Photonic crystal hydrogel sensor for detection of nerve agent

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Abstract. Nowadays the photonic crystal hydrogel materials have shown great promise in the detection of different chemical analytes, including creatinine, glucose, metal ions and so on. In this paper, we developed a novel three-dimensional photonic crystal hydrogel, which was hydrolyzed by sodium hydroxide (NaOH) and immobilized with butyrylcholinesterase (BuChE) by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDC). They are demonstrated to be excellent in response to sarin and a limit of detection(LOD) of 1×10^{-9} mg mL^{-1} was achieved.

1. Introduction

There is currently an urgent need for efficient, rapid detection of nerve agents. Nerve agents are a class of highly toxic organophosphates which disrupt nerve conduction by blocking acetylcholin esterase (AChE), an enzyme that normally destroys acetylcholine, a neurotransmitter. Among various sensor technologies, photonic crystal hydrogel is a novel technology which now attracts more and more attentions due to its unique ability for the detection [1-3].The term “photonic crystals(PhC)” relates to a periodical arrangement of a regularly shaped material consisting of nanoparticles [4]. A much more rugged polymerized crystalline colloidal array (PCCA) was prepared by embedding the crystalline colloidal array (CCA) within a hydrogel matrix. These three-dimensionally ordered CCAs can intensely Bragg diffract light in the ultraviolet, visible, and infrared regions of the spectrum. The diffracted wavelength of the photonic crystal hydrogel shifts as the hydrogel volume changes due to the alterations in the spacing between lattice planes [5-7].

Liu et al. developed a molecularly imprinted photonic crystal 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 [8]. Walker and Asher attached AChE to photonic crystal, and AChE binds organophosphorus compounds irreversibly, creating an anionic phosphonyl species. These AChE-PhC acted as dosimeters for parathion concentrations as low as 4.26×10^{-15} mol L^{-1} [9]. Although AChE has good recognition ability to organophosphates, exploration of other cheaper and more efficient enzymes is still necessary.

In this study, we developed a method of synthesizing monodisperse polystyrene colloids of diameters between 100~440 nm. These monodisperse colloidal particles readily self-assembled into robust three-dimensionally ordered CCAs. We then polymerized a hydrogel around the CCA to form a PCCA. The PhC is immobilized with BuChE from duck blood, and was characterized 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. Experimental Section

2.1. Preparation of photonic crystal hydrogel

Figure 1 depicts the preparation of the photonic crystal hydrogel. Monodisperse polystyrene spheres were synthesized by emulsion polymerization with sodium-dodecyl sulphate (SDS, Beijing Chemical Works) as emulsifier. After 8 h of reaction, all the suspension were centrifuged for purification. The particle sizes were measured using quasi-elastic light scattering (QELS; Malvern Nano Series) and the morphology was measured by scanning electron microscope (SEM; HITACHI Quanta 250 FEG). Acrylamide (Amresco), N,N′-methylenebisacrylamide (Sigma-Aldrich), a colloid suspension (polystyrene spheres) in nanopure water, and 10% diethoxyacetophenone (DEAP, Alfa Aesar) in DMSO (Sigma-Aldrich) were mixed and injected between two quartz disks. The colloidal particles self-assembled into a CCA, leading to a liquid film that diffracts light. The film was exposed to 365-nm UV light (Ultra-Violet Products Ltd) for 2 h. A polyacrylamide hydrogel network formed around the CCA, resulting in a PCCA. The diffraction of the PCCA was monitored using a fiber-optic diode spectrometer with a tungsten halogen light source (Avaspec-2048TEC, Avantes) using a reflectance probe.

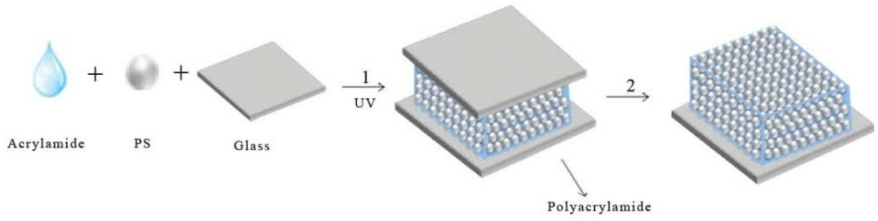

Figure 1. Preparation of photonic crystal hydrogel.

2.2. Functionalization of PCCA

The PCCA was then hydrolyzed in a solution of NaOH (Beijing Chemical Works) containing 10% v/v N,N,N’,N’-tetramethylethylenediamine (TEMED, Sigma-Aldrich) at 25 °C for different times. The hydrolyzed PCCA was washed for 2 h with 150 mM NaCl (Beijing Chemical Works) [10]. The surface profile of hydrolyzed PCCA was measured using scanning electron microscope (SEM; HITACHI Quanta 250 FEG). The diffraction of the PCCA was monitored using a fiber-optic diode spectrometer.

To immobilize BuChE onto the hydrolyzed PCCA, 2000 units BuChE was dissolved into Tris buffer (0.15 mol L^{-1}, pH=7.4), and the hydrolyzed PCCA was incubated in it for 48 h before immersed into 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDC▪HCl) solution for 2 h. After that, the PCCA 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 BuChE-PCCA was cut into small pieces before usage.

2.3. Measurement of Sarin agents

The diffraction of BuChE-PCCA was recorded using Avaspec-2048TEC optical fiber spectrometer (Avantes, Netherlands). The BuChE-PCCA was incubated into nanopure water for 15 min, and the diffraction spectrum was recorded as a reference. To detect Sarin agent, the concentration of Sarin solution was adjusted from 1×10^{-9} mg mL^{-1} to 1×10^{-2} mg mL^{-1} using nanopure water, and its pH was

adjusted to NaOH solutions, the BuChE-PCCA was immersed into the Sarin solution for 10 min, and the diffraction spectrum was recorded.

3. Results and discussion

3.1. Characterization of PCCA

Monodisperse, highly charged polystyrene colloidal particles with diameters between 100–400 nm were synthesized that listed in Table 1. Because of electrostatic interactions, all of these colloids readily formed CCAs, which exhibited sharp diffraction peaks in the UV/Vis region. It is known that the diffraction of PCCA depends on the self-assembly procedure. With the increasing diameter of colloidal particles, the diffraction of PCCA red shifted (Fig. 2), which agreed with the Bragg's law.

Table 1. Characterization of polystyrene nanospheres with different dosage of emulsifier by QELS

number	1	2	3	4	5	6	7
SDS/g	0.100	0.125	0.150	0.175	0.200	0.225	0.250
Diameter/nm	441.2	404.3	355.7	289.6	223.5	180.0	101.0
PDI	0.007	0.060	0.094	0.069	0.079	0.007	0.035

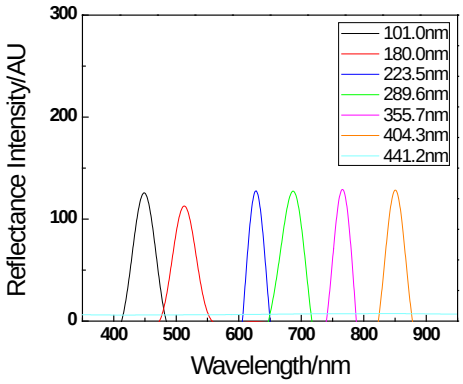

Figure 2. The diffraction spectra of PCCA in related to the colloidal diameter.

3.2. Characterization of Functionalized PCCA

For immobilizing BuChE onto the PCCA, the amide group of the photonic crystal hydrogel needed to be converted into carboxylic group via hydrolysis with NaOH. Figure 3a showed SEM images of functionalized PCCA. The polystyrene spheres in the hydrogel appeared neat and showed high ordering with unclose packed structure. Via monitoring the diffraction spectrum of functionalized PCCA, we found that 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. 3b). We found that modification could make PCCA volume increased. Too short modification time may make the modification inadequately. Too long modification time can give rise to the deterioration in PCCA mechanical strength, because the PCCA volume increased too much. For chemical sensing, the PCCA is required to have proper mechanical strength, so 40 min was optimized as the appropriate modification time.

BuChE is a substitute of AChE, which can serve as a stoichiometric bioscavenger of organophosphate AChE inhibitors in the human body to detoxify organophosphorus molecules. BuChE obtained from duck blood is cheaper than AChE. The unimmobilized PCCA diffracted the light at 632 nm, when BuChE was immobilized on the photonic crystal hydrogel via condensation with EDC, the diffraction of BuChE-PCCA red shifted (Fig.4). With the immobilization of BuChE, the hydrogel became more hydrophilic and the free energy of mixing increased, thus the diffraction red shifted.

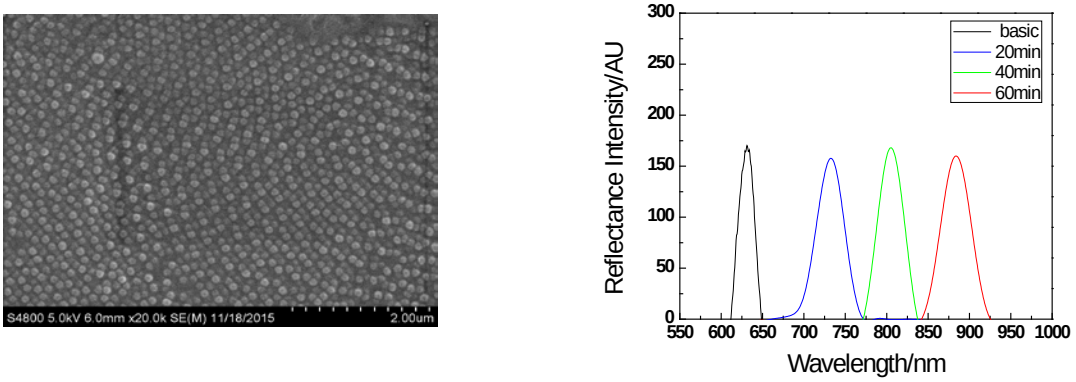

Figure 3.(a) SEM of the functionalized PCCA; **(b)**The diffraction spectrum of PhC in related to the hydrolysis time.

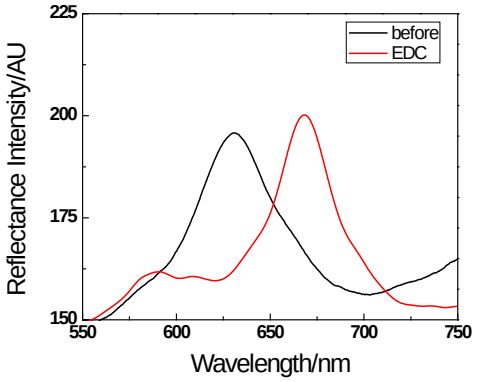

Figure 4. Diffraction of the PCCA before and after immobilization with BuChE.

3.3. Sensor Properties of BuChE-PCCA

Figure 5 showed the diffraction response of unimmobilized PCCA to sarin. Figure 6 showed the diffraction response of BuChE-PCCA to sarin. The results showed that as Sarin concentration increased from 1×10^{-9} mg mL^{-1} to 1×10^{-2} mg mL^{-1}, the diffraction of the unimmobilized PCCA blue shifted. However, the diffractions of the BuChE-PCCA which response to Sarin concentration from 1×10^{-9} mg mL^{-1} to 1×10^{-7} mg mL^{-1} red shifted compared with the diffractions of BuChE-PCCA in nanopure water, and blue shifted for Sarin concentration from 1×10^{-6} mg mL^{-1} to 1×10^{-2} mg mL^{-1}. A LOD of 1×10^{-9} mg mL^{-1} was achieved. A BuChE-free PCCA was exposed to Sarin solutions in the same way as a control experiment 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 [4].The molecular recognition agent for the sensor was the BuChE, which bound sarin irreversibly, creating an anionic phosphonyl species. This charged species creates a Donnan potential, which swells the hydrogel network, which increases the embedded particle array lattice spacing and causes a red-shift in the wavelength of light diffracted [9].But as the concentration of sarin increased, the amount of red-shift decreased continuously. While in this case, the response of the PhC was due to the HF formed when Sarin inhibited the BuChE [11]. 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 decrease of red-shift.

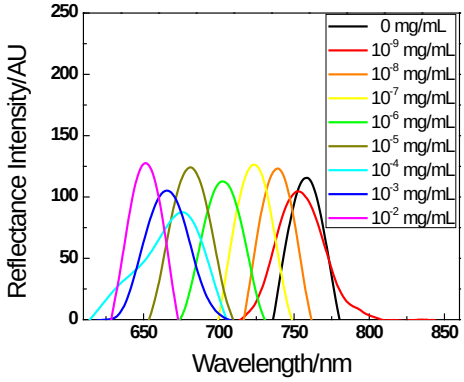

Figure 5. Diffraction spectra of the BuChE-free PCCA at various concentration of Sarin solutions.

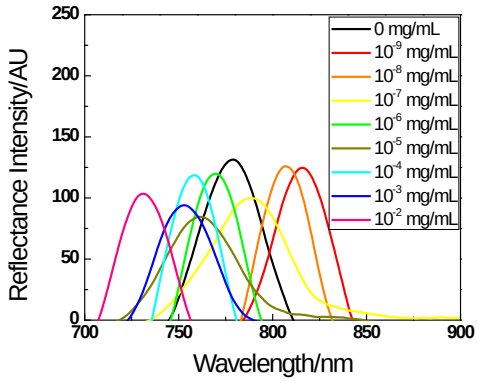

Figure 6. Diffraction spectra of the BuChE- PCCA at various concentration of Sarin solutions.

4. Conclusions.

We developed a novel material to determine various concentrations of sarin, which were immobilized with BuChE by EDC onto functionalize PCCA. After chemical modification of hydrogel backbone and immobilization, the diffraction of the functionalized photonic crystal hydrogel red shifted compared with the diffractions of BuChE-PCCA in nanopure water in response to Sarin concentration from 1×10^{-9} mg mL^{-1} to 1×10^{-7} mg mL^{-1} and blue shifted for Sarin concentration from 1×10^{-6} mg mL^{-1} to 1×10^{-2} mg mL^{-1}. This detection limit was 1×10^{-9} mg mL^{-1} lower than current enzyme detection methods for organophosphorus agents. We were developing this sensor for use in detecting other organophosphorus agents in this field.

References

[1] Moshe M B, Alexeev V L and Asher S A, Fast responsive crystalline colloidal array photonic crystal glucose sensors, *Anal. Chem*. **78** (2006) 5149–5157

[2] Reese C E and Asher S A. Photonic Crystal Optrode Sensor for Detection of Pb^{2+} in High Ionic Strength Environments. *Analytical Chemistry*, **75**(2003) 3915-3918

[3] Holtz J H and Asher S A. Polymerized Colloidal Crystal Hydrogel Films as Intelligent Chemical Sensing Materials. *Nature*, **389**(1997) 829-832

[4] Fenzl C, Wilhelm S, Hirsch T and Wolfbeis O S, Optical Sensing of the Ionic Strength Using Photonic Crystals in a Hydrogel Matrix, *Applied Materials & Interfaces*, (2013) 173–178

[5] Kamenjicki M, Kesavamoorthy R and Asher S A, Photonic Crystal Devices, *Ionics*. (2004), 233–236

[6] Ge J P and Yin Y D, Responsive photonic crystals, *Angew. Chem. Int .Ed*. **50** (2011) 1492-1522

[7] Kamenjicki M and Asher S A. Epoxide Functionalized Polymerized Crystalline Colloidal Arrays. *Sensors and Actuators B*, **106**(2005) 373-377

[8] Liu F, Huang S Y, Xue F, Wang Y F, Detection of organophosphorus compounds using a molecularly imprinted photonic crystal, *Biosens. Bioelectron*. **32** (2012) 273–277

[9] Walker J P and Asher S A, Acetylcholinesterase-Based Organophosphate, *Analytical Chemistry*, (2005) 1596–1600

[10] Walker J P, Kimble K W and Asher S A, Photonic Crystal Sensor for Organophosphate Nerve Agents Utilizing the Organophosphorus Hydrolase Enzyme, *Anal Bioanal Chem*, (2007) 2115–2124

[11] Hu X B, Li G T, Li M H, Huang J, Li Y, Gao Y B and Zhang Y H, Ultrasensitive specific stimulant assay based on molecularly imprinted photonic hydrogels, *Adv. Funct. Mater*, **18** (2008) 575–583