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Epoxide functionalized polymerized crystalline colloidal arrays

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Abstract

We report the development of a novel method for functionalizing polymerized crystalline colloidal arrays (PCCA). This new method enables us to easily incorporate molecular recognition agents into the PCCA to produce chemical sensing materials that report on analyte concentration via diffraction of visible light. We copolymerize glycidyl methacrylate with acrylamide and *N,N′*-methylenebisacrylamide around crystalline colloidal arrays. The incorporated epoxide groups are available for further PCCA functionalization through reactions with thiol, amine or hydroxyl groups. We fabricated a Pb^{2+} sensor, which utilizes a crown ether recognition agent, as well as a glucose sensor, which utilizes glucose oxidase.
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Keywords: Crystalline colloidal array; Polymerized crystalline colloidal array; Sensors; Hydrogels; Glycidyl methacrylate

1. Introduction

We recently developed a new class of chemical sensing materials known as intelligent polymerized crystalline colloidal arrays (IPCCA) [1–8]. These materials are composed of an array of colloidal particles immobilized within a hydrogel (PCCA). The array spacing is ~ 200 nm such that it diffracts visible light. We fabricate chemical sensing material by adding molecular recognition agents to the PCCA, which actuate hydrogel volume changes upon reaction with analytes [1–8]. The resulting hydrogel volume changes are read out by the consequent changes in the diffraction wavelength. We attached the molecular recognition agents either by introducing them during PCCA polymerization, or by attaching the agents after PCCA polymerization. For example, we previously fabricated a lead sensing IPCCA by copolymerizing a vinyl crown ether into the PCCA [1,5–8]. In contrast, we fabricated a glucose sensing IPPCA by attaching glucose oxidase by using avidin–biotin coupling subsequent to polymerizing the biotin group into the PCCA [1–3].

We utilize more complex functionalization in our fabrication of glucose sensing boronic acid IPCCA, where we hydrolyzed the PCCA to form carboxyl groups, which we subsequently used to attach molecular recognition groups [7,8]. Only non-ionic molecular recognition groups can be copolymerized into the PCCA; charged groups would disorder the colloidal particle array. Thus, we require post polymerization molecular functionalization strategies for developing IPCCA sensors.

In this paper, we describe a new method for incorporating molecular recognition agents into the PCCA. This method involves copolymerization of glycidyl methacrylate, which has a good balance of hydrolytic stability and reactivity with amine groups, and does not destroy the order of the CCA. This synthesis provides epoxy groups along the hydrogel surface, which readily react with thiol, amine or hydroxyl groups [9,10].

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2. Experimental

Highly charged, 120 nm diameter monodisperse polystyrene colloidal particles (10% wt. suspension) were prepared by emulsion polymerization, as described elsewhere [11,12]. Excess ions and surfactants were removed from the colloidal suspensions by dialysis against deionized water. The dialyzed suspension was further cleaned by shaking with an ion-exchange resin (Bio-Rad). The suspension became iridescent due to Bragg diffraction from the CCA.

In order to fabricate the PCCA, acrylamide (50 mg, Fluka) and glycidyl methacrylate (20 μl, Aldrich) were used as monomers, and *N,N'*-methylene-bisacrylamide (3 mg, Fluka) was used as a crosslinker (Fig. 1). Ten microlitres of a 10% solution of diethoxyacetophenone (Acros Organics, v/v) UV photoinitiator in DMSO was added to the suspension. This mixture was injected into a cell consisting of two quartz plates, separated by 80 μm spacer (DuraSeal). The cell was exposed to UV light from a Black Ray model B-100, UVP Inc. mercury lamp (365 nm maximum wavelength) to initiate polymerization. After a 30 min exposure, the cell was opened and the resulting hydrogel film, which adheres to one of the quartz plates, was washed in deionized water in order to remove all unreacted compounds. This PCCA functionalized with epoxy groups is then immediately reacted with the desired species containing terminal amine groups (Fig. 2).

The maximum molar ratio of glycidyl methacrylate to acrylamide that was incorporated in the hydrogel was 15% due to the limited solubility of glycidyl methacrylate in water [13]. We observed phase separation at a 20 mol% of glycidyl methacrylate. The concentration of the reactive epoxide groups was determined from absorption measurements of an amine-containing dye, Disperse Orange 3, which shows an absorption maximum around 450 nm in water. This dye was reacted with the glycidyl PCCA for 2 h, and the resulting gel was washed overnight in deionized water. The concentration of dye inside the hydrogel was determined to be ∼3 mM using a Perkin–Elmer Lambda 9 UV/VIS/NIR spectrophotometer.

We fabricated a lead sensor by exposing the PCCA to a 0.08 M aqueous solution of 4′-aminobenzo-18-crown-6 (Aldrich). The response of this IPCCA was measured by using an Ocean Optics USB2000 Fiber Optic Spectrometer. The wavelength diffracted from the IPCCA sensor monitors the IPCCA's linear dimension. The IPCCA diffraction wavelength shift reached its equilibrium value within 2 min for 1–10 mM analyte concentrations, or within 10 min for analyte concentrations <0.1 mM, since the response is diffusion limited.

We fabricated a glucose-responsive PCCA by covalently attaching glucose oxidase (Sigma, G7016, 50,000 units) to the epoxy-functionalized PCCA. The PCCA was exposed to a 9×10^{-5} M aqueous solution of glucose oxidase at room temperature overnight under continuous stirring. The hydrogel was then washed with deionized water. We examined the time dependence of the diffraction red-shift of our glucose oxidase sensor under constant purging with N_2 in order to exclude oxygen from the solution.

We also used the glycidyl PCCA to fabricate a photoresponsive PCCA by covalently attaching an amine-containing azobenzene derivative to the epoxy-funtionalized PCCA. The PCCA was exposed to a 7 mM solution of a newly synthesized water-soluble azobenzene derivative (Fig. 2) and kept in dark under stirring for 2 h. A gradual red-shift of the diffraction

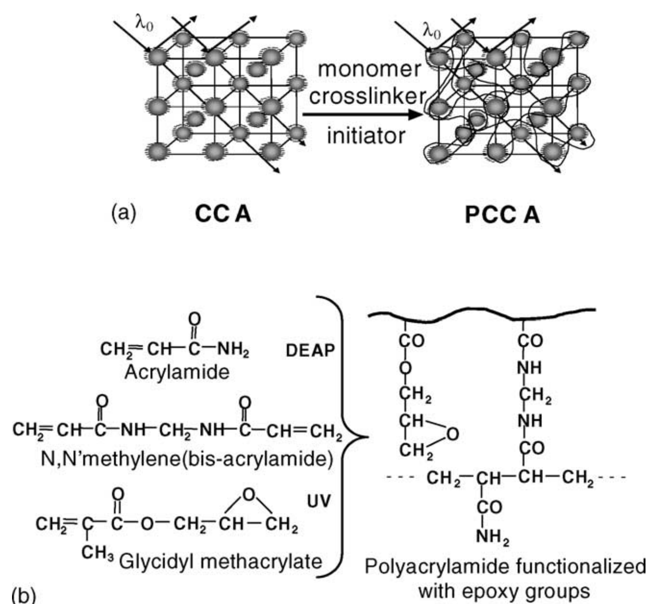


Fig. 1. (a) Formation of polymerized crystalline colloidal array (PCCA) by polymerization of hydrogel around crystalline colloidal array (CCA) of 120 nm polystyrene colloidal particles. (b) Functionalization of PCCA with epoxide groups.

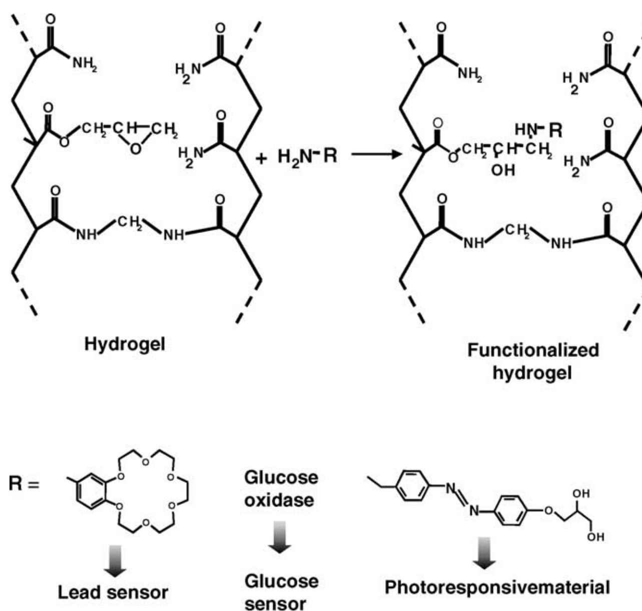


Fig. 2. Epoxy-functionalized PCCAs are reacted with species containing terminal amines.

peak upon UV illumination was observed due to the *trans* → *cis* isomerization of azobenzene. Excitation with visible light resulted in the reverse *cis* → *trans* isomerization and a diffraction peak blue-shift to the original position. Therefore, information could be written using UV light and stored until visible light excitation erased it. Detailed properties of this photosensitive PCCA are discussed elsewhere [14].

3. Results and discussion

3.1. Crown ether Pb^{2+} IPCCA

In previous work, we fabricated a Pb^{2+} IPCCA sensor by copolymerizing acryloyamidebenze-18-crown-6 with acrylamide and bisacrylamide [1,5–8]. In the present study, we copolymerized glycidyl methacrylate to create a PCCA, which has the capability of binding any recognition agent containing amine, thiol, or hydroxide functionalities. We synthesized an analogous Pb^{2+} sensing IPCCA by reacting the glycidyl PCCA with 4′-aminobenzo-18-crown-6. Fig. 3 shows the response of this IPCCA sensor to Pb^{2+} in the form of dissolved $Pb(NO_3)_2$. The diffraction red-shifts with increasing Pb^{2+} concentrations in a manner similar to that observed previously. [1,5–8].

Fig. 3b shows that the diffraction red-shift increases as the epoxide group concentration increases presumably due to attachment of larger amount of 4′-aminobenzo-18-crown-6. The IPCCA reached a saturated response within 1 min of analyte addition under constant stirring.

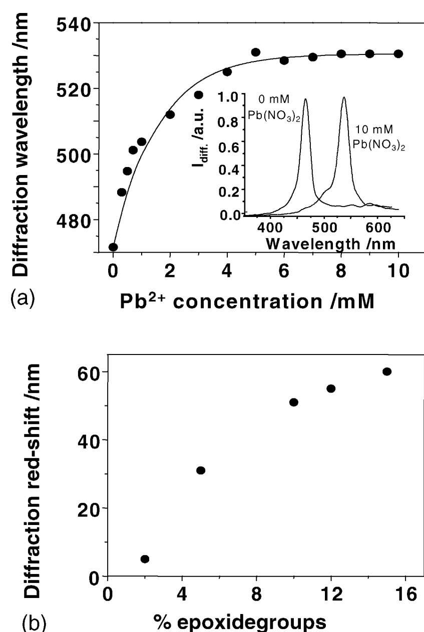


Fig. 3. (a) Diffraction wavelength dependence on Pb^{2+} concentration for a crown ether functionalized PCCA, which contained 15% epoxide groups. The inset shows the 60 nm diffraction red-shift of this IPCCA in the presence and absence of 10 mM $Pb(NO_3)_2$. (b) Dependence of diffraction red-shift on epoxide concentration of the functionalized PCCA in 10 mM $Pb(NO_3)_2$.

This IPCCA red-shift results from hydrogel swelling due to a Donnan potential established by the increased concentration of mobile counterions to the Pb^{2+} chelated to the 18-crown-6 [1,3,5,8]. The slope of the calibration plot for Pb^{2+} is determined by the association constant of the crown ether, the concentration of crown ethers in the IPCCA and the hydrogel crosslink density, which determines the IPCCA elasticity [1,3,5,8]. We used 2% *N,N*′- methylenebisacrylamide crosslinking to give us a robust 80 μm thick gel.

This IPCCA fully responds to Pb^{2+} within a few min. For example, Fig. 4 shows that the diffraction of the top surface of an IPCCA gives a saturating 30 nm red-shift due to a 1 mM $Pb(NO_3)_2$ solution within 2 min under constant stirring. In these measurements the diffraction peak was monitored, until no further change in diffraction occurred. The IPCCA reaches its equilibrium diffraction more slowly at low analyte concentrations (<0.1 mM) because the response is diffusion limited.

3.2. Glucose oxidase IPCCA

We previously fabricated a glucose IPCCA sensor based on glucose oxidase, fabricated by attaching biotin to the PCCA, and using the well-known avidin–biotin interaction to attach avidinated glucose oxidase to the hydrogel. [1,3] In the present work, we describe a method, which more simply binds glucose oxidase through its exposed amine groups.

Glucose oxidase is an ellipsoidal flavin-containing glycoprotein with dimensions of 60 Å × 52 Å × 37 Å. The monomer is folded into two structural domains, one binds flavin and the other involved with substrate binding. At neutral pH, the oxidized flavin is uncharged, but the reduced flavin is anionic [1,3,15,16].

Glucose oxidase converts glucose to gluconic acid in a two-step process. [15,16] In the first step, glucose is converted to gluconic acid, and the enzyme is reduced. In the second step, the enzyme is reconverted to its oxidized form by oxygen

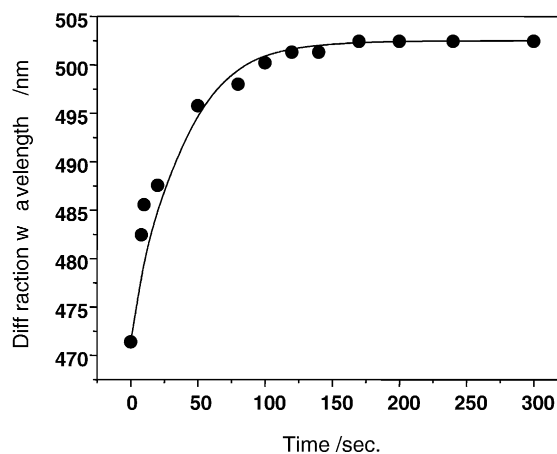
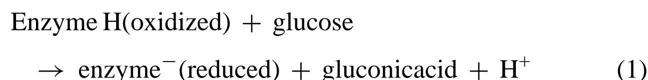
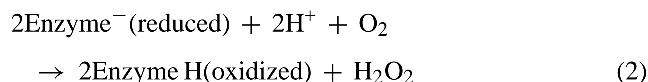


Fig. 4. Time dependence of diffraction of Pb^{2+} responsive IPCCA upon exposure to 1 mM Pb^{2+}. The PCCA contains a 15% molar ratio of epoxide groups.

present in the solution, producing hydrogen peroxide as a byproduct:



$$\text{Enzyme H(oxidized)} + \text{glucose} \rightarrow \text{enzyme}^{-}\text{(reduced)} + \text{gluconicacid} + \text{H}^{+} \quad (1)$$



$$\text{2Enzyme}^{-}\text{(reduced)} + 2\text{H}^{+} + \text{O}_2 \rightarrow \text{2Enzyme H(oxidized)} + \text{H}_2\text{O}_2 \quad (2)$$

We showed previously [1,3] that the reaction of glucose with the glucose oxidase IPCCA reduced the flavin prosthetic group, which becomes an anion, resulting in a Donnan-potential induced hydrogel expansion in low ionic strength solutions.

Fig. 5a shows the UV–vis absorption spectra of a dehydrated glycidyl acrylamide PCCA, which eliminates the visible diffraction peak. Fig. 5a compares the absorption of the dried glycidyl gel and a similar gel reacted with glucose oxidase. The glucose oxidase FAD prosthetic group absorption bands at 380 nm and 450 nm demonstrate the attachment of the glucose oxidase protein.

Fig. 5b shows that the glucose oxidase IPCCA (containing a 15% molar ratio of glycidyl methacrylate) monotonically red-shifts with increasing glucose concentrations. The diffraction red-shift saturates with an 86 nm red-shift in the presence of 10 mM glucose. This behavior is similar to that of our glucose oxidase sensors attached through avidin–biotin coupling. This indicates that the glycidyl attachment has not deactivated the glucose oxidase.

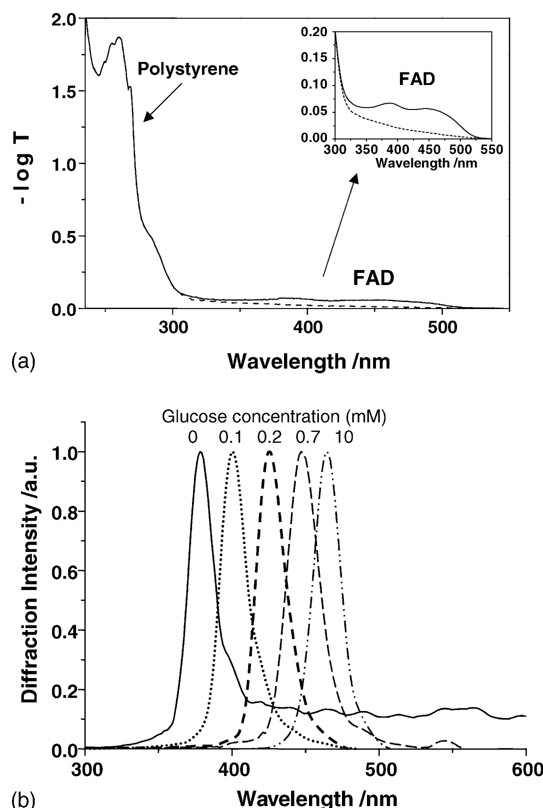
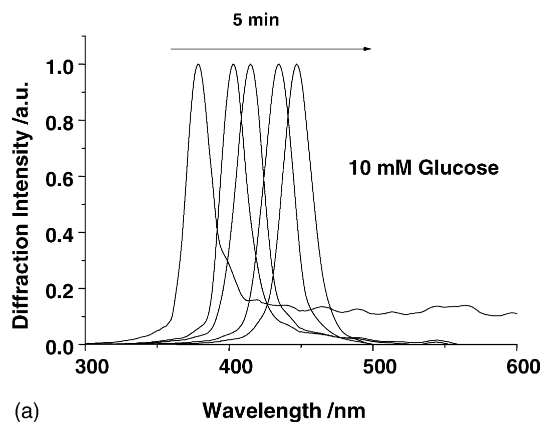


Fig. 5. (a) Absorption spectra of dehydrated glycidyl acrylamide hydrogel (- - -) before and after (—) reaction with glucose oxidase. The FAD absorption peaks indicate the successful attachment of glucose oxidase. (b) β-D(+)Glucose concentration dependence of diffraction for glucose oxidase IPCCA.



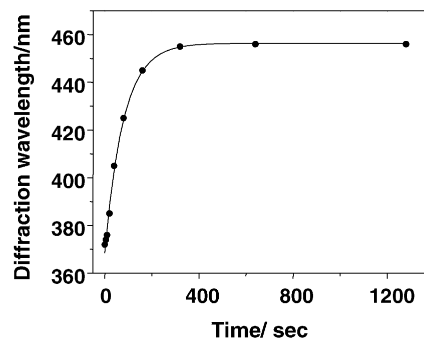


Fig. 6. (a) Time dependence of diffraction of glucose oxidase IPCCA upon exposure to 10 mM glucose solution. (b) The response saturates in less than 5 min.

We examined the time dependence of the diffraction red-shift of our glucose oxidase sensor under constant purging with N_2 in order to exclude oxygen from the solution, thereby preventing re-oxidation of the reduced enzyme. Fig. 6 shows the time dependence for a 10 mM glucose solution. The response saturated within 5 min. Measurements for 0.1 mM and 100 mM glucose solutions showed saturation at 8 min and 30 s, respectively. The response rate is determined by the mass transport of substrate into the gel, the rate of enzyme reaction and the collective diffusion constant of the hydrogel. The sensor response time was very similar to our previously reported material, in which the coupling was done through the avidin–biotin chemistry. [1]

4. Conclusion

Our new method of creating functionizable PCCA by copolymerizing glycidyl methacrylate into the PCCA makes it convenient to attach molecular recognition groups. The

only requirement for attachment is the presence of either thiol, amine or hydroxyl groups on the recognition agent. This advance will help in developing IPCCA sensing materials for additional analytes.

Acknowledgments

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Marta Kamenjicki received her Ph.D. degree from the University of Pittsburgh in 2004, where her doctoral work focused on the development of photoresponsive polymerized crystalline colloidal arrays and chemical sensors. She is continuing her work with these materials as an assistant professor of Chemistry at Penn State Altoona. She received her B.A degree in Chemistry from Wittenberg University in Springfield, Ohio.