A novel photonic sensor for the detection of chloramphenicol

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Abstract A novel photonic sensor using a self-cross-linked imprinting close-packed opal (CPO) as a recognition element was developed for the detection of chloramphenicol (CAP). The self-cross-linked imprinting CPO film was consisted of monodisperse molecular imprinted – N-hydroxymethylacrylamide particles (MI-HAM particles) with self-assembling and self-crosslinking properties. A dilute dispersion of these particles was self-assembled to formation of the highly ordered CPO structure, and at the same time the neighboring particles reacted to form covalent bond to stabilize the CPO structure at the air/dispersion. Thus the obtained self-cross-linked imprinting CPO was characterized by a highly stable three-dimensional (3D) CPO structure without the interference of bulk hydrogel matrix, in which numerous CAP recognition sites were dispersed through the molecular imprinted process. The inherent high affinity of the recognition sites allowed the self-cross-linked imprinting CPO to recognize CAP with high specificity, and changes of the periodic structure enabled the self-cross-linked imprinting CPO to transfer the recognition events into readable optical signals. Then the self-cross-linked imprinting CPO film was packed into a reaction cell with an optical spectrometer to establish a novel photonic sensor. A linear relationship was found between the diffraction intensity decrease and CAP concentration in the range from 2 ng mL⁻¹ to 512 ng mL⁻¹, whereas there was no obvious optical change for CAP analogues, thus indicating that the sensor had selective and sensitive response to CAP molecules. Furthermore, the sensor was applied successfully to detect CAP in drinking water samples. As a result, the developed sensor might offer a potential alternative in routine supervision for residue due to its convenience, low cost, reusability, and high sensitivity as well as selectivity.

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1. Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic which was widely used in both human and poultry medicine (X.M. Yang et al., 2015; R.R. Yang et al., 2015). However, it has serious side-effects on human beings, such as bone marrow suppression, “gray baby syndrome”, aplastic anemia acute leukemia and so on (Que et al., 2014; X.M. Yang et al., 2015; R.R. Yang et al., 2015). Thus, many countries including USA, Canada and China have controlled CAP
Molecular imprinting polymers are synthetic materials with artificially imprinted template molecules (Y. Liu et al., 2009; N. Liu et al., 2009). In addition, the utility of colloidal arrays can be cross-linked making use of a suitable cross-linker (Zhao et al., 2009; Zhang et al., 2012). However, addition of the cross-linker solution may disturb the preformed ordered structure, and the cross-linking reaction usually causes a heterogeneous distribution. Alternatively colloidal particles with functional groups such as vinyl groups, were synthesized and then cross-linked under the special polymerization condition (Chen et al., 2013). Unfortunately this method was time-consuming and difficult to control. A facile method was later developed, in which colloidal particles incorporated with N-hydroxymethylacrylamide (HAM) have cross-linked simultaneously when the spheres assembled into colloidal array taking advantage of the self-cross-linking property of HAM (Chen et al., 2014). Based on this principle, monodisperse spheres composed of a copolymer of poly(N-isopropylacrylamide) (PNIPAM) and HAM were synthesized to build a stable colloidal crystal for sensing temperature (Zhou et al., 2009). Therefore, the introduction of HAM into the imprinting CPO would be a promising way to solidify the ordered structure due to its simple and time-saving characteristics.

Herein, a self-cross-linked imprinting CPO was fabricated using molecular imprinted particles with HAMs (MI-HAM particles) for development of a new photonic CAP sensor without the interference of bulk hydrogel. As shown in Fig. 1, the MI-HAM particles that can be assembled to form the CPO structure and at the same time mutually reacted to form covalent bonds to stabilize the CPO structure. Thus, the obtained self-cross-linked imprinting CPO possessed not only a larger specific surface area with recognition sites for specifically adsorbing CAP molecules, but also the stable high-ordered CPO structure for creating optical signals along with the CAP recognition process. Meanwhile, the developed sensor was employed to detect the fragile inverse opal, which may further destroy the hydrogel. Moreover, relying on the bulk hydrogel response would prolong the sensing time, which was caused by the slow swelling rate of the bulk hydrogel (Y. Liu et al., 2009; N. Liu et al., 2009). Recently, we have developed an imprinting CPO by the assembly of molecular imprinted microgel particles that could easily identify a small change in Bragg diffraction and rapidly detect 17β-estradiol (Sai et al., 2015). However, the imprinting CPO still needs the bulk hydrogel matrix to stabilize the fragile ordered structure of the microgel particles array, which was time-consuming and complex process. Moreover, the hydrogel matrix may significantly reduce the extent of the volume phase transition of the microgel particles (Chen et al., 2013; Guan and Zhang, 2011).

During the past decade, cross-linking of colloidal array has drawn great attention and some efforts have been made for the stabilization of the highly ordered principle. One way is that the particles in colloidal arrays can be cross-linked making use of a suitable cross-linker (Zhao et al., 2009; Zhang et al., 2012). However, addition of the cross-linker solution may disturb the preformed ordered structure, and the cross-linking reaction usually causes a heterogeneous distribution. Alternatively colloidal particles with functional groups such as vinyl groups, were synthesized and then cross-linked under the special polymerization condition (Chen et al., 2013). Unfortunately this method was time-consuming and difficult to control. A facile method was later developed, in which colloidal particles incorporated with N-hydroxymethylacrylamide (HAM) have cross-linked simultaneously when the spheres assembled into colloidal array taking advantage of the self-cross-linking property of HAM (Chen et al., 2014). Based on this principle, monodisperse spheres composed of a copolymer of poly(N-isopropylacrylamide) (PNIPAM) and HAM were synthesized to build a stable colloidal crystal for sensing temperature (Zhou et al., 2009). Therefore, the introduction of HAM into the imprinting CPO would be a promising way to solidify the ordered structure due to its simple and time-saving characteristics.

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![Figure 1](https://example.com/figure1.png)

**Figure 1** The schematic diagram of the novel photonic sensor system.

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CAP in real samples. As far as we concerned, there has been few reports on the sensor based on the self-cross-linked imprinting CPO for the detection of CAP or other relevant veterinary drug residues.

2. Materials and methods

2.1. Chemical and reagents

CAP, chloramphenicol succinate (CAP succinate), penicillin, thiamphenicol, and methyl methacrylate (MMA) were purchased from Aladdin (Shanghai, China). Potassium peroxydisulfate (KPS), methanol, acetic acid, HAM, sulfuric acid and other chemicals were supplied from Tianjin Regent Corp. (Tianjin, China). MMA was treated with activated carbon for 24 h prior to use. KPS was recrystallized using deionized water prior to use. The deionized water used throughout the study was passed through ion-exchange columns. Microscope slides (7 cm x 3 cm x 0.12 cm) were purchased from Haimen Condor Experimental Equipment Factory (Haimen, China). Before use, the slides were immersed in H2SO4·H2O2 mixture (7:3, V/V) for 12 h, followed by rinsing with deionized water in an ultrasonic bath for 3 times. All solvents and chemicals were of reagent quality and were used without further purification.

2.2. Instrumentation

As shown in Fig. 1, the schematic diagram of the photonic sensor system basically consists of a Y-shape fiber optical reflection probe (R400-7, Ocean Optics, Dunedin, FL, USA), high resolution fiber-optic spectrometer (HR2000, Ocean Optics, Dunedin, FL, USA), a tungsten halogen light source (HL2000, Ocean Optics, Dunedin, FL, USA), a U-shape brown glass dish and a computer. The surface, the shape and the size of self-cross-linked imprinting CPO were studied using the Thermo-4800 high-resolution field emission scanning electron microscopy (SEM).

2.3. Preparation of the self-cross-linked imprinting CPO

In a 50 mL glass flask, the CAP (65 mg) and HAM (0.05 mg) were dissolved in MMA (3 mL) and then were stored for 12 h at room temperature (RT) for sufficient complexation. The mixture and KPS (60 mg) were poured into 100 mL flask with a reflux condenser, a teflon stirrer powered by a mechanical stirrer, a temperature sensor and a nitrogen inlet. After degassing and nitrogen purging for about 10 min to remove oxygen, the flask was allowed to polymerization at 80 °C with constant stirring rate of 300 rpm and the presence of nitrogen gas for 45 min. These MI-HAM particles were fully dispersed in deionized water and then were injected into a clean staining jar. After complete evaporation of deionized water, a CPO structure was formed and the self-crosslink reaction between neighboring MI-HAM particles was carried out at the same time. The self-cross-linked imprinting CPO film was finally obtained by the removal of the template molecules CAP with a mixture of acetic acid and methanol (15:1, V/V) until no CAP molecules were detected by a UV-vis spectrophotometer. At the same time, a self-cross-linked non-imprinting CPO was also fabricated with the same manner but under conditions of the absence of CAP. The self-cross-linked imprinting CPO and the self-cross-linked non-imprinting CPO were respectively equilibrated in deionized water before recording reflection measurements.

2.4. The photonic CAP sensor analytical performance

Technical details on the photonic sensor system used this work have been shown in Fig. 1. The prepared self-cross-linked imprinting CPO was fixed into a piece of U-shape brown glass dish, which served as a reaction cell under the optical fiber probe that linking with a tungsten halogen light source and high resolution fiber-optic spectrometer. The sensitivity of the sensor was examined by adding different concentrations of CAP in methanol solutions. After detection, the used self-cross-linked imprinting CPO film was rinsed with a mixture of acetic acid and methanol (15:1, V/V) solution for recovery. Analogues of CAP including CAP succinate, thiamphenicol and penicillin at different concentrations in methanol solution were detected respectively for measuring the selectivity and specificity. A series of tests on the sensor regarding repeatability and reproducibility were undertaken at the same conditions.

The data analysis was performed by normalizing the changes of Bragg diffraction intensity with the following Eq. (1):

\[ \Delta I = \left( I_0 - I_b \right) - \left( I_x - I_b \right) \]

where \( \Delta I \) is the change of the peak intensity of the response, \( I_0 \) and \( I_b \) are the reflection peak height of the spectrum and baseline respectively, and \( I_x \) and \( I_b \) represent the reflection peak height values and the baseline values, respectively, after adding CAP solution.

2.5. Real sample preparation for analysis

Drinking water sample was obtained from the Haihe River in Tianjin. A Millipore filter (0.45 μm) was used to filter the water sample, then CAP standard (20 ng mL\(^{-1}\), 60 ng mL\(^{-1}\) and 180 ng mL\(^{-1}\)) was spiked for analysis.

3. Results and discussion

3.1. Preparation of self-cross-linked imprinting CPO films

The self-cross-linked imprinting CPO films were prepared with MI-HAM particles by a self-assembly approach. Firstly, the MI-HAM particles were synthesized by emulsion-free polymerization. The size of the particles can be adjusted in the range of 180–250 nm by changing the polymerization conditions. Concretely, larger sized particles were synthesized by using a more amount of MMA, a less amount of KPS, a slower rotation speed or lower temperatures. In contrary, smaller size particles were obtained using a smaller MMA, larger KPS, higher temperatures or faster rotation speed. In our work, monodisperse particles with an average diameter of 210 nm were used to form CPO structure by a vertical deposition method on glass substrates. During the formation of the CPO structure, constant temperature, humidity and quiet environment were necessary in order to produce a highly ordered CPO structure avoiding the irregular deposition layer conformation. In addition, it should be noted that the CPO structure can be cross-linked by itself. In the process of CPO structure
formation, the MI-HAM particles were gradually close to each other by the self-assembly and reacted each other to form covalent bonds to stabilize the CPO structure based on the self-crosslinking property of HAM with the evaporation of water from the particles dispersion. After removal of CAP templates with weak acid solution, the stable self-cross-linked imprinting CPO with numerous recognition sites was fabricated. Generally, the fabrication of molecular imprinted PCs needs considerable time. For imprinted IOPC materials (Zhou et al., 2012; Hu et al., 2006; Wu et al., 2008), it usually takes hours, days, or even months to prepare the material. For our previous material (Sai et al., 2015), the extra process of hydrogel matrix synthesis was required spending tens of minutes. Therefore, the preparation of the self-cross-linked imprinting CPO was an easy and time-saving process as the material had self-assembled and self-crosslinking functions.

Fig. 2 shows SEM images of the self-cross-linked imprinting CPO. The highly ordered monodispersity and the CPO arrangement were clearly observed in Fig. 2A. The monodispersity offered the self-assembly and the production of face-centered cubic (FCC) ordered layers of particles. In addition, there were linkages between neighboring particles for the stabilization of the CPO structure. Fig. 2B demonstrated a random particle touching another six in one layer, evidenced by a highly 3D ordered with a hexagonal symmetry of the imprinting CPO. The self-cross-linked imprinting CPO were arranged over the entire surface with the (1 1 1) plane of the FCC lattice, parallel to the substrate, making it easier for the target molecules to pass in and out. Therefore, the target molecules can be washed off and adsorbed easily. As a result, these special structure characteristics enabled the material not only to immediately create optical signals due to the Bragg diffraction effects, but also to rapidly and sensitively respond to target analytes due to their large integrated area and the easy accessibility of drugs into the recognition sites within the self-cross-linked imprinting CPO.

3.2. The photonic sensor analytical performance for the determination of CAP

When a series of concentrations of CAP were injected into the sensor, the optical signal changes are shown in Fig. 3. As shown in Fig. 3, the diffraction intensity decreased by 1.718 a.u upon exposure to 2 ng mL\(^{-1}\) of CAP. It was found that the sensor was very sensitive to CAP molecules. With an increase of the CAP concentration, the diffraction intensity decreased gradually, and then reached the plateau value around 512 ng mL\(^{-1}\) of CAP due to saturation of accessible imprinting recognition sites. Relationship between CAP concentration and \(D_I\) (the reducing value of the diffraction intensity) was calculated. It was learned that the photonic sensor exhibited an great linear relationship between CAP concentration and \(D_I\) with \(R\) value of 0.99122, which was better than our previous work (Sai et al., 2015). This phenomenon may be due to the avoidance of the bulk hydrogel interference in this study. As a result, this sensor can realize quantitative detection of CAP with the help of spectrometer, which establishes a good foundation in order to detect CAP in real samples.

**Figure 2** Morphology of the self-cross-linked imprinting CPO: (A) SEM image was taken at 50,000× and (B) fractured surface showing the FCC array of particles 100,000×.

**Figure 3** The typical responses of the photonic sensor using the self-cross-linked imprinting CPO and the regression curve of the photonic sensor using the self-cross-linked imprinting CPO.
3.3. Possible reaction mechanisms of the photonic sensor

To further clarify the molecular recognition property of the photonic sensor, the self-cross-linked non-imprinting CPO was used as a recognition element instead of the self-cross-linked imprinting CPO. As shown in Fig. 4, the diffraction intensity responded slightly and changed with irregular fluctuation along with the change of CAP concentration, which was greatly different from the response event of the self-cross-linked imprinting CPO in Fig. 3. This may be explained by the absence of the CAP imprinting sites in the self-cross-linked non-imprinting CPO. Therefore, the sensing properties of the photonic sensor were due to the numerous recognition sites derived from CAP imprinting and obeyed the recognition mechanism of molecular imprinted technology. On the other hand, when applying the bulk imprinting polymer without CPO structure as a recognition element, we found that the diffraction peak disappeared, leading to an ill-defined optical signal (not shown), which may be caused by the absence of Bragg diffraction effects. Thus, just using the self-cross-linked imprinting CPO would be able to play a role in the sensor. When the CAP solution was absorbed on the self-cross-linked imprinting CPO, the recognition event with high affinity can occur and cause reduction of diffraction intensity subsequently in Fig. 3. The phenomenon would be illustrated by the following reasons. Firstly, CAP molecules entering into the self-cross-linked imprinting CPO can form a multitude of simultaneous hydrogen bonds, electrostatic attraction and associated weak interaction between the internal of imprinting CPO and CAP based on the molecular imprinting recognition. These bonds formation made the colloidal particles of the CPO swell. Thus, with the increase of CAP concentration, the size of particles increased, and resulted in an increase in the inter-planar spacing followed by the reflection intensity reducing. The results of our analysis were consistent with those of some reports (Hall et al., 2005; Guo et al., 2012). Moreover, the increase in the effective refractive index can be one of the factors causing the decrease of the Bragg diffraction intensity. The response to CAP adsorption can be resulted in a decrease in the average refractive index ($n_a$), causing a decrease in diffraction peak intensity. The effective refractive index $n_a$ of the CPO is calculated as follows:

$$n_a = \left[ n_{\text{particle}}^2 f_{\text{particle}} + n_{\text{water}}^2 (1 - f_{\text{particle}}) \right]^{1/2} \tag{2}$$

In this case, $n_{\text{particle}} = 1.492$ and $n_{\text{H2O}} = 1.333$ (Waterhouse and Wat, 2007). Therefore, the refractive index ratios of the self-cross-linked imprinting CPO ($n_{\text{particle}}/n_{\text{H2O}} = 1.119$) were all less than the value of 2.9, which is required for the realization of a full gap. Therefore, they exhibit only pseudo photonic band gaps. The theoretical requirements to achieve a complete band gap in a CPO structure included a refractive index comparatively higher than 2.9. In short, the swelling of the particles caused a decrease in the refractive index of the spheres ($n_{\text{swell particle}} < n_{\text{particle}}$), so $n_{\text{particle}}/n_{\text{H2O}} < 1.119$ became small. Therefore, the molecular recognition event based on molecular imprinting would affect some of structural characteristics of CPO, which finally evolved into optical signal changes.

3.4. Selectivity study

The selectivity study of the photonic sensor was investigated by using two structurally similar antibiotics (CAP succinate as well as thiamphenicol), and one structurally unrelated antibiotic (penicillin). Compared to the results in Fig. 3, CAP succinate and thiamphenicol induced slight optical changes of diffusive intensity (Fig. 5). This could be easily explained by their close homologous to CAP and having the same functional group in the specific position, thus forming a similar recognition behavior with CAP as shown in Fig. 6. The selectivity of CAP-imprinted photonic sensors was investigated by several groups previously, and similar results were also observed. For example, Kara et al studied selectivity of a surface plasmon resonance nanosensor using molecular imprinted nanoparticles and found that thiamphenicol could bind the sensor with constants 8.86 for CAP/ thiamphenicol (Kara et al., 2013). On the other hand, there was a low affinity of the structurally unrelated penicillin, causing almost no
perceptible optical signal changes of the sensor. Furthermore, it was learned that the average cross selectivity of the sensor for penicillin turned out to be 1.1%, even that for CAP succinate and thiamphenicol that have similar structure to CAP were found to be less than 13%. Therefore, it was suggested that the sensor possessed highly specific and sensitive recognition ability for CAP.

3.5. Response characteristics and reproducibility

The sensing characteristics of the photonic sensor were investigated by the adding CAP solution (256 ng mL\(^{-1}\)) for 0–15 min. As shown in Fig. 7A, the sensing time of the sensor reached 7 min and represented a dynamic and continuous response process. This rapid response of our sensor would be caused by the following reasons. Firstly, the small microgel MI-HAM particles can easily identify small change in Bragg diffraction compared with the bulk hydrogel response. In addition, its homogeneous layers and the high surface-to-volume ratio of the self-cross-linked imprinting CPO made the CAP molecules diffuse into the material and occupy the binding sites easily. In addition, the established photonic sensors to determine CAP usually require a longer detection time (Estevez et al., 2012; Ciminellin et al., 2013). For the IOPC bulk hydrogels for CAP (Zhou et al., 2012), the response achieved stability within 8 min. For a surface plasmon resonance sensor (Yang et al., 2016), it usually takes tens of minutes to separate the analyte before determination. For the high-throughput suspension arrays sens or (Y. Liu et al., 2009; N. Liu et al., 2009), it generally consumes more than 30 min to get a result.

As the self-cross-linked imprinting CPO was of a highly cross-linked polymeric nature, the corresponding sensors also showed good physical stability and chemical inertness. The recoverability of the sensor was studied by an elution and rebinding method. From Fig. 7B, it can be observed obviously that the sensor possessed a good recoverability in 6 cycles and, the relative standard deviation (RSD) was just within 4.9%. Therefore, the novel photonic sensor can be used repeatedly several times and still maintain its responsive property.

![Structural formulae of CAP and other antibiotics used in this study.](image-url)
3.6. Application

The developed sensor was used to analyze CAP in drinking water samples. As shown in Fig. 8, there were obvious diffraction intensity decreases according to different CAP spiked concentrations. Therefore, the application of the sensor platform made the detection of significant amounts of CAP residuals in liquid sample possible.

4. Conclusion

A novel photonic sensor by using the self-cross-linked imprinting CPO as a recognition element was described. The constructed sensory system is characterized by (a) self-cross linking to get rid of the interference from the bulk hydrogel; (b) low cost, easy construction and simple operation; and (c) rapid, sensitive and selective response. Moreover, the sensor was successfully applied in the real samples for sensing CAP. Therefore, the described assay not only provided an attractive alternative to create optical diffraction-based chemical or biological sensors, but also expanded the applications of CPO films in other areas such as drug separation, surface patterning and clinical assays. Further research should be focused on the improvement of the sensing sensitivity and selectivity of the sensor for other solid samples testing, such as seafood, livestock and poultry meat.

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