Colorimetric Glucose Sensor

Simple and Precise Preparation of a Porous Gel for a Colorimetric Glucose Sensor by a Templating Technique**

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An inverse opal structure having a periodically ordered interconnecting porosity endows hydrogels with structural colors (i.e., colors that arise from physical optics as opposed chromophores) based on the Bragg diffraction of light.[1] Additionally, the porous hydrogels exhibit a rapid change in volume responding to environmental variations.[1,2] Consequently, the structural colors of the gels are quickly synchronized with their characteristic volume change. Such gels have the potential for applications in chemical sensing.[1c] Herein, we report the precision design and simple preparation of a colorimetric sensor for level of glucose in blood by using structurally colored gel.

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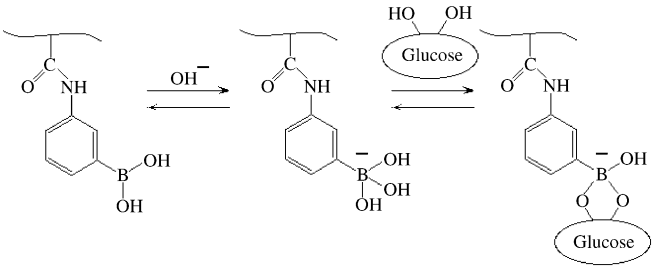
Most existing glucose sensors use glucose-sensitive proteins, such as glucose oxidize enzyme, immobilized on films or in hydrogels.[3] However, such proteins will denature under prolonged storage and use, because of their high fragility towards environmental changes. Additionally, their response depends on the dissolved oxygen concentration. For commercial applications, especially, there is a strong demand for a more stable and simpler sensor. Synthetic compounds are among the leading candidates for such a system.[4]

It is well known that phenylboronic acid and its derivatives can form covalent complexes with glucose reversibly. In alkalescent aqueous solutions the phenylboronic acid compounds are in equilibrium between the undissociated and the dissociated states (Scheme 1). In this system, complexes with



Scheme 1. Equilibria associated with complex formation between phenylboronic acid derivative and glucose under a basic condition.

glucose molecules are mainly formed with the dissociated state. Thus, the addition of glucose in the system causes a shift in the equilibrium in the direction of increasing the presence of charged phenylborates. Hence, if one prepares a hydrogel of a phenylboronic acid derivative, complexation with glucose causes the volume of the hydrogel to increase through positive osmotic pressure in the hydrogel.[4a,b,e] In this project, we attempted to introduce this mechanism into the structurally colored gel to produce a colorimetric glucose sensor. It follows that such a gel can be expected to display glucose-dependent changes in structural color.

A periodically ordered interconnecting porous gel exhibiting a desired structural color can be prepared by using a closest-packing colloidal crystal as a template (Scheme 2).[1] A pregel solution can easily be infiltrated into an open structure between each contact particle of the closest-packing colloidal crystal as a narrow vessel for making the gel. After the removal of the crystal component from the gel obtained, the porous gel exhibits a brilliant structural color under white light illumination[1b] and undergoes fast and drastic changes in color in response to a variety of environments.[1a]

The interval of time required for the volume of a gel to change is governed by the collective diffusion of the polymer network forming the gel.[5a,b] The time is proportional to the square of a characteristic length of a gel such as the diameter for a spherical or cylindrical gel and the thickness for a slab gel. Thus, the period needed for volume change reduces as the size of the gel becomes smaller. A bulk gel forms a dense polymer skin layer, which prevents the permeability of solvent molecules when a drastic change in the environment comes about.[5c] The result is a very slow deswelling of the

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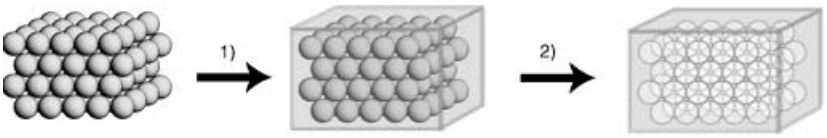

Scheme 2. Preparation of a periodically ordered interconnecting porous gel by using a closest-packing silica colloidal crystal as a template. 1) Pregel solution, polymerization; 2) immersion into aqueous HF to remove the silica component.

bulk gel. In contrast to the bulk gel, porous gel exhibits a very fast response time, attributable to the interconnecting porous structure which makes solvent discharge easier.

The peak values of reflection spectra, λ_{max}, for the porous gel are obtained by Equation (1):[1,6]

$$\lambda_{max} = 1.633\,(d/m)\,(D/D_0)\,(n_a^2 - \sin^2\theta)^{1/2} \quad (1)$$

in which d is the diameter of a colloidal particle, m is a constant, D/D_0 is the equilibrium swelling degree of the gel (D and D_0 are diameters of the gel in the equilibrium state at a certain condition and in the reference state, respectively.), n_a is the refractive index of the porous gel at a certain condition, and θ is the angle measured from the normal to the plane of the gel. In this report, d and θ are fixed at 220 nm and 0°, respectively, to reduce the parameters for this experiment. Consequently, we have only to experimentally determine the environmental dependence of the gel's swelling ratio (D/D_0), and refractive index to presurmise its observed value of λ_{max}. The swelling ratio can be estimated by monitoring the diameter of a cylindrical gel prepared in a capillary with a diameter of 100 μm. In the present case, D_0 is the inside diameter of the capillary. The change in the refractive index of the porous gel with varying conditions is then measured by a refractmeter. Although the rate of change in n_a for the porous thermosensitive chemical gel composed of *N*-isopropylacrylamide (NIPA) was less than 1% when the temperature was changed from 15 °C to 60 °C, the swelling ratio changed by about a few times.[1b] Therefore, the swelling ratio is dominant over λ_{max} of the observed reflection spectrum for the porous NIPA gel.

It follows from what has been said that a periodically ordered interconnecting porous gel based on NIPA and a phenylboronic acid derivative would reveal a shift in λ_{max} to a higher wavelength with an increase in the glucose concentration at certain temperature. To fabricate a visual sensor, λ_{max} should land somewhere between 400 nm to 700 nm. To obtain a sensor exhibiting visible color in any conditions, the value of D/D_0 should be kept between about 0.9 and 1.5 based on equation (1). As our prototype, we designed a colorimetric sensor for checking urine or blood glucose levels. If a person's blood glucose level on an empty stomach is more than about 11.0 mM, the person is diagnosed as diabetic. When the blood glucose level is between 7.8 mM to 11.0 mM, the person is borderline diabetic. In such a case, a sensor that signals these concentrations through distinctive colors would be useful in diagnosis. As, red, yellow, and green are used internationally in traffic signals, we introduced this color variation in our gel system in an easy-to-understand manner, thus aiming for a structurally colored gel that is yellow when the glucose concentration is from 7.8 mM to 11.0 mM, and red when the concentration is more than 11.0 mM. To realize this system, it was crucial that the desired swelling ratio of the gel could be systematically obtained by using precise recipes. If $n_a = 1.4$, D/D_0 should be from 1.15 to 1.17 for the gel to display yellow (580–590 nm) at the appropriate glucose concentration.

According to the theory,[7] the swelling ratio of a gel comes to equilibrium when the osmotic pressure difference between the inside and outside of the gel becomes zero. The total osmotic pressure of the gel is determined by the mixing between monomers and solvent, the elastic contribution, and ion concentration differences in and out of the gel. Based on the assumption that gelation proceeds perfectly, it is technically possible to prepare a gel membrane that exhibits a desired swelling ratio by controlling the recipe.[8] It follows that a porous gel reflecting a specific color at a certain condition may also be systematically obtained.

With the desired properties in mind, we carefully selected the monomer species, their concentrations and ratio, the concentration of cross-linker, and the solvent for making the gel. We used 3-acrylamidophenylboronic acid (AAPBA) as a sensing monomer to prepare the glucose-sensitive gel.[4a,b] Figure 1 shows the degree of swelling of NIPA-*co*-AAPBA

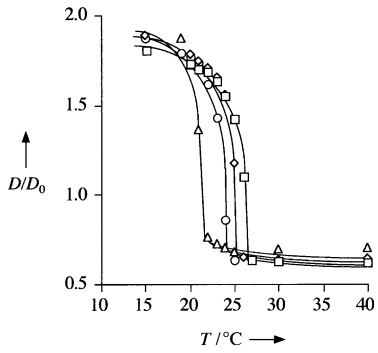

Figure 1. Degree of swelling of poly(NIPA-*co*-AAPBA) gel as a function of temperature in a CHES buffer aqueous solution. The total monomer concentrations ([NIPA] + [AAPBA]) in the recipes for these gels were fixed at 1.65 M, while [AAPBA] was changed: (□) [AAPBA] = 50 mM, (◇) [AAPBA] = 75 mM, (○) [AAPBA] = 100 mM, (△) [AAPBA] = 165 mM. [BIS] was also fixed at 2 mol% of the total monomer concentration. DMSO was used as the solvent.

gels that were prepared by using dimethylsulfoxide (DMSO) as a function of temperature in a 2-(cyclohexylamino)-ethanesulphonic acid (CHES) buffer. The monomer ratio between NIPA and AAPBA was changed, while the total monomer concentration ([NIPA] + [AAPBA]) and the cross-linker concentration were fixed. The ionic strength and pH value of the buffer were adjusted at 0.15 and 9.0, respectively. Although all gels underwent drastic volume changes, the transition temperature became lower as the AAPBA concentration was increased. It is known that transition temperature of NIPA copolymer gel having ionic sites increases as the amount of ionic sites increases.[7b,c] However, the swelling

behavior of NIPA-*co*-AAPBA gels is not consistent with that of the typical ionized NIPA gel.[7d] This may be due to the hydrophobicity of the AAPBA monomer or to the hydrogen bonding between monomers.

Swelling curves measured on a series of NIPA-*co*-AAPBA gels in buffer solutions including different amounts of glucose are shown in Figure 2. An increase in glucose

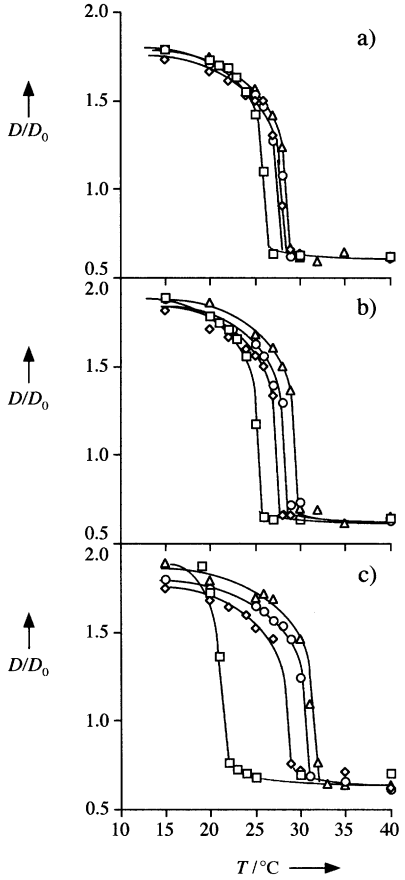

Figure 2. Degree of swelling of poly(NIPA-*co*-AAPBA) gels as a function of temperature in a CHES buffer aqueous solution including different concentrations of glucose: (□) [glucose] = 0 mM, (◇) [glucose] = 5 mM, (○) [glucose] = 10 mM, (△) [glucose] = 20 mM. The total monomer concentrations ([NIPA] + [AAPBA]) in the recipes for these gels were fixed at 1.65 M, while [AAPBA] was changed: a) [AAPBA] = 50 mM, b) [AAPBA] = 75 mM, c) [AAPBA] = 165 mM. [BIS] was also fixed at 2 mol% of the total monomer concentration. DMSO was used for the solvent.

concentration shifts the transition temperature upward in every case. The change in the volume with increasing the glucose concentration can be observed clearly at certain temperatures. The region where the remarkable change can be observed widens with as the quantity of AAPBA increases.

The ratio of monomer concentration to cross-linker concentration for a pregel solution is a strong determinant of the swelling ratio of a gel obtained.[8] The more cross-linkers there are in a solution with a given amount of monomers, the smaller the swelling ratio of the gel is in its swollen state. The swelling ratio of NIPA-*co*-AAPBA gel at lower temperatures also decreased with the amount of the cross-linker. As indicated above, D/D_0 should be within the range between 1.15 to 1.17 for glucose concentrations at from 7.8 mM to 11 mM to obtain the desired property at about room temperature. Hence, for the gels displayed in Figure 2, it was clear that we needed to increase the amount of the cross-linker in the recipe.

1,4-Dioxane (DOx), the polarity of which is significantly lower than that of DMSO, was also used as a solvent. However, the gel obtained by DOx was not sensitive to the concentration of glucose (Figure 3). The hydrogen bond

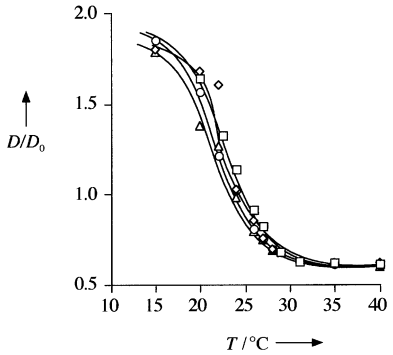

Figure 3. Degree of swelling of poly(NIPA-*co*-AAPBA) gel as a function of temperature in a CHES buffer aqueous solution including different concentration of glucose: (□) [glucose] = 0 mM, (◇) [glucose] = 5 mM, (○) [glucose] = 10 mM, (△) [glucose] = 20 mM. [NIPA] was 1.55 M of the recipe for the gel, while [AAPBA] was 100 mM. [BIS] was 2 mol% of the total monomer concentration. DOx was used for the solvent.

between NIPA and an acidic monomer such as an acrylic acid can be formed during polymerization in a nonpolar solvent.[9] The copolymer gels obtained often have micro-domain structures produced by the hydrogen bond: the structures are maintained even after the displacement of the nonpolar solvent to water. It is possible to infer that the boronic acid site in the gel must be inactive to react with glucose, as the acid part participates in forming the micro-domain through the hydrogen bond.

Eventually, we arrived at the following recipe for the pregel solution: [NIPA] = 1485 mM, [AAPBA] = 165 mM, [*N*,*N*′-methylene-bis-acrylamide (BIS: cross-linker)] = 5 mol% of a total monomer concentration ([NIPA] + [AAPBA]), and [*N*,*N*-azobisisobutyronitril (AIBN: initiator)] = 0.4 mol% of [NIPA] + [AAPBA]. DMSO was used for the solvent. The swelling ratios of the gel obtained by the recipe as a function of glucose concentration at 28 °C are shown in Figure 4a. The swelling curve suggests favorable terms for our desired property of exhibiting traffic signal colors. Practically, the tailor-made porous gel displays a yellow color when the glucose level is borderline diabetic (Figure 4b). The color of the gel turns red in the higher glucose concentrations associated with a clear-cut diagnosis of diabetes.

In conclusion, we demonstrated the creation of a colorimetric glucose sensor that can provide the desired monitoring of glucose levels by the naked eye. This new approach to

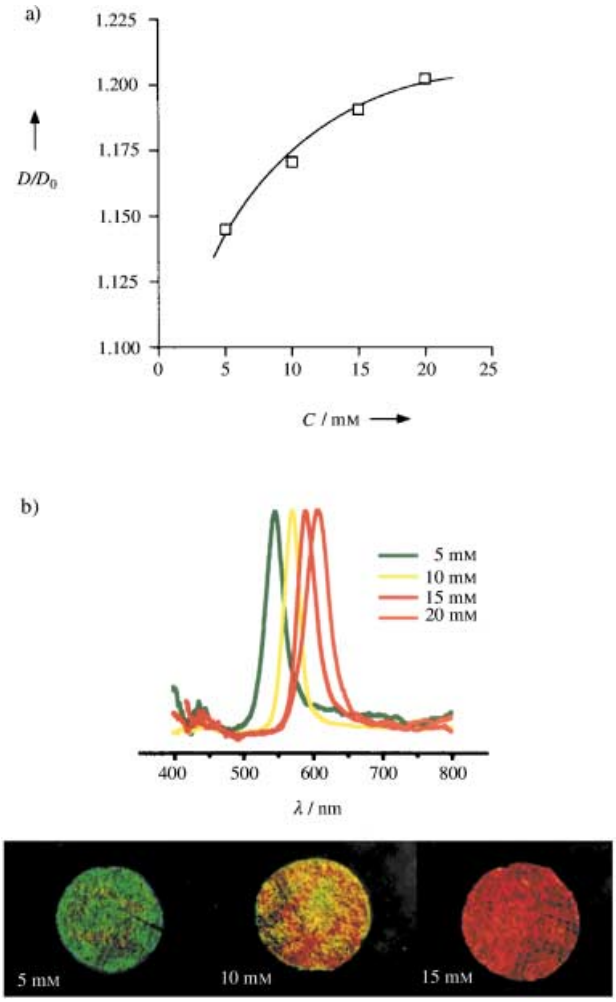

Figure 4. a) Degree of swelling of poly(NIPA-*co*-AAPBA) gel as a function of glucose concentration in a CHES buffer aqueous solution at 28 °C. [NIPA] was 1.485 M in the recipe for the gel, while [AAPBA] was 165 mM. [BIS] was 5 mol % of the total monomer concentration. DMSO was used for the solvent. b) Reflection spectra and photographs of periodically ordered interconnecting porous poly(NIPA-*co*-AAPBA) gel in a CHES buffer aqueous solution including different concentrations of glucose at 28 °C.

preparing colorimetric porous gel can be applied to many sensor systems. Similar colorimetric systems for sensing glucose, composed of a liquid crystal[4d] or a nonclosest packing colloidal crystal immobilized by polymer gels,[4e] were reported. However, our system is easier to fabricate and to control than those systems. Furthermore, the time required for porous gels to reach swelling equilibrium is shorter. These points give our method the advantage for development and use in sensor systems.

Experimental Section

To prepare the porous gels, we used closest-packing colloidal crystals composed of silica sphere particles 220 nm in diameter (Nippon Shokubai). The growth of the crystal was reported.[1b] The resulting crystal structure was confirmed by an atomic force microscope and a scanning electron microscope. Glucose-sensitive gels were prepared by free-radical polymerization as follows. First, certain amounts of NIPA, AAPBA, BIS, and AIBN, were dissolved in degassed and nitrogen-saturated DMSO or DOx to a final volume of 50 mL. The solutions were then infiltrated into the colloidal crystal in a Petri dish, and the polymerizations were conducted at 60 °C for 24 h. Afterward the samples were immersed in a 3wt % HF aqueous solution to remove the SiO_2. Cylindrical gels for swelling measurement were also prepared in glass micropipettes of 100 μm diameter. The resulting gels were washed carefully with distilled water for 1 week. Swelling was measured by monitoring the diameter of the cylindrical gel in a CHES buffer by using an inverted microscope. The reflection spectra of the disk-shaped porous gels (5 mm in diameter) were obtained by an Ocean Optics USB2000 fiber optic spectrometer. The temperature in the measurements was controlled by using a circulating water temperature control system.

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