and their indiscriminate use by certain nations and terrorist groups have increased the world wide concern, and the efforts of the scientific communities toward the detection of these deadly chemicals.

Among various sensor technologies, PhC is a cutting edge technology which now attracts more and more attentions due to its unique ability for the naked-eye detection [1–6]. Most of PhC sensors utilize a colloidal crystal array (CCA) encapsulated inside a hydrogel network which Bragg diffracts light in the visible spectral region [7–10]. Due to their unique nanostructure, PhC presents special properties and capabilities and therefore leads to an outstanding potential for sensing applications [11–15].

Considering the difficulties in storage and accessibility of real nerve agents, current works primarily concentrate on the detection of other organophosphates, such as degradation products of nerve agents, stimulants of nerve agents and organophosphorus pesticides. Particularly, the monitoring of degradation products of nerve agents provided an indirect path to the detection of nerve agents. Liu et al. developed a molecularly imprinted PhC (MIPhC) for the detection of methyl phosphonic acid (MPA), which was released from the hydrolysis of nerve agents, with the LODs of 3.5×10^{-6}, 2.5×10^{-5}, 7.5×10^{-5} and 7.5×10^{-5} mol L^{-1} for Sarin, Soman, VX and R-VX respectively [16]. Another MIPhC for ethyl phosphate (EPA) could detect 0.5×10^{-3} mol L^{-1} EPA with apparent red shift of reflectance [17]. When it comes to the detection of the stimulants of nerve agents, Jang et al. [18] used nanostructured rugate porous silicons (PSi) containing multiple photonic band gaps to detect triethyl phosphate (TEP), diethyl chlorophosphate (DCP), dimethyl methylphosphonate (DMMP) and diethyl ethylphosphonate (DEEP). Chen et al. developed PhC nanobeam cavities with high-quality factors and sensitivity to change the dielectric properties of their surroundings, therefore this material was constructed into an ultrasensitive chemical sensor for nerve agent simulant methyl salicylate gases and a LOD of 1.5 ppb was achieved [19]. Jung and Kim et al. synthesized a colorimetric azopyridine and its recyclable mesoporous silica-immobilized nanoparticles for selectively sensing DCP over a series of other phosphate compounds and the corresponding color changes are easily recognizable by the naked-eye [20]. As for the detection of pesticides, Bui and Seo modified a polymerized crystalline colloidal array (PCCA) thin film with beta-cyclodextrin (β-CD) polymer as a capping cavity for the selective detection of paraoxon-ethyl and parathion-ethyl chemical agents [21]. The diffraction peak of the β-CD modified PCCA thin film showed a red shift according to the change of paraoxon-ethyl and parathion-ethyl concentrations at a

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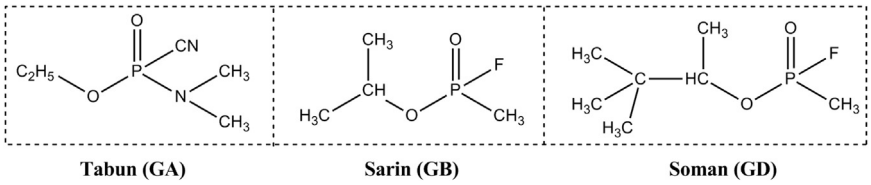

Fig. 1. The structures of Sarin, Soman and Tabun.

fast response time (10 s) and a LOD of 2.0 and 3.4 ppb respectively. Walker and Asher attached AChE to PhC, and AChE binds organophosphorus compounds irreversibly, creating an anionic phosphonyl species. This charged species created a Donnan potential, which swelled the hydrogel network and increased the embedded particle array lattice spacing and caused a red-shift in the wavelength of light diffracted. These AChE-PhC acted as dosimeters for parathion concentrations as low as 4.26×10^{-15} mol L^{-1} [22]. After that, their group also attached organophosphorus hydrolase (OPH) to PhC [23]. OPH catalyzed the hydrolysis of methyl paraoxon at basic environment, producing *p*-nitrophenolate, dimethylphosphate, and two protons. The protons lowered the pH and created a steady-state pH gradient. Protonation of the phenolates attached to the hydrogel made the free energy of mixing of the hydrogel less favorable, which caused the hydrogel to shrink and blue-shifted the diffracted light. The LOD is 2×10^{-7} mol L^{-1} paraoxon.

Although AChE has good recognition ability to organophosphates, exploration of other cheaper and more efficient enzymes is still necessary. On the other hand, detection of real nerve agents by PhC sensor has not been reported yet. Herein, we reported an effort to detect Sarin agents by PhC sensor. The PhC is immobilized with BuChE from duck blood, and was characterized by scanning electron microscope (SEM) and optical fiber spectrometer. The LOD of Sarin agent was also calculated. The simple method for the detection of Sarin agent represented its potential in response to other nerve agents.

2. Materials and methods

2.1. Materials

Tris(hydroxymethyl)aminomethane (Tris), N,N,N',N'-tetramethylethylenediamine (TEMED), N,N'-methylenebisacrylamide (BIS), DEPBT, acrylamide (AM), 2, 2-diethoxyacetophenone (DEAP), ammonium persulfate ($NH_4S_2O_8$), dimethyl sulfoxide (DMSO) and NaOH were purchased from Acros (USA). Styrene was purchased from Sigma (USA). S-butyrylthiocholine iodide (BuChI) was purchased from Fluka (USA). 1-Propanol, sodium dodecyl sulfate (SDS), L-Cysteine hydrochloride, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Aladdin Co. Ltd. All of above chemicals were of analytical grade. BuChE (MW 360,000, 8.4 units/mg of solid) from duck blood was purchased from Beijing 6901 Biochemical Plant in China; Sarin agent was donated by Institute of Chemical Defence; ion-exchange resin (AG501-X8(D), 20–50 mesh, mixed bed) was purchased from Bio-Rad (USA). Deionized water (Resistivity: 18.25 MΩ cm, Aquapro) was used for the experiment.

2.2. Instruments

The diffraction spectra were recorded by an Avaspec-2048 TEC optical fiber spectrometer (Avantes, Netherlands). The UV polymerization was carried out in a SCIENTZ D3 UV cross-linker (Ning Bo, China). SEM images were taken by a HITACHI S4800 field emission scanning electron microscope (Tokyo, Japan) with an accelerating voltage of 15 kV.

2.3. Preparation and functionalization of PhC

Polystyrene (PS) colloidal particles were prepared by mixing 100 mL ultrapure water, 0.25 g $NH_4S_2O_8$, and certain amount of SDS in a 250 mL four neck bottle. The mixture was stirred at 200 rmp, and bubbled under N_2 at 75 °C. Styrene was added slowly (1 drop every two seconds) for 9 h. The suspension was centrifuged, and the obtained PS colloidal particles were washed thoroughly with pure water for 3–4 times. Through controlling the amount of SDS with 0.225, 0.200, 0.175 and 0.150 g respectively, PS colloidal particles with 180 nm, 240 nm, 290 nm and 355 nm was obtained for further study.

The PhC was prepared and functionalized by a modified method according to Walker and Asher's report [22]. Fig. 2 describes the process of preparation and functionalization of PhC. The PhC was optimized by changing the colloidal diameter, colloidal concentration, acrylamide fraction, BIS fraction of prepolymerization solution respectively. To prepare optimal PhC, 2 mL 10% PS colloid suspension, 2.85 mg DEAP, 1.5 g ion-exchange resin, 400 mg AM (200 mg/mL) and 10 mg BIS (5 mg/mL) were mixed together and then oscillated overnight. After the removal of ion-exchange resin, the solution was added to the surface of a clean glass slide, and a cover glass was covered on. The polymerization was carried out at room temperature under 365 nm for 2 h. The obtained PhC was immersed into NaOH solution (containing 10% v/v TEMED) for 40 min to get free carboxylic acid group, and then washed with pure water.

To immobilize BuChE onto PhC, 0.25 g (2100 units) BuChE was dissolved into 10 mL Tris buffer (0.15 mol L^{-1}, pH=7.4), and the PhC was incubated in it for 48 h before immersed into DEPBT solution for 2 h. After that, the PhC was re-incubated into the enzyme solution for another 2 h, and finally was washed in Tris solution for 48 h to clean up the unreacted BuChE. The functionalized PhC was cut into small pieces (1.5×1.5 cm^2) before usage.

2.4. Measurement of enzyme activity for the functionalized PhC

The activity of BuChE on the functionalized PhC was determined using a modified Ellman's assay. BuChI, a mimic of butyrylcholine, was selected as substrate. Firstly, 0.15 mol L^{-1} Tris buffer (pH 8.0) was used to prepare 1.8×10^{-4} mol L^{-1} DTNB solution. Then 1×10^{-3} to 6×10^{-3} mol L^{-1} BuChI were respectively dissolved in the DTNB solution. After that, the functionalized PhC was placed into a series of BuChI solutions in sequence, UV absorbance at 412 nm was monitored over a 30 min time period.

2.5. Detection of Sarin agents

The reflectance of PhC was recorded using Avaspec-2048TEC optical fiber spectrometer (Avantes, Netherlands). The functionalized PhC (1.5×1.5 cm^2 in size) was incubated into deionized water for 30 min, and the diffraction spectrum was recorded as a reference. To detect Sarin agent, the concentration of Sarin solution was adjusted from 10^{-4} mol L^{-1} to 10^{-11} mol L^{-1} using deionized water, and its pH was adjusted to 7.0 using 0.1 mol L^{-1} NaOH solutions, the functionalized PhC was immersed into the Sarin solution for 30 min, and the diffraction spectrum was

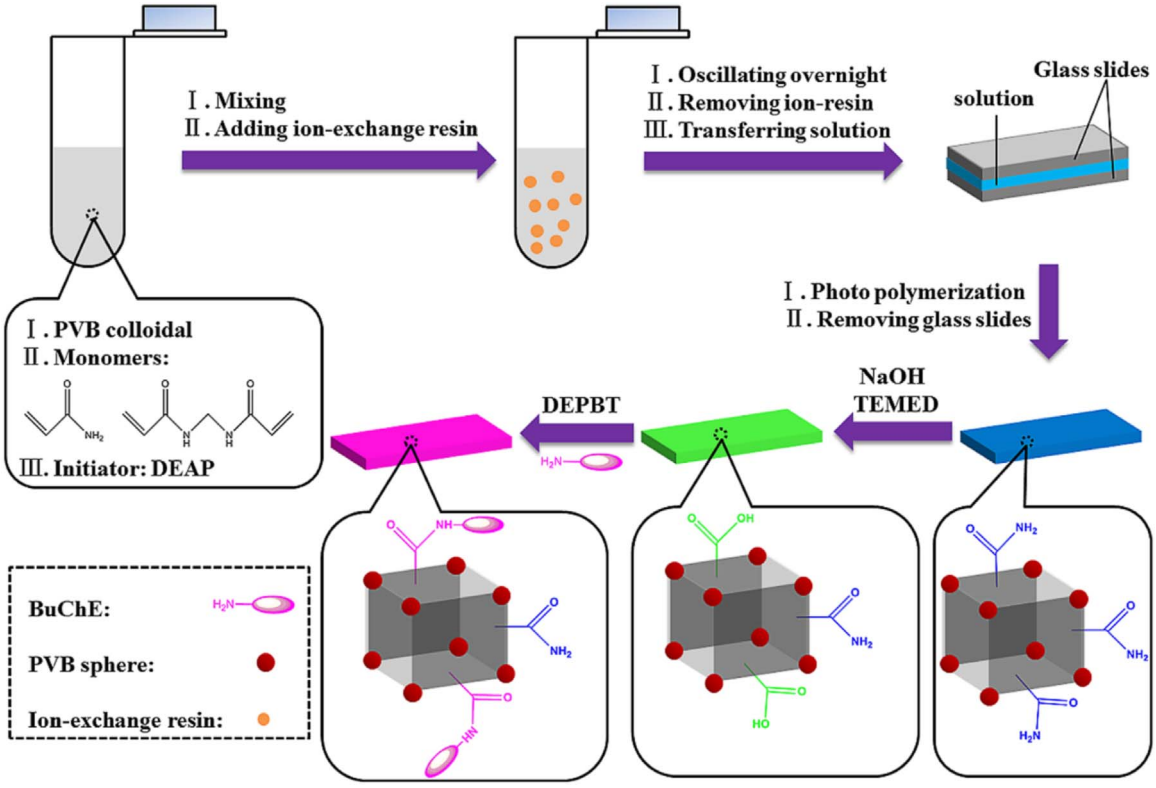

Fig. 2. Preparation and functionalization of PhC.

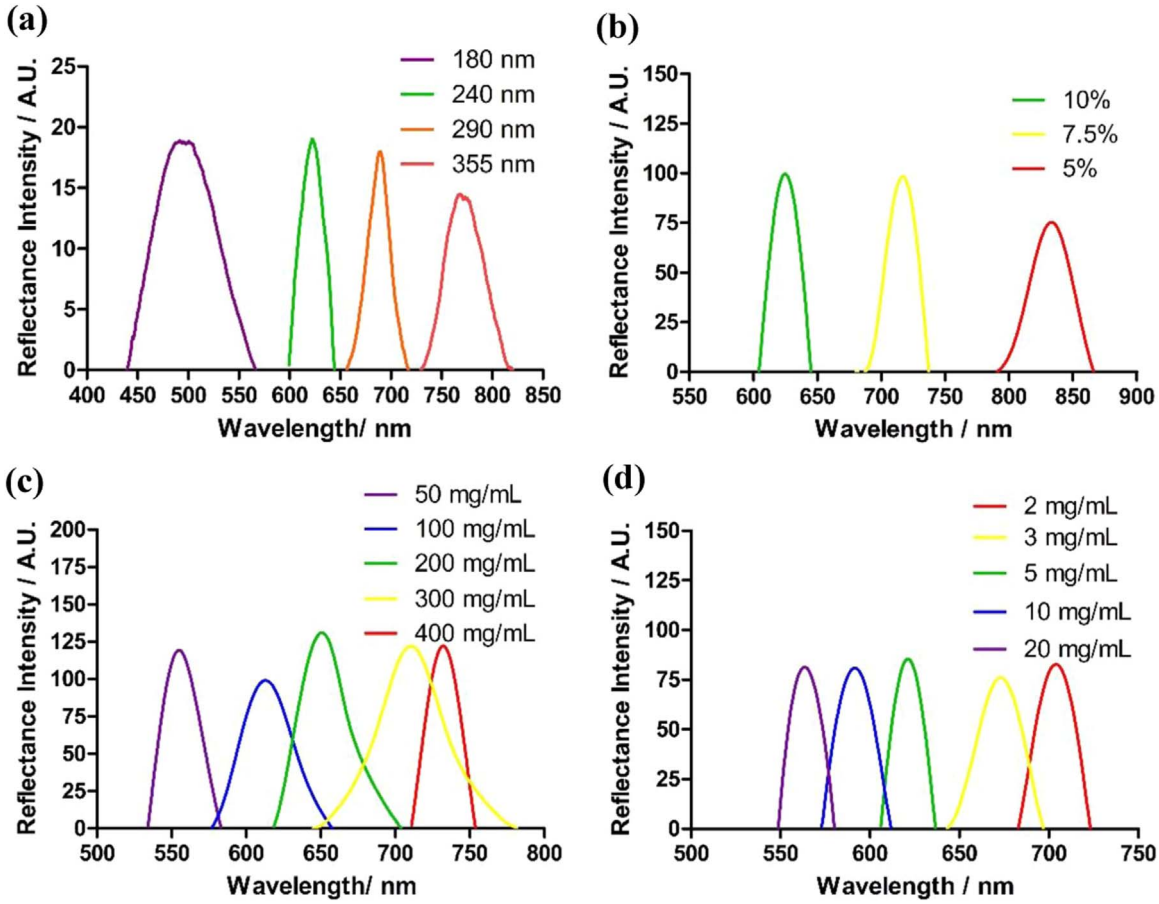

Fig. 3. The diffraction spectra of PhC in related to the colloidal diameter (a), colloidal concentration (m/m, b), acrylamide fraction in the hydrogel (c), BIS fraction in the hydrogel (d) respectively and the amount of the other components keeps same.

recorded.

3. Results and discussion

3.1. Preparation and Characterization of the functionalized PhC

The recipe of PhC was firstly optimized for better optical performance. It is known that the diffraction of PhC depends on the self-assembly procedure. With the increasing diameter of colloidal particles, the diffraction of the PhC red shifted (Fig. 3a), which agreed with the Bragg's law. But when the colloidal concentration increased, the diffraction blue shifted (Fig. 3b), the higher PS colloidal concentration, the smaller space between the colloidal particles within the PhC hydrogel, thus the diffraction blue shifted. It was also noticed that with the increasing of acrylamide fraction, the amount of crosslinker did not change, thus the crosslinking degree decreased, and the swell ability increased which induced the red shift of diffraction (Fig. 3c). When the fraction of BIS increased, the crosslinking degree increased, the hydrogel shrunk and made the diffraction blue shift (Fig. 3d). According to the above results, in order to obtain the diffraction spectrum in visible region, PhC hydrogel was made from a recipe which contained 240 nm PS colloid suspension (10% w/w), 200 mg/mL AM and 5 mg/mL BIS in a 2 mL suspension.

Before the immobilization of BuChE, the amide group of the PhC hydrogel needed to be converted into carboxylic group via hydrolysis with NaOH, the longer the hydrolysis time, the more amide groups were converted to carboxylic groups, and the hydrogel became more hydrophilic and red shifted the diffraction (Fig. 4a). Before hydrolysis, pH value didn't change the osmosis pressure of hydrogel because it didn't have influence on the interaction of adjacent amide groups, thus pH value did not affect the diffraction of the PhC significantly. But after the hydrolysis, with the increasing of pH, the carboxylic groups of the PhC hydrogel were ionized and increased the osmosis pressure, thus the diffraction red shifted. The structure of hydrolyzed PhC hydrogel was very similar to PAM-acrylic acid (AA) co-polymerized PhC hydrogel. While the pKa of AA is 4.25 at 25 °C, we surmised that the actual pKa of hydrolyzed PhC hydrogel was consistent with the pKa of AA. As a consequence, the hydrolyzed PhC hydrogel swelled most around pH 4.25 following with a sudden jump in diffraction peak from pH 4 to pH 5 (Fig. 4b). And higher ionic strength decreased the osmosis pressure, which led to the shrinking of the hydrogel and blue shift of the diffraction peak (Fig. 4c).

The morphology of the functionalized PhC was shown in Fig. 5a. The unfunctionalized PhC diffracted the light at 632 nm, when BuChE was immobilized on the PhC hydrogel via condensation with DEPBT, the functionalized PhC diffracted the light at 685 nm (Fig. 5b). With the immobilization of BuChE, the hydrogel became more hydrophilic and the free energy of mixing increased, thus the diffraction red shifted. Instead of EDC which is usually used for the immobilization of enzyme, DEPBT was selected as condenser for its higher efficiency for the synthesis of linear and cyclic peptides [24,25].

BuChE is a substitute of AChE, it can serve as a stoichiometric bioscavenger of organophosphate AChE inhibitors in the human body to detoxify organophosphorus molecules [26–29]. BuChE obtained from duck blood is cheaper than AChE. In order to identify the BuChE activity on the PhC, the functionalized PhC was applied to hydrolyze BuChI solution into thiocholine, which reacted with DTNB to form a yellow product. The standard curve that depicted the relationship of the concentration of hydrolysis product and absorbance was set up by a substitute of thiocholine, L-cysteine hydrochloride (Fig. S1). According to Lamber-Beer's law, the absorbance could be converted into the concentration of substance. Herein, one unit represented the required amount of enzyme to hydrolyze 1.0 μmol of substrate into thiocholine per min at 37 °C under pH 8.0. According to Michaelis–Menten kinetics analysis (Fig. S2), the Michaelis constant K_m was calculated as 1.2×10^{-3} mol L^{-1}. The maximum hydrolysis rate V_{max} was determined as 3.7 μmol min^{-1}, which means a total of 3.7 units of BuChE were immobilized onto the PhC hydrogel. In a former report which immobilized AChE onto the PhC via condensation with EDC, 2.4 units of AChE was immobilized [22]. Such a difference might be attributed to the usage of different substrate and enzyme during this study.

3.2. Detection of Sarin agents

To detect Sarin agent, the functionalized PhC was subjected to 10^{-11} mol L^{-1}–10^{-4} mol L^{-1} Sarin solutions in sequence, and the diffraction peak blue shifted with the increasing of Sarin concentration. A linearity (r^2=0.977) was observed between the amount of diffraction and the logarithm of Sarin concentration from 10^{-11} mol L^{-1} to 10^{-4} mol L^{-1} (Fig. 6). A LOD of 10^{-15} mol L^{-1} was achieved. A BuChE-free PhC was exposed to Sarin solutions in the same way as a control experiment, whereby no significant diffraction shift was observed, and this indicated that the resulting diffraction shift was not triggered by the amide or carboxyl groups on the polymer backbone.

The blue shift of diffraction in response to Sarin agent is different from that of Walker and Asher's report [22], in their case AChE bound parathion and actuated a volume increase proportional to the concentration of analyte because of the formation of an anionic phosphonyl species. The increase of hydrogel volume made the red shift of diffraction light. Their AChE-PCCA sensor relied upon a volume phase transition of the hydrogel due to changes in the ionic free energy of the system. While in this case, the response of the PhC was due to the HF formed when Sarin

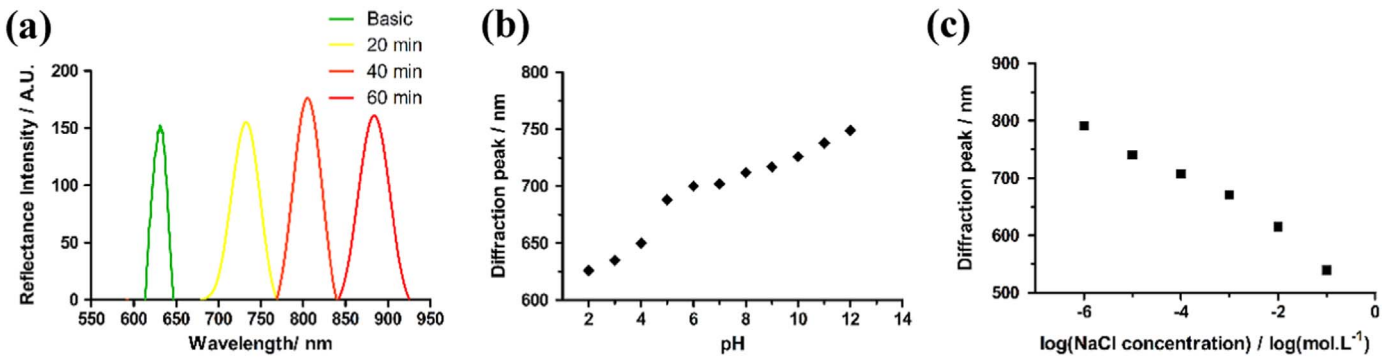

Fig. 4. The diffraction spectrum of PhC in related to the hydrolysis time (a); the diffraction peak of unfunctionalized PhC as a function of pH (b) and ionic strength (c) after hydrolysis, the other environments are same.

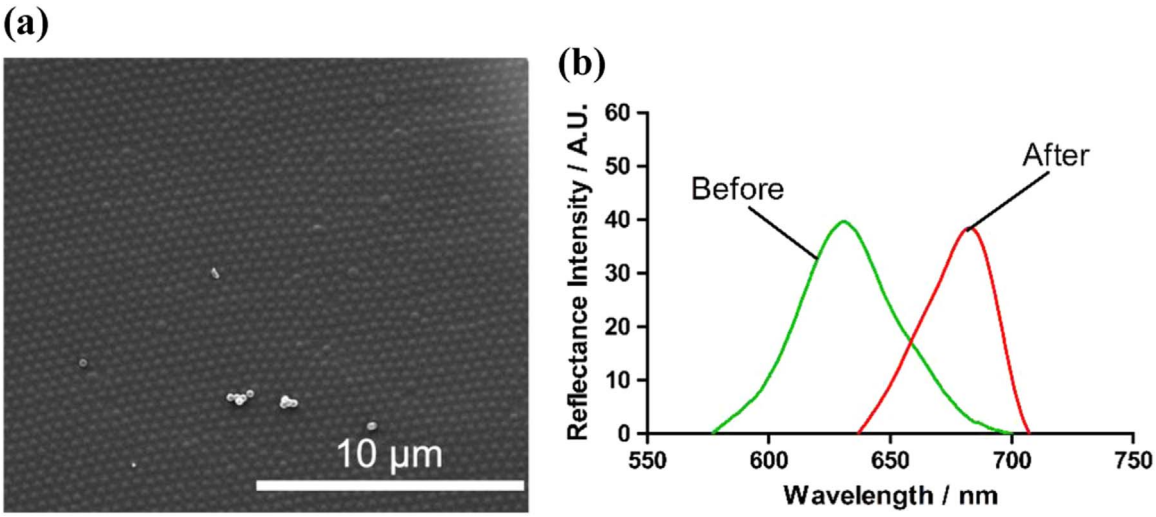

Fig. 5. (a) SEM of the functionalized PhC; (b) diffraction spectrum of the PhC before and after functionalization with BuChE.

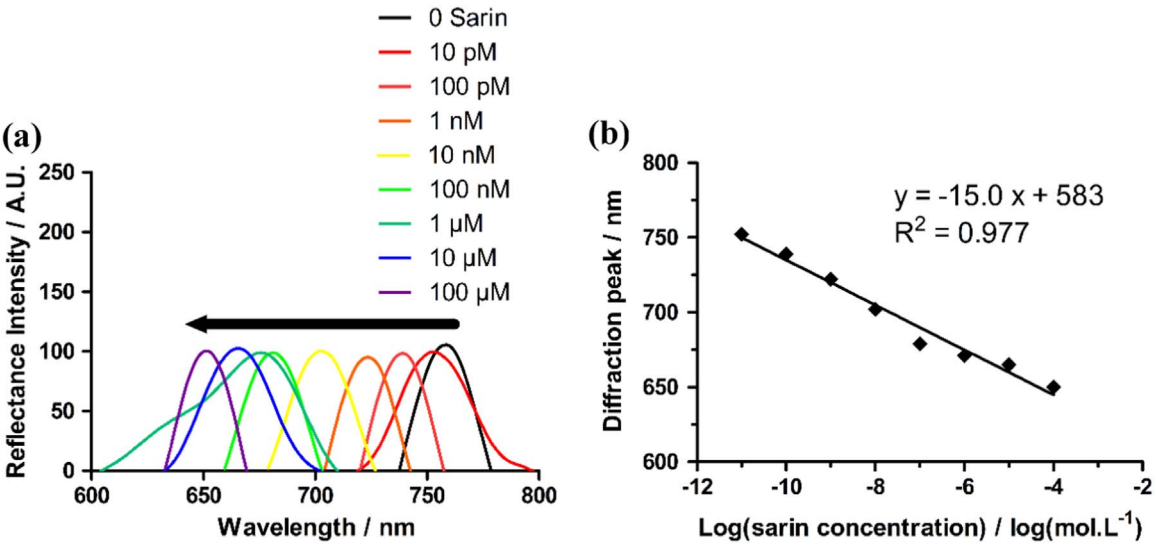

Fig. 6. (a) Diffraction spectra of the functionalized PhC at various concentration of Sarin solutions; (b) the diffraction peak of functionalized PhC in response to Sarin in aqueous solutions.

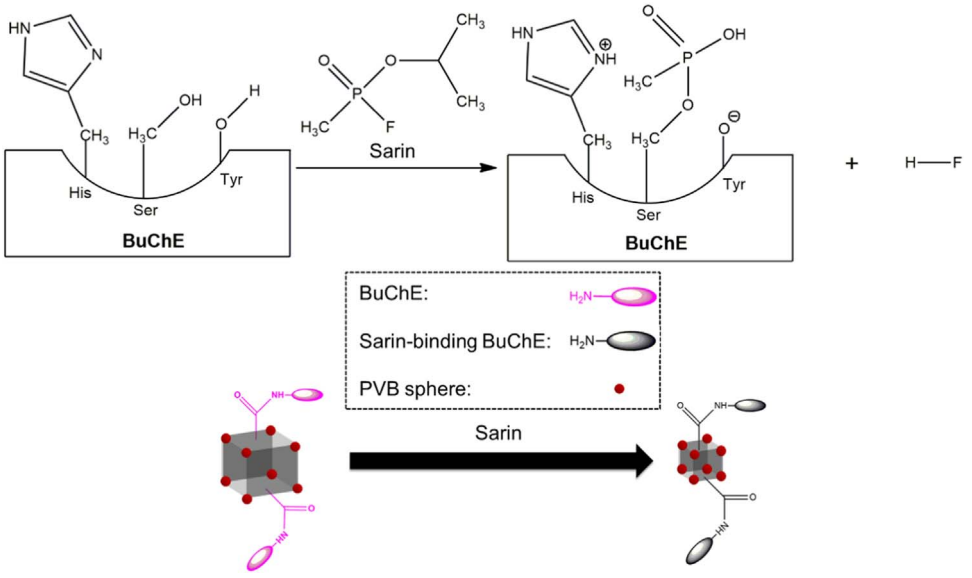

Fig. 7. The reaction mechanism of Sarin with BuChE.

inhibited the BuChE (Fig. 7) [30]. The formation of HF made the solution acidic, and thus caused the decreasing of pH value and the increasing of ionic strength, both of which were able to make the diffraction wavelength blue shift.

4. Conclusions

In order to detect Sarin agent, we developed a method to functionalize PhC by the immobilization of BuChE with DEPBT condenser. Compared with the widely used AChE, more active BuChE (50%) can be immobilized on the PhC. The diffraction of the functionalized PhC blue shifted in response to the Sarin agent. HF was formed when Sarin agent inhibited BuChE, which induced the decrease of pH value and the increase of ionic strength, thus blue shifted the diffraction. A LOD of 10^{-15} mol L^{-1} was achieved, and in the 10^{-11} mol L^{-1} to 10^{-4} mol L^{-1} Sarin solutions, a linearity of LgC_{sarin} to the amount of diffraction blue shift was achieved. This functionalized PhC achieved a simple way for the detection of real nerve agent.

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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.talanta.2016.06.045.

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