Physically Controlled Cross-Linking in Gelated Crystalline Colloidal Array Photonic Crystals

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ABSTRACT We prepared a poly(vinyl alcohol) (PVA) gelated crystalline colloidal array (GCCA) through physical cross-linking. PVA hydrogel was formed by utilizing a chilling−thawing method while the CCA was physically immobilized within the PVA hydrogel matrix. After being chilled at 2 °C for 24 h, the gel could be formed without disturbing the CCA. With the repetition of chilling−thawing cycle, the hydrogel network was reinforced. This photonic crystal material could be shaped as needed and efficiently diffracts visible light, and the diffraction wavelength can be tuned anywhere within the visible spectrum by simply varying the CCA concentration. The GCCA represents sol−gel reversibility as the temperature is cycled. It has been observed that the GCCA retained its ability of diffraction after rehydration, and the sample could be stored for long periods of time. We further functionalized the PVA hydrogel with Chitosan (CS), and the pH sensing behavior of the PVA/CS GCCA was observed. It revealed that the sensitivity of the PVA/CS GCCA correlates with the CS concentration.

KEYWORDS: crystalline colloidal array • poly(vinyl alcohol) • hydrogel • sensor • chitosan;

INTRODUCTION

Since the concept of photonic crystal was put forth, a great deal of research has been done in related fields (1, 2). Photonic crystal materials control light propagation because of the periodic variations of their optical dielectric constants. With their unique properties, such as uniform submicrometer scale sizes, photonic crystal materials have been extensively applied as functional optical devices (3−7).

Among the applications, there is intense interest in the fabrication of polymerized crystalline colloidal array (PCCA) photonic materials. This optical diffraction device consisting of a crystalline colloidal array (CCA) polymerized within a hydrogel that responds to certain analytes (4). As the hydrogels change volume in response to the changes in environmental conditions, the diffraction wavelength shifts according to Bragg's law (8).

The three-dimensional periodic CCA solution consists of self-assembled ~100 nm highly charged polystyrene (PS) spheres due to their electrostatic repulsion. Since the CCA could be disordered in the presence of ionic species, monomers or oligomers used in polymerization were limited (4, 9, 10). Thus, physically cross-linked hydrogel was utilized as CCA container (11).

Poly(vinyl alcohol) (PVA) is a water-soluble semicrystalline polymer of extensive interest because of its biocompatibility, nontoxicity, noncarcinogenic, and water permeability (12). Most PVA solutions such as PVA/water are well-known to form thermoreversible gels as hydroxyl groups of PVA produce inter/intramolecular hydrogen bondings. PVA hydrogels are hydrophilic three-dimensional networks connected by physical or chemical bonds which have been utilized in various applications involving controlled drug delivery (13, 14), tissue engineering (15), contact lenses (16, 17), and artificial organs (18).

Physically cross-linked PVA hydrogel has been proved as a porous polymer network involves different phenomena as phase separation during gelation (19), which was investigated as a host for nanofillers, different fillers affecting the PVA matrix in different ways (20). Asher's group fabricated CCA within a thermoreversible PVA/H_2O/DMSO gel through traditional freezing−thawing method (11), which is the most widely used process to prepare PVA hydrogels (21). It was proved that CCA array would aggregate in PVA aqueous solution during the freezing process, and disorder occurred probably because of crystallizes of freezable bound water (22).

In this research, we have found another way out by utilizing a chilling−thawing method instead of the freezing−thawing process (23, 24). The gelated crystalline colloidal array (GCCA) was prepared by chilling the CCA/PVA mixture at 2 °C for 24 h and then thawed at room temperature for 2 h. This chilling−thawing cycle was repeated 3−10 times to enhance the mechanical property of the GCCA hydrogel. The resulting GCCA efficiently diffracted visible light, and could demonstrate thermoreversible and rehydratable behavior. We also modified the GCCA hydrogel with Chitosan (CS). The PVA and CS complex has been widely studied elsewhere and applied in the biomedical field because of its good biocompatibility, biodegradability, nontoxicity, and availability (25). As a result of this modification, the PVA/CS GCCA hydrogel showed desired pH sensitivity, which significantly shifts the diffraction wavelength. Combined with

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Received for review February 13, 2010 and accepted April 08, 2010

DOI: 10.1021/am100130n

the biofunctionality of CS, GCCA photonic materials will therefore provide the biological signals required to support biosensing in vitro.

EXPERIMENTAL SECTION

CCA Preparation. Monodisperse polystyrene (PS) colloidal spheres were prepared by emulsion polymerization as reported elsewhere (26). The CCA solution was obtained by dialyzing 20% w/w suspensions of ~100 nm PS colloidal particles against pure water (18 MΩ cm^{-1}). The CCA solution became iridescent because of Bragg diffraction as the colloidal particles were adequately cleaned and self-assembled.

PVA GCCA Preparation. In a typical preparation of GCCA, Poly(vinyl alcohol) (PVA, 98% hydrolyzed, M_w = 1750, Shanghai Tianlian Industry of Fine Chemicals Co., Ltd., 100 mg/mL, dissolved in pure water at 90 °C, stirred for 6 h) and CCA solutions were mixed (v/v = 1/1) and dialyzed against water for 10 days. The resulting pregel was then injected into special molds and gelled at 2 °C for 24 h to form PVA GCCA. After thawing for 2 h, the GCCA hydrogel can be removed from the cast.

Rehydratability. We cut 3 layers of parafilm (~120 μm thick, Chicago, IL) carefully to make a 10 mm × 30 mm ×360 μm spacer, and the spacer was clamped between a set of glass slides to provide a uniform GCCA thickness. The GCCA pregel was injected into the cast, and then the sample went through 5 chilling−thawing cycles. The hydrogel film was placed in vacuum oven for 72 h. After dehydration, the GCCA was rehydrated by direct immersion in pure water at room temperature to reach swelling equilibrium. The glassware used in all experiments was cleaned in a RCA solution (5:1:1 mixture of water, hydrogen peroxide (30%) and ammonia (29%)) at 75 °C for 30 min.

Thermal Reversibility. We observed the state transition of PVA GCCA produced through chill−thaw cycling. A 8% PVA aqueous solution and approximately 5% PS colloidal particles were formulated. The pregel was poured into a culture flask and chilled for 24 h at 2 °C into GCCA. After thawing at room temperature for 2 h, the sample was heated in 60 °C water bath for 30 min to form gel−sol transfer. The sol−gel transition was thermally cycled ten times, and gelation was confirmed by upside-down method (27).

PVA/CS GCCA Preparation. The PVA/CS/CCA mixture (PVA/CS GCCA pregel) was prepared as follows: PVA GCCA pregel and Chitosan (CS, degree of deacetylation ≥85%, Sigma-Aldrich, 100 mg/mL, dissolved in 2 vol% acetic acid) were mixed with various PVA/CS volume ratios (1/1, 2/1, 3/1, and 6/1), and ion-exchange resin (Bio-Rad, AG 501-X8 (D) resin) was added. The mixture was shaken overnight and also went through the chilling−thawing cycle (the same as those applied to the PVA/CCA system) to form the hydrogels. The route for the fabrication of GCCA is illustrated in Scheme 1.

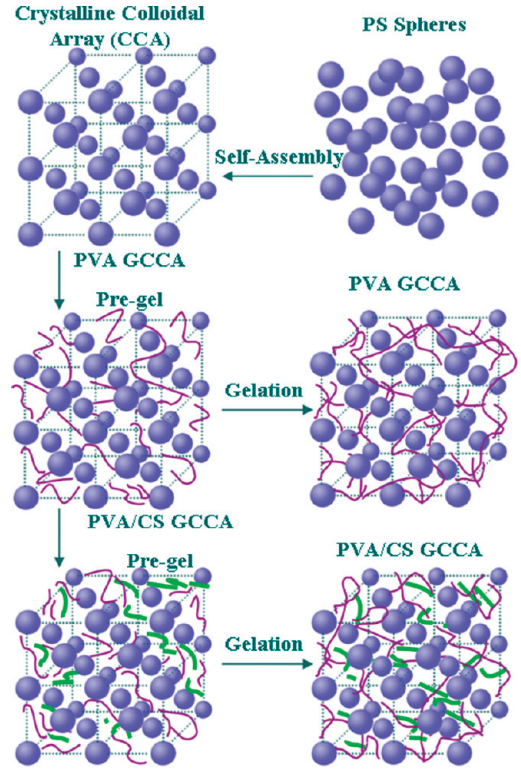
Swelling Properties. Swelling degrees (SDs) of hydrogels were measured by immersing the fresh made samples (wet) in buffer solutions at different pH values after being weighted. The samples were gently wiped with filter paper to remove the surface solution when taken out from the solutions, then weighted. The SD was calculated as follows: SD (%) =(W^*/W) × 100, where W is the weight of the original hydrogel and W^* is the weight of the swollen hydrogel.

Characterization. UV−vis absorption spectroscopy was performed with a Unico UV-2102PC spectrophotometer. Diffraction measurements were conducted utilizing an Ocean Optics USB2000−UV−vis Spectrometer. During measurements, the GCCA samples were oriented normal to the incident light beam.

RESULTS AND DISCUSSION

This research was mainly carried out to combine PVA hydrogel with crystalline colloidal array (CCA) so as to form

Scheme 1. Schematic Representation of GCCA Preparation



a gelated crystalline colloidal array (GCCA) photonic crystal material without any organic solvent. In contrast to traditional photopolymerized crystalline colloidal array (PCCA), physically cross-linked GCCA, independent of photoinitiator, has simpler chemical components, and can therefore be shaped as needed in opaque cast without restriction by UV radiation. Although photopolymerization could be finished in several minutes, the subsequent modifying and swelling test of prepared PCCA should be very carefully handled. Furthermore, after photopolymerization, the PCCA needs to be washed to remove excessive monomer or photoinitiator.

PCCA sensors are individually handmade; although the preparation was under the same conditions, there is imperfect reproducibility from one batch to another, and the thickness of PCCA was limited because of the optical density of the PCCA precursor solution. Figure 1 shows a GCCA sample stored in a 30 mL jar. GCCA could be prepared in a large scale, which may be divided into pieces with identical properties as needed, as a result reducing the differences between batches.

Figure 1 shows a GCCA sample stored in a 30 mL jar. Diffraction of GCCA can be tuned anywhere within the visible spectrum as required by varying the colloidal concentration (see the Supporting Information, Figure S1).

To avoid the occurrence of CCA disordering resulting from the presence of water crystallites when frozen, a chilling−thawing method was utilized, which well-preserved the ordering of PS array inside PVA hydrogel matrix during gelation. Subsequently, GCCA with preferable mechanical properties was formed after 3−10 chilling−thawing cycles.

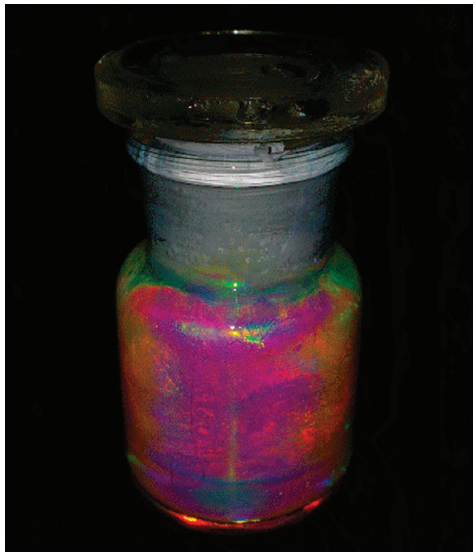


FIGURE 1. GCCA sample stored in a 30 mL jar.

Table 1. Impact of CCA on PVA Gelation

cycles	6% GCCA	6% PVA	8% GCCA	8% PVA	10% GCCA	10% PVA
1	sol	sol	sol	sol	gel	gel
3	sol	sol	gel	sol	gel	gel
5	sol	sol	gel	sol	gel	gel
10	gel	sol	gel	sol	gel	gel

We examine the critical gelation concentration of GCCA and PVA solution (28). The details of the formulations are presented in Table 1. In the presence of CCA, the critical gelation concentration of GCCA is 6%, whereas that of PVA is 8%. We discovered that cross-linking tends to occur at higher temperatures in GCCA pregel than in PVA aqueous solution. During the gelation process, more chilling-thawing cycles were needed to form gel in PVA aqueous solution, which indicated that the presence of CCA ordered structure facilitates PVA crystallization (29). Thus, the GCCA can be prepared at lower PVA concentration.

Rehydratability. We noticed the diffraction of PVA GCCA blue-shifted as the repetition of chill−thaw process was increased or the chilling time was extended, and disappeared after thorough dehydration.

Figure 2 shows the reflectance spectra of PVA GCCA before dehydration and rehydrated after 1, 2, 4, 48 h, respectively. Diffraction recurred within 30 min and the diffraction intensity increased during reswelling. Only a ~6 nm red-shift was obtained after 1 h, indicating that the equilibrium of reswelling was achieved within 1 h. The film was placed in a dish with excess water, and the reflectance spectra of the films could be observed after a number of weeks. The recovered hydrogel retained its mechanical strength, which therefore was capable of a long-term storage after dehydration.

We assume that the rehydrated GCCA formation consists of the following two processes: (i), during the chilling process, cross-linking points were formed. PVA was cross-linked by microcrystals and polymer dominates over amounts of bound water (19). (ii), free water evaporated from the hydrogel during dehydration introduced crystallites, which

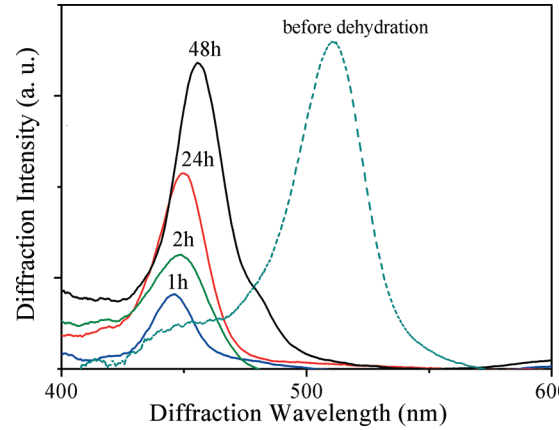


FIGURE 2. Diffraction dependence of PVA CCCA on rehydration time.

shrank the hydrogel matrix besides disordering the unfold chains (30, 31). This process increased the degree of crystallinity, consequently decreased the equilibrium reswelling ratio, which explains the blue-shift after dehydration. Furthermore, the process reveals that such diffraction shifting exactly reflects the swelling behavior of GCCA hydrogels.

Thermal Reversibility. The gel−sol−gel transition of PVA GCCA was studied by repeating thermal cycles. Fresh dialyzed pregel was chilled at 2 °C for 48 h to form GCCA. Gelation was examined by upside-down method. After 2 h thawing at room temperature, we took a photograph and recorded spectra. The sample was further thawed in a water bath at 60 °C for 30 min, which caused the GCCA to melt into liquid. After the sample was cooled to room temperature, another photograph was taken and additional spectra recorded.

In Asher's work (11), a PVA/DMSO/H_2O gel was utilized to immobilize CCA, which follows a different gelation mechanism from that of PVA/H_2O system (32). A decrease in diffraction intensity was observed after the thermal cycles, which was attributed to the ionic impurities leaching from the sealer. However, phase separation occurred during the gelation process, causing PVA to form intra- and intermolecular hydrogen bonds between appropriate adjacent OH groups extensively while the redundant solvent remained unfrozen. The gel melted in the subsequent heating process, and the solvent could diffuse into polymer-rich phase. Diffraction ebbed away probably due to insufficient remixing after the gel−sol transition. Besides, DMSO would transfer from the gel, which would affect the swelling and storage of the gel, and the reversibility would be lost at the same time.

The chilling−thawing method could avoid the phase separation. Figure 3 shows the photographs during ten thermal cycles, the ability of diffraction of the sample did not weaken as the thermal cycles even increased to ten times, indicating that PVA GCCA prepared through chilling−thawing method represents a desirable thermal reversiblility. The diffraction data show the good reversibility of the GCCA sample (see the Supporting Information, Figure S2).

PVA/CS GCCA. To make GCCA sensitive for sensing purposes, we introduced chitosan (CS) into the gel system. Considering the insolubility of CS ($pKa = 6.5$) in an alkaline

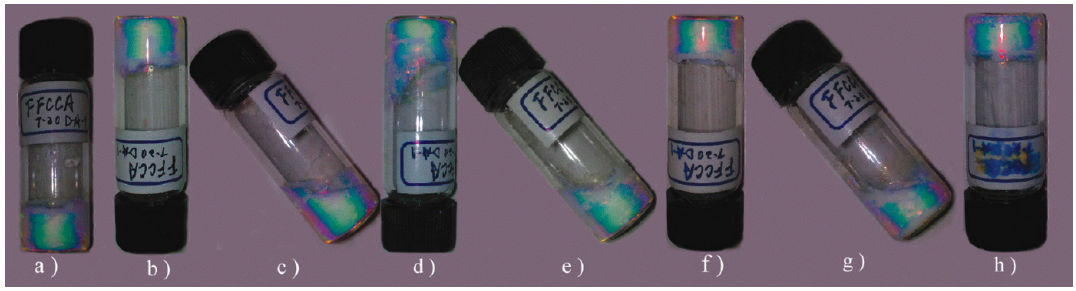


FIGURE 3. Photographs of thermal reversibility show the sol state (a) before first and after (c) first, (e) second, and (g) fifth thermal cycle, and the gel state after (b) first, (d) second, (f) fifth, and (h) tenth gelation, respectively.

environment, we measured the pH values of PVA aqueous solution, CCA solution, and GCCA pregel to be 7.0, 4.6, and 6.5, respectively, making sure that GCCA is suitable for the CS solution.

We originally attempted to directly mix CCA with PVA/CS solution, and a large amount of sediment occurred immediately, presumably because the free NH_2 groups on CS chain protonated as NH_3^+, which adsorbed with negatively charged PS sphere.

It was examined that PVA could adsorb a layer on latex particles, whose thickness increases with time (33). Therefore, we mixed PVA GCCA pregel with CS solution under gentle stirring, and no significant sediment was found.

When the ratio of PVA to CS ranged from 6/1 to 3/1, the mechanical strength of the hydrogels was quite agreeable. When the ratio of PVA to CS was decreased from 2/1 to 1/1, the hydrogel was undesirable and inapplicable after five thermal cycles. The increase in the CS ratio decreases the mechanical strength, which indicated that the cross-linking points of the hydrogel were mainly formed by PVA (34).

It can be seen in Figure 4a, at lower CS concentration, PVA/CS GCCA diffraction spectra red-shifts from ~467 nm to ~551 nm as the pH values decrease from 8.4 to 1.0. As the CS concentration was increased, the diffraction red-shifts from ~468 nm to ~633 nm (Figure 4b). The latter findings reveal that a decrease in PVA/CS ratio from 6/1 to 3/1 causes an increase in the pH sensitivity, as shown in Figure 4c.

Figure 5 shows the swelling degree of PVA/CS GCCA (w/w = 3/1 and 6/1) at various pH values. The swelling degrees of PVA/CS GCCA hydrogels depend on pH value of the buffer solution. However, at pH 7.4 and 8.4, the hydrogels shrank from initial weight. These results reveal that the PVA/CS hydrogel swelled markedly and absorbed large amount of water into its network, when the surrounding pH value is decreased. At different PVA/CS ratio, the effect of pH on the swelling behavior was similar to that on diffraction wavelength.

According to Bragg's diffraction equation, $\lambda_0 = 2n_a d_{hkl} \sin\theta$, where λ_0 is the diffracted wavelength in air, d_{hkl} is the interplanar spacing, n_a is the average refractive index of the system, and θ is the Bragg angle ($\theta = 90°$). Because the unpolarized light is propagated along the [111] direction of the face centered cubic (fcc) lattice, the observed diffraction wavelength is then related to the lattice parameter of the cubic unit cell through $a_c = 3^{1/2} d_{111}$, whereas the nearest neighbor distance is $a = a_c 2^{-1/2}$. Thus, the equation can be

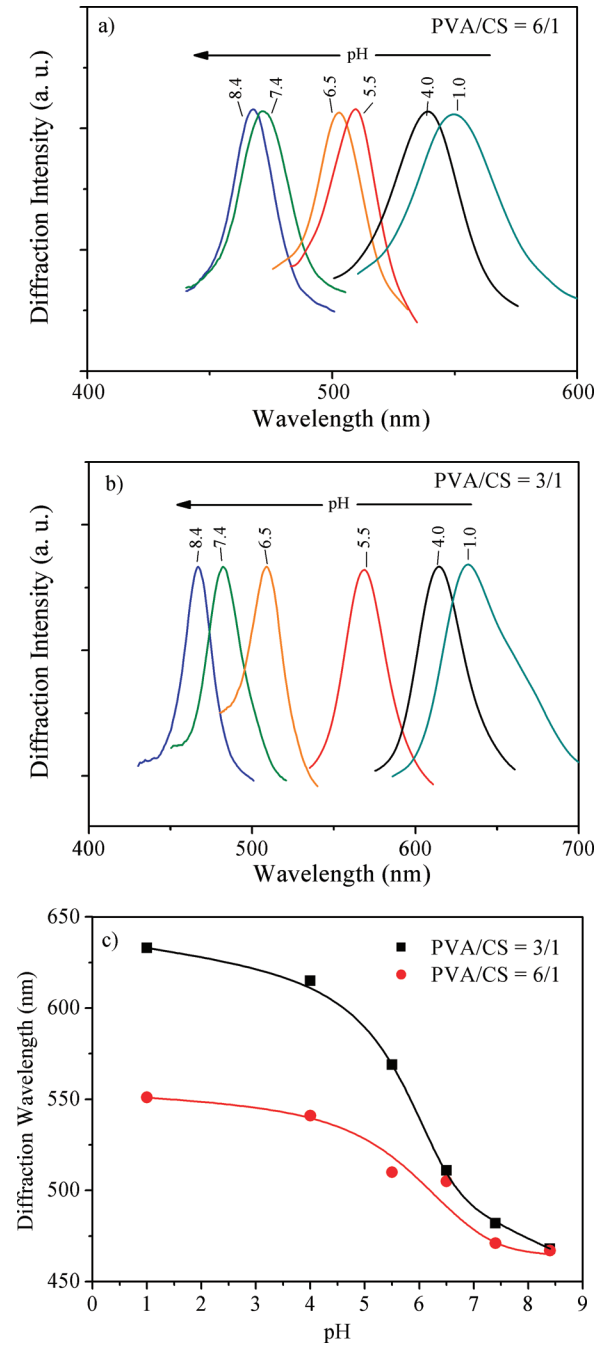


FIGURE 4. Response of PVA/CS GCCA to pH at room temperature: (a) PVA/CS = 6/1; (b) PVA/CS = 3/1; (c) comparison of diffraction response from PVA/CS = 6/1 and PVA/CS = 3/1 (lines added to aid the eye).

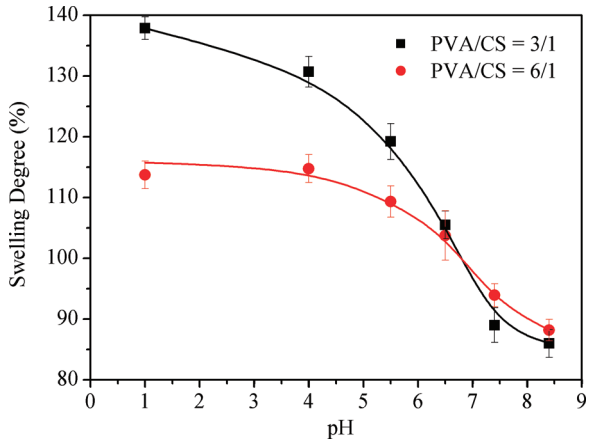


FIGURE 5. Comparison of swelling degree of PVA/CS = 3/1 and 6/1 at various pH values (lines added to aid the eye).

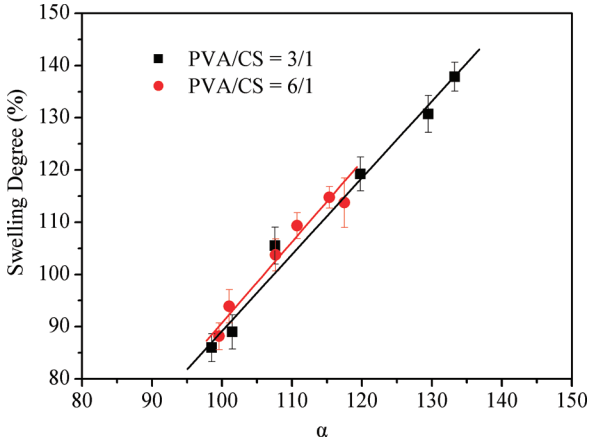


FIGURE 6. Plots of swelling degree against α at PVA/CS = 3/1 and 6/1.

rewritten as $\lambda_0 = 2n_a(2/3)^{1/2}a\sin\theta$. We plotted swelling degree against the linear deformation factor, $\alpha = \lambda^*/\lambda$, where λ^* and λ are the diffracting wavelengths at the normal state and at the swelling state, respectively. Figure 6 shows a typical plot of swelling degree versus α. Good linearity is shown, indicating that the diffraction wavelength proportionate to the swelling ratio of the GCCA hydrogels. By these plots, we calculated that ~0.3 % change in the hydrogel weight causes ~1 nm shift of diffraction wavelength.

The mechanism of pH sensitive swelling involves the protonation of amine groups of CS under low pH condition, which leads to chain repulsion, and simultaneous diffusion of proton and counterions together with water inside the gel (35). According to ideal Donnan equilibrium, the chemical potential of an ionic species inside the hydrogel must be equal to that outside. Thus, the osmotic pressure is caused by the concentration difference of counterions between the gel and the external solution; as a result, the swelling occurred. The curve also indicates an effective PVA/CS GCCA p*K*a ~ 6.8. Besides, in the presence of CS, GCCA also showed little thermosensitivity.

The GCCA is easy to prepare and the diffraction behavior can be easily tuned by varying the CCA concentration. PVA/CS are both biodegradable, and the combination has shown desirable attributes in the presence of CCA. We anticipate that this hydrogel/CCA system can be a promising candidate as photonic crystal sensing material.

CONCLUSIONS

We prepared a gelated colloidal crystalline array (GCCA) from PVA hydrogel/CCA composites. This GCCA is easy to prepare because of its simple formula. The physically cross-linked PVA hydrogels show reversible sol−gel transition as the temperature is cycled. The GCCA efficiently diffracts the visible light, even after rehydration. We also functionalized the hydrogel with Chitosan to make the hydrogel stimuli sensitive. The pH response of PVA/CS GCCA leads to a ~165 nm diffraction red-shift, which could be distinguished by the naked eye. Further modification would be done to make the GCCA multisensitive.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (20925621, 20976054), the Key Project of Science and Technology for Ministry of Education (107045), the Innovation Program of Shanghai Municipal Education Commission (09ZZ58), the Program of Shanghai Subject Chief Scientist (08XD1401500), the Shuguang Scholar-Tracking Foundation of Shanghai (08GG09), and the Shanghai Leading Academic Discipline Project (B502).

Supporting Information Available: Additional information about UV−vis spectra of GCCA with different colloidal concentration and diffraction spectra of GCCA during the thermal reversibility test as noted in text (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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AM100130N