Swelling and Shrinking Kinetics of Totally Synthetic, Glucose-Responsive Polymer Gel Bearing Phenylborate Derivative as a Glucose-Sensing Moiety

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ABSTRACT: A glucose-responsive polymer gel bead with a diameter in the range of several hundred micrometers, bearing a phenylborate derivative (3-acrylamidophenylboronic acid) as a glucose-sensing moiety, was successfully prepared by inverse phase suspension polymerization. The kinetics of the glucose responsive swelling and shrinking process of the gel bead was studied by monitoring changes in the size and shape of the gel under a microscope. The equilibrium swelling volumes of the gel determined under various temperatures and glucose concentrations revealed the presence of critical temperatures and glucose concentrations to induce discontinuous volume phase transitions of the gel. The glucose-induced swelling process was accompanied by the appearance of a marked swelling boundary (swelling front) intervening between the core collapsed phase and the outer swollen layer of the gel, and the swelling curve as a function of the square root of the time exhibited a sigmoidal (nonlinear) feature, both indicating that the process is essentially rate determined by the relaxation process of the polymer chains due to the hydration. The swelling rate was significantly affected by the bead size and the terminal glucose concentration. An accelerated disappearance of the swelling front was observed immediately before the gel reaches the equilibrium swelling, which was highlighted by an increased bead size, implying that increasing elastic and osmotic pressures generating from the swollen layer affect the swelling kinetics. The glucose-induced shrinking process involved the formation of a skin layer on the gel surface followed by a radical structural change. The observed, appreciably long-termed preservation of a quasi-swollen state on a sub-millimeter scale gel bead with a distinctive skin layer may propose the potential applicability of the gel to chemical valve systems discretely switching for solute release.

Introduction

Significant progress in the study of stimuli-responsive polymer gels over these past decades has revealed a wide variety of potential and successful application forms of these gels. Numerous types of stimuli such as heat,[1−4] pH,[5,6] light,[7] and electric fields[8] have been demonstrated to induce abrupt changes in the physicochemical properties of a polymer gel. In the list of the stimuli types thus far shown to induce responses, however, few examples can be found of chemical stimuli, i.e., concentration changes of certain molecules in the environment, although the design of such chemical-stimuli responsive systems could still expand the range of their applicable fields.

One challenging molecule that may have a great impact on medical applications may be glucose, i.e., change in blood sugar level. Many attempts have indeed been devoted to the establishment of a glucose-responsive polymer (gel) system, aimed at the development of self-regulated insulin delivery devices to treat diabetes.[9] The common methodologies to construct such systems can be categorized into two major types.[10] One is the utilization of an enzymatic reaction between glucose oxidase (GOD) and glucose,[11−14] and the other is based on the competitive and complementary binding properties to concanavalin A (Con A) of synthetically glycosylated insulin and glucose.[13−19] However, these protein-based components that could potentially be denatured are quite subjective to the environmental changes and thus limit the reliable functionality of the system when used and stored for an extended period of time.

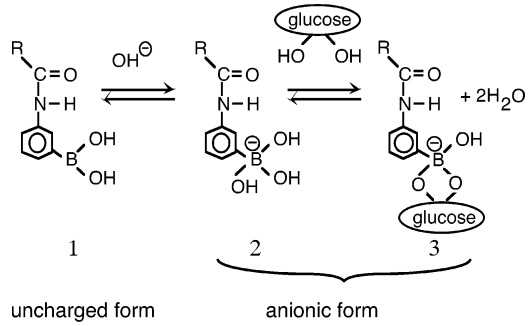

Figure 1. Equilibria of (alkylamido)phenylbronic acid in an aqueous solution in the presence of glucose.

In this context, the use of a somewhat nonnatural or synthetic component as a glucose-sensing moiety may offer a new prospect for achieving more advanced systems. Phenylboronic acid may serve as a potential candidate for such an objective.[20,21] As shown in Figure 1, phenylboronic acid compounds in water exist in equilibrium between the uncharged, and thus relatively hydrophobic, form and the charged, and thus relatively hydrophilic, form. Upon the addition of glucose, the charged form of phenylborate can form a stable complex with glucose through reversible covalent bonding,[22−24] whereas the complex between the uncharged form of phenylborate and glucose is quite unstable with a high

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susceptibility to hydrolysis.[25] Because the complex formed between the charged phenylborate and glucose itself is also anionically charged, the further addition of glucose induces a shift in the above-described equilibrium in the direction of the increasing charged (hydrophilic) forms of phenylborate and vice versa.

Consequently, by introducing this property of phenylborates into an amphiphilic polymer gel structure, such as that of the poly(*N*-isopropylacrylamide) (PNIPAAm) gel, one should obtain a functional polymer gel that undergoes a reversible volume change (volume phase transition) driven by the change in the counterions' osmotic pressure synchronized with the change in glucose concentration.[26] Our previous work has reported the first preparation of such a polymer gel that is composed of PNIPAAm and 3-acrylamidophenylboronic acid (AAPBA) (9:1 in molar ratio: NB10 gel). A sufficiently on−off regulated releasing profile of insulin from a disk-shaped NB10 gel has been repeatedly achieved.[26] In addition, the observed quick response and the effectively shut-off release of insulin in a synchronizing manner with the change in the external glucose concentration implied the formation of a dehydrated skin layer on the gel surface, serving as a highly sensitive releasing "switch".

Accordingly, this study is intended to provide a detailed investigation of the glucose-responsive kinetics of this unique class of gels. Some obstacles need to be overcome for the quantitative kinetic evaluation of a gel with an anisotropic shape (nonspherical). As the nature of polymer gels, the response rate is dependent on the size and shape of the gel.[27] Also, the shear energy originating from the latent anisotropy of the gel is not negligible.[28] With these factors in mind, the use of a smaller-sized, spherical (isotropic) gel reveals great advantages such that quick swelling and shrinking of the gel can be achieved or that the swelling and shrinking behaviors can be readily observed under a microscope and evaluated three-dimensionally with no regard to the shear relaxation process. Hence, in the present study, we prepared spherical NB10 gel beads by an inverse phase suspension polymerization technique. For the static-state information, the equilibrium swelling volumes of the NB10 gel at various glucose concentrations and temperatures were determined, which were quantitatively correlated with the disassociation degree of the phenylborate moieties assessed from the potentiometric titration. The kinetics of the glucose-induced swelling of the gel was discussed in comparison with that induced by a temperature change. The effects of the bead size as well as the varied terminal glucose concentrations on the swelling rates were then examined. Furthermore, for a more profound understanding of the swelling kinetics, both the collapsed phase in the gel core and the surrounding swollen layer, which emerge during the swelling process, were independently analyzed. Finally, the glucose-induced shrinking process of the gel was investigated, and the possible kinetics was described in detail.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAAm) was purchased from Kojin (Japan) and recrystallized from *n*-hexane. 3-Acrylamidephenylboronic acid (AAPBA) was supplied from Wako (Japan) and was used as received. *N*,*N*′-Methylenebis(acrylamide) (MBAAm), tetramethylethylene diamide, ammonium persulfate, and liquid paraffin were all purchased from Wako (Japan) and were used without further purification.

Preparation of Gel Beads by Inverse Phase Suspension Polymerization. Gel beads were prepared by inverse phase suspension polymerization using liquid paraffin as the organic phase. 80 mL of liquid paraffin was initially bubbled with argon for 1 h in a round-bottomed flask immersed in a water bath controlled at 15 °C. The initiator solution was prepared by dissolving 40 mg of ammonium persulfate in 1 mL of distilled water. NIPAAm (2.565 g), AAPBA (0.504 g), MBAAm as a cross-linker (0.041 g), and tetramethylethylene diamide as an accelerator (0.750 g) were dissolved in 16 mL of distilled water (monomer solution). Both solutions were degassed for 5 min, bubbled with argon, and then incubated in an ice bath for 10 min. These incubations were critical to inhibit any occurrence of immediate polymerization in the highly concentrated monomer solution upon the addition of the initiator solution. Subsequently, 0.25 mL of the initiator solution was added to the monomer solution. After a quick stirring, it was poured into the flask. The reaction was allowed to proceed under an argon atmosphere at 15 °C and stirred at the rate of 400 rpm for 1 h. The collected gel beads were washed several times with THF to remove the liquid paraffin and the remaining monomers. The diameter of the obtained beads was approximately 0.2−0.8 mm in the stored solution (THF) and was significantly dependent on the amount of the added accelerator.

Potentiometric Titration. Potentiometric titration of the bead gel was performed for various glucose concentrations at 25 °C under an argon atmosphere, using an automatic titrator system (DL25, Mettler Toledo, Germany) equipped with a pH electrode (Glass electrode DG111-SC, Mettler Toledo, Germany). The sample was stirred at ca. 100 rpm during the titration. The calibration of the electrode was carried out at 25 °C with carbonate and phthalate buffers (Beckman). Purified gel beads via dialysis against deionized water and then freeze-dried (10 mg) were suspended in 40 mL of freshly prepared 0.01 N NaOH solution (150 mM NaCl) adjusted to a series of glucose concentrations. The water was purified by a Milli-Q instrument (Millipore Corp.). The titrant (0.01 N HCl, 150 mM NaCl) was added through a 10 mL bullet in 0.1 mL portions when the drift reached the rate of ±0.01 pH unit over the monitoring time of 10 min. The average molar content of the phenylborate moiety (AAPBA) in the gel bead was calculated to be 10.8 mol % (8.2×10^{-3} mol/g), from the amount of the added titrant during the titration and the dried state weights of the gel, assuming an incorporation of the cross-linking moiety (MBAAm) in the same molar content as in the feed (1 mol %).

Observation of Swelling−Shrinking Behavior of Gel Beads. The gel beads stored in THF were transferred to an excess amount of distilled water and incubated for at least 1 day to thoroughly exchange the solvent from THF to distilled water. As a means to readily observe the shape-changing behavior of the gel while controlling the temperature, two concentric laboratory dishes of different sizes were placed on the microscope stage, serving as a two-chambered cell. A gel bead was put in the inner chamber filled with each experimental buffer solution. The temperature of the solution was sufficiently controlled by applying a thermocontrolled water flow in the outer chamber. Prior to each experiment, the gel beads were kept in the solution under the controlled temperature for a sufficient period of time in order to reach their equilibrium swelling. Investigations of the glucose responsive behaviors of the gels were carried out by exchanging the solution in the inner chamber with the different glucose concentrations conditioned buffers. The images of the gel were observed under a microscope (Nikon, Diaphot) equipped with a black−white CCD camera (Hamamatsu Photonics, C2400). The obtained images were processed using analytical software (Hamamatsu Photonics, image processor Argus C5510) and then recorded by a video recorder.

Results and Discussion

Equilibrium Swellings of NB Gel. The NB gel is a copolymer gel mainly composed of poly(*N*-isopropylacryl-

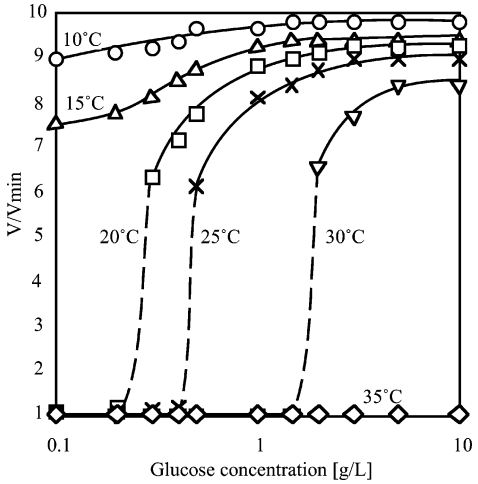

Figure 2. Equilibrium swelling volume (the ratio to the volume at 40 °C: $V_{min} = 387\ \mu m$) of an NB gel bead in a pH 9 CHES buffer solution ($I = 0.15$) as a function of glucose concentrations and temperatures.

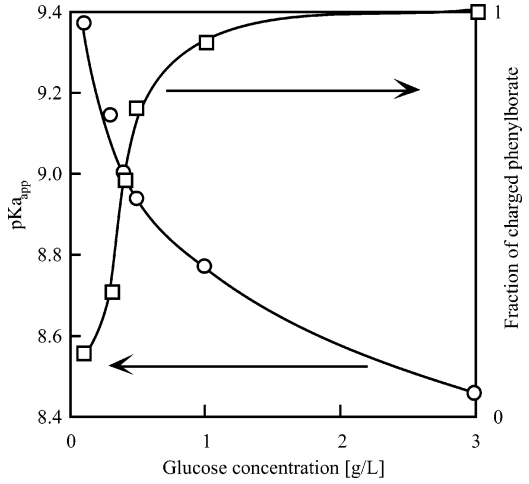

Figure 3. Changes in the apparent pK_a (p$K_{a,app}$) of the phenylborate group in an NB gel bead (left-hand side) at 25 °C, and the fraction change of the charged phenylborate (right-hand side) at pH 9 and 25 °C, as a function of glucose concentration, as determined from the potentiometric titration.

amide) (NIPAAm) which itself undergoes a thermosensitive phase transition, with a pendent phenylborate derivative (AAPBA), which reversibly dissociates in response to change in the pH or glucose concentration. Hence, this type of gel is capable of responding to both physical (temperature) and chemical stimuli (pH or glucose).

The theoretical basis for the volume-changing behavior of a polymer gel has been well-established by Tanaka et al. with modified Flory−Huggins formula.[29,30] The equilibrium swelling degree of a polymer gel is determined by the osmotic pressures' balance among those originated from the following three factors: (1) rubbery elasticity due to the cross-linked network, (2) the change in mixing free energy (between the polymer segment and the solvent molecule), and (3) the osmotic pressure induced by the presence of the counterions, particularly when the gel contains fixed charges. The third term, the counterions' osmotic pressure, has been theoretically as well as experimentally shown to be most influential.[30] For example, the introduction of 1 mol % acrylic acid (carboxyl anion) into the PNIPAAm gel network brings about as much as 20% increase in the swelling degree at 25 °C.[30]

Figure 2 shows the equilibrium swelling volumes of the NB gel as a function of the glucose concentration for various temperatures in a pH 9 CHES buffer solution ($I = 0.15$). While the gel is in the completely shrunken state at 35 °C regardless of the glucose concentration and in the sufficiently swollen state at 10−15 °C, in an intermediate temperature range (20−30 °C), the gel undergoes marked volume changes at each critical glucose concentration. Figure 3 indicates the change in the apparent pK_a (p$K_{a,app}$) of the phenylborate group in the NB gel (left-hand side) along with the change in the fraction of the charged phenylborate (right-hand side) (pH 9, 25 °C) as a function of the glucose concentration that were assessed from the potentiometric titration. The p$K_{a,app}$ decreases with increased glucose concentration because of the stabilization of the charged phenylborates (2 and 3: Figure 1) through the complexation with glucose. Consequently, the fraction of the charged phenylborate at pH 9 (25 °C) increases with increased glucose concentration. For the glucose concentration of 0.4 g/L, where the gel's volume appears to start increasing in Figure 2, the fraction reaches 0.61, indicating that this value of the fraction corresponds to a critical amount of the charged phenylborate necessary to expand the network of the NB gel at 25 °C. On the basis of the calculated phenylborate (AAPBA) content (10.8 mol %) from the titration result, the converted molar ratio of the critical amount of the charged phenylborate (to initiate the gel's swelling at 25 °C) to the entire gel's structure becomes 6.6 mol %. This value, indeed, agrees with a fact that the PNIPAAm-based copolymer bearing 10.5 mol % phenylborate moiety shows its LCST of about 25 °C when the fraction of the charged phenylborates becomes 0.59 mol % (the converted molar ratio: 6.2 mol % (data not shown)). Also revealed in Figure 2 is that, with increased temperature, the critical glucose concentration at which the gel starts swelling shifts toward a higher glucose concentration. This is due to the increased contribution of the second factor (increased mixing free energy) mentioned above. According to an equation of state for a polymer gel obtained from the aforementioned three factors, a physical meaning of changing temperature is synonymous with that of change in mixing free energy (the second factor).[29] Specifically, when the constituent polymer segment possesses the LCST (lower critical solution temperature) as in the present case (PNIPAAm main chain), an increased temperature causes an increased mixing free energy, thus resulting in a decreased volume of the gel (a less hydration of the network). Therefore, a higher glucose concentration or an increased fraction of charged phenylborates is necessary to expand the network when the temperature is increased as observed in Figure 2. The same reason accounts for a slightly decreased swollen-state volume of the gel with increased temperature as can be seen in the lower glucose concentration range in Figure 2.

Swelling Process of NB Gel. Figure 4 shows images of (a) temperature- and (b) glucose-induced swellings of the gel. In both cases, distinct swelling boundaries (swelling fronts) between the gel core, in the collapsed phase, and the outer swollen layer were observed. The size of the core collapsed phase decreased with time, while the surrounding swollen layer continued to grow until finally the entire phase made the transition to the swollen phase. After disappearance of the swelling front,

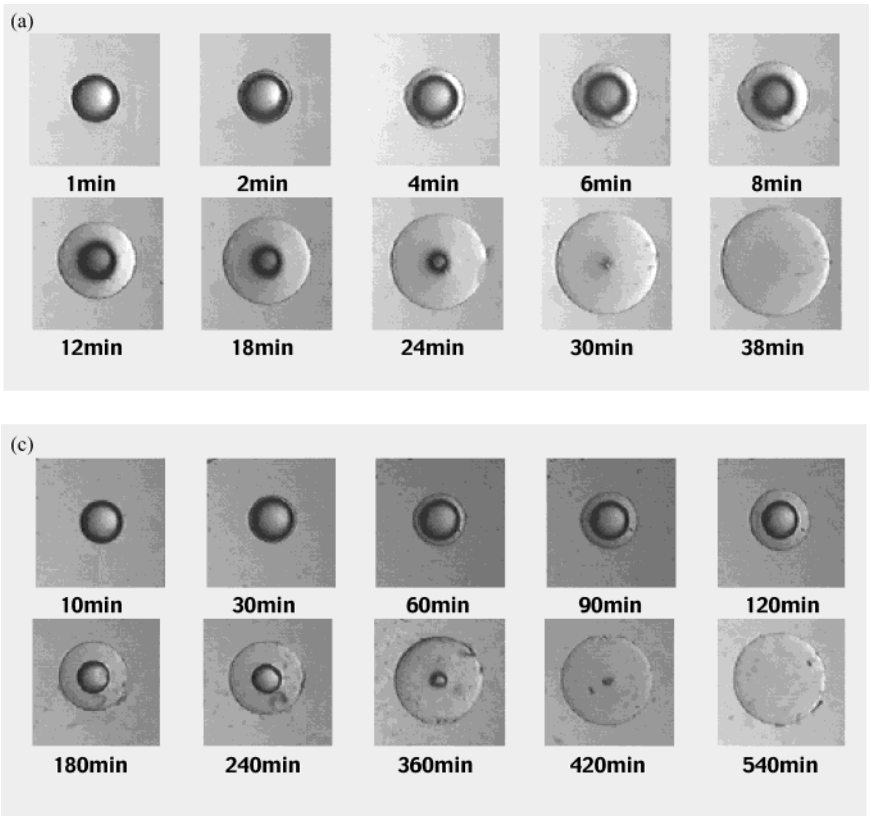

Figure 4. Images of swelling behavior of an NB gel bead induced by (a) a decreased temperature from 30 °C to 15 °C in a pH 9 CHES buffer saline ($I = 0.15$) in the absence of glucose and (b) an increased glucose concentration from 0 to 5 g/L at 25 °C in a pH 9 CHES buffer saline. The initial diameter of the gel in the completely collapsed state was 400 μm.

the gel did not demonstrate any further significant size change, indicating that the gel had reached its equilibrium swelling as soon as the collapsed phase disappeared. The swelling rate of the glucose-induced process is, however, much slower than that of the temperature-induced one. During the temperature-induced swelling process, the heat quickly and uniformly propagates throughout the entire volume of the gel. Consequently, the gel promptly swells due to the hydration of the polymer chains when the temperature becomes lower than the LCST of the network. On the other hand, for the glucose-induced swelling process, glucose molecules first diffuse into the gel and then bind to the phenylboronic acid groups, which causes hydration of the polymer chains. Figure 5 shows a series of terminal glucose concentration-induced swelling curves of the gel, all with an initial glucose concentration of 0 g/L. The swelling degrees are standardized with the value of the completely shrunken state in order to show the differences in the equilibrium swelling degrees for the varied terminal glucose concentrations. For the terminal glucose concentration of 3 and 5 g/L, where the phenylborate groups become completely charged in the equilibrium states, the same terminal (equilibrated) volumes of the gel are achieved. However, the swelling rate has been increased for the terminal glucose concentration of 5 g/L compared to that for the terminal glucose concentration of 3 g/L, requiring approximately 600 and 800 min to reach its equilibrium swelling, respectively. The observed different swelling rates between the two processes (terminal glucose concentrations of 3 and 5 g/L) imply that the relaxation process (relaxation constant) of the polymer chains depends on the glucose concentration effectively. Apparently, the relaxation

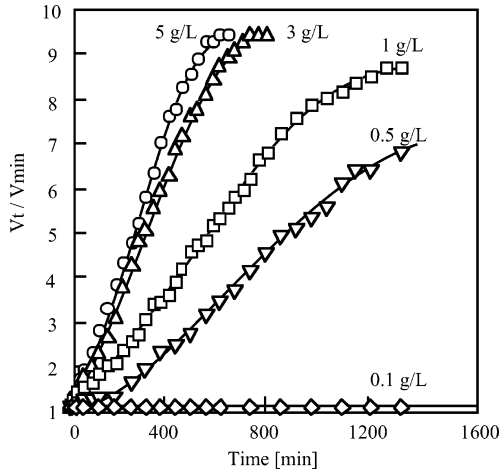

Figure 5. Series of terminal glucose concentrations: (◇) 0.1, (▽) 0.5, (□) 1, (△) 3, and (○) 5 g/L, induced swelling curves of an NB gel bead, all with the initial glucose concentrations of 0 g/L, as a function of the linear time at 25 °C in a pH 9 CHES buffer saline ($I = 0.15$). The initial diameter of the gel in the completely collapsed state was 400 μm.

process of the polymer chain is rate-determining for these glucose-induced swelling processes as will be discussed in the following paragraph. The slightly smaller value of the equilibrium swelling for the terminal glucose concentration of 1 g/L, as compared to those with higher terminal glucose concentrations such as 3 and 5 g/L, is due to the decreased fraction of anionically charged phenylborates (0.95: Figure 3) and thus a corresponding decrease in osmotic pressure arising from the mobile counterions. For the terminal glucose concentration of 0.5 g/L (fraction of the charged

phenylborates: 0.77), the gel swelled quite slowly, with both collapsed and swollen phases remaining even after 24 h. Moreover, for the terminal glucose concentration of 0.1 g/L (fraction of the charged phenylborates: 0.14), the gel remained in a completely collapsed state even after several days, apparently reaching its equilibrium shrinking state. Thus, it was demonstrated that for a decreased terminal glucose concentration, a longer period of time is required for the gel to reach its equilibrium swelling. This means that one can readily control the swelling rate of the gel with varied signal amplitude. Note that the continuous swelling of a submillimeter scaled gel bead at a sufficiently slow rate is obviously relevant for the sustained release of bioactive compounds, such as insulin, entrapped in the collapsed-state gel.

In general, the swelling process of a polymer gel with absorbing a solvent occurs in the following steps: (1) diffusion of the solvent molecules into the polymer network, (2) relaxation of the polymer chains due to solvation, and (3) diffusion of the polymer chains into the solvent. The swelling behavior of the gel significantly differs according to which step is the rate-determining one. When step 2 is the rate-determining one, in particular, when the relaxation rate of the polymer chains (step 2) is slower than the diffusion rate of the solvent molecules (step 1), the gel exhibits two phases partitioned with a relaxation boundary intervening between the core collapsed phase and the outer swollen layer. This boundary layer is called the swelling front, and it advances at the constant rate into the gel core while absorbing the solvent (case II transport). For a gel that swells according to solvent penetration as in the case II transport, the swelling kinetics can be described by the following equation:

$$M_{st}/M_{s\infty} = 1 - (1 - k_0 t/C_s a)^N \quad (N = 1, 2, 3) \quad (1)$$

where M_{st} and $M_{s\infty}$ are the cumulative amounts of solvent absorbed into the gel at time t and at an infinite time, respectively, k_0 is the relaxation constant of the polymer chains, C_s is the solvent concentration in the swollen region, and a is the radius of the gel. N is a number dependent on the shape of the gel, which equals 1, 2, or 3 for a membranous, cylindrical, or a spherical shape, respectively. According to the equation, it can be predicted that, with a spherical shape, the swelling curve of the gel as a function of the square root of the time exhibits a sigmoidal (nonlinear) feature, unlike the diffusion-determined (case I diffusion) case where the curve should exhibit a linear feature. Figure 6 shows a series of terminal glucose concentration-induced swelling curves of the gel as a function of the square root of the time. Regardless of the terminal glucose concentration, all the curves exhibit sigmoidal (nonlinear) features. Thus, it should follow from what has been described above that the glucose-induced swelling kinetics of the gel, which showed a marked swelling front and a sigmoidal (nonlinear)-shaped swelling curve as a function of the square root of the time (Figure 6), is a process essentially rate-determined by the relaxation process of the polymer chains accompanied by hydration (case II transport). Indeed, the occurrence of the case II transport was confirmed from a direct observation of a change in the radius of the collapsed phase during the glucose-induced swelling process in the following section.

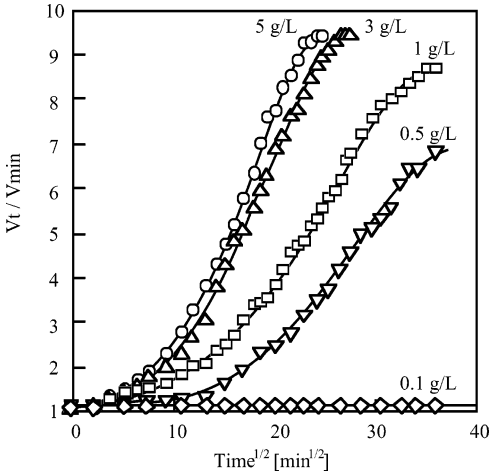

Figure 6. Series of terminal glucose concentrations: (◇) 0.1, (▽) 0.5, (□) 1, (△) 3, and (○) 5 g/L, induced swelling curves of an NB gel bead, all with the initial glucose concentrations of 0 g/L, as a function of the square root of the time at 25 °C in a pH 9 CHES buffer saline ($I = 0.15$). The initial diameter of the gel in the completely collapsed state was 400 μm.

Kinetics of Collapsed Phase and Swollen Layer. Thus far, the swelling kinetics of the gel were investigated on the basis of the volume change. In particular, the kinetics of the glucose-induced swelling processes were explained by the following steps: (1) binding of glucose molecules to phenylborate moiety in the gel at the boundary region, (2) hydration of polymer chains due to complex formation between charged phenylborates and glucose, and (3) relaxation (swelling) of polymer chains synchronized with a phase transition occurring at the swelling front. For a more profound investigation of the glucose-induced swelling behavior, we took notice of the respective kinetics of the collapsed phase and the swollen layer. These two regions were independently evaluated from (1) a change in the radius of the collapsed phase [R_c (collapsed region)/R_{max}] and (2) a change in the radius of the swollen layer [R_s (swollen region)/R_{max}] (Figure 7). In Figure 7, the curves ranging from around 0.5 to 0 on the vertical axis indicate a change in the radius of the collapsed phase, and the curves ranging from 0 to 1 on the same axis indicate a change in the radius of the swollen layer. The radius of the collapsed phase constantly decreases until reaching around 0.2 on the vertical axis, and then it rapidly decreases, more remarkably with increasing terminal glucose concentration. The observed, constantly decreasing radius of the collapsed phase in the early stage of the swelling clearly indicates that the process indeed follows the case II transport. On the other hand, the radius of the swollen layer increases in a constant manner until reaching around 0.7 on the vertical axis, where the rate increases at an accelerated pace until the gel reaches the equilibrium swelling. Similar phenomena have indeed been reported by several other researchers.[29,31−33] Siegel et al.[33] found such an acceleration of the swelling rate in the late stage of the hydration process of a disk-shaped (with an aspect ratio about 40) copolymer gel composed of methyl methacrylate (MMA) and (*N*,*N*-dimethylamino)ethyl methacrylate (DMA). The mechanism was explained as follows: (1) as the initially dried-state gel disk absorbs water, two swelling fronts from both surfaces advance into the gel core, (2) the glassy gel core constrains the swelling to occur in the direction normal to the fronts,

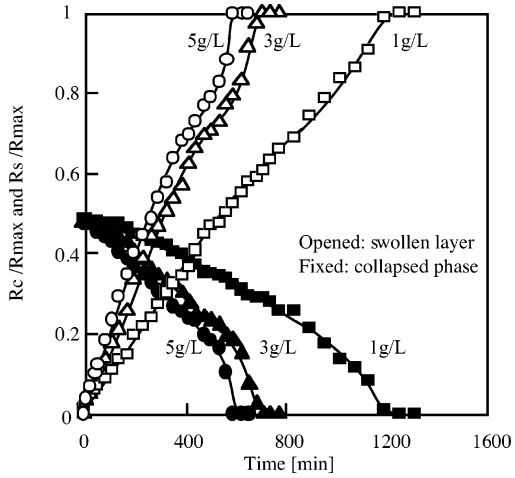

Figure 7. Radius changes of the collapsed phase [R_c (collapsed phase)/R_{max}] (fixed symbols) and the swollen layer [R_s (swollen layer)/R_{max}] (opened symbols) in the glucose-induced swelling process of an NB gel bead for various terminal glucose concentrations: (squares) 1, (triangles) 3, and (circles) 5 g/L, all with an initial glucose concentration of 0 g/L, as a function of the linear time at 25 °C in a pH 9 CHES buffer saline (I = 0.15). The initial diameter of the gel in the completely collapsed state was 400 μm, and that of the completely swollen state (R_{max}) was 867 μm.

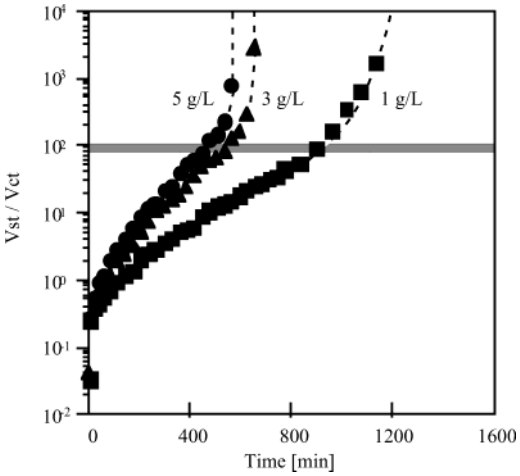

Figure 8. Changes in volume ratio of the swollen layer to the collapsed core in the glucose-induced swelling process of an NB gel bead, for varied terminal glucose concentrations: (●) 1, (▲) 3, and (■) 5 g/L, all with an initial glucose concentrations of 0 g/L, as a function of time at 25 °C in a pH 9 CHES buffer saline (I = 0.15).

(3) the two fronts will meet at the center of the gel disk, and eventually the glassy core vanishes, and (4) swelling can then commence in three dimensions, leading to an acceleration of the swelling rate (super case II transport). In the present case, however, it appears that the commencement and completion of the acceleration of the swelling and the accelerated disappearance of the collapsed core simultaneously take place. Figure 8 may provide an interpretation for the phenomena, where the changes in the volume ratio of the swollen layer to the core collapsed phase are plotted for the varied terminal glucose concentrations. The value exponentially increases with the decreased volume of the core collapsed phase. Noteworthy is that, for all cases, the value can be practically observed in the same 10^2 range on the vertical axis, at the times where the acceleration phenomenon appears to commence in Figure 7. Although for the terminal glucose concentration of 1 g/L, where the fraction of charged phenylborates in the equilibrium state is slightly low (0.95) compared to cases for the higher terminal glucose concentrations of 3 and 5 g/L, the effect of the terminal glucose concentration on the critical range of the value in Figure 8 may not be significant. Thus, these results suggest that the swelling kinetics may be affected by the elastic as well as the osmotic pressure balance between the glassy gel core and the outer swollen layer. That is, when the elastic pressure, which arises when the swelling front undergoes a phase transition, or the osmotic pressure, which is due to the counterions produced by the anionically charged phenylborates, increase to a critical level, a disruption throughout the entire volume of the collapsed phase may be induced, resulting in a rapid disappearance of the collapsed phase. This view can be most readily observed in the behavior of a gel bead with increased size. Figure 9 shows the glucose-induced (from 0 to 5 g/L) swelling profiles for a gel with varied bead sizes. In these experiments, the terminal glucose concentration has been fixed at 5 g/L for all cases. Therefore, the osmotic pressures generated by a unit volume of the swollen layer should be regarded as the same for all cases, enabling us to observe more directly the aforementioned pressure balance (between the elastic and the osmotic pressures) form the changes in the volume ratio of the swollen layer to the core collapsed phase. As can be seen in Figure 9, with an increased bead size, a more rapid acceleration of the swollen-layer growth and the collapsed-phase disappearance took place when the core size decreased to a certain size. With the increased bead size, the gel possesses both a larger volume of the swollen layer and a larger volume of the core collapsed phase. This may cause a more critical pressure balance between the two phases, resulting in an increasingly rapid disruption of the gel core during the final stage of the gel's swelling. Figure 10 shows the same plots as Figure 8 for varied bead size conditions. As expected, regardless of the bead size, the values at the times where the accelerations occur

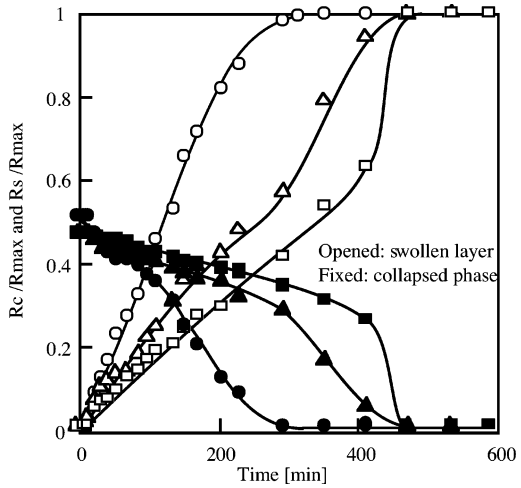

Figure 9. Radius changes in the collapsed phase [R_t (collapsed region)/R_{max}] (fixed symbols) and the swollen layer [R_t (swollen region)/R_{max}] (opened symbols) in the glucose induced (from 0 to 5 g/L) swelling process of an NB gel with varied bead size, as a function of time at 25 °C in a pH 9 CHES buffer saline (I = 0.15). The initial diameters of the gel in the completely collapsed state were (●) 278, (▲) 400, and (■) 556 μm. The corresponding values of R_{max} were 544, 867, and 1189 μm, respectively.

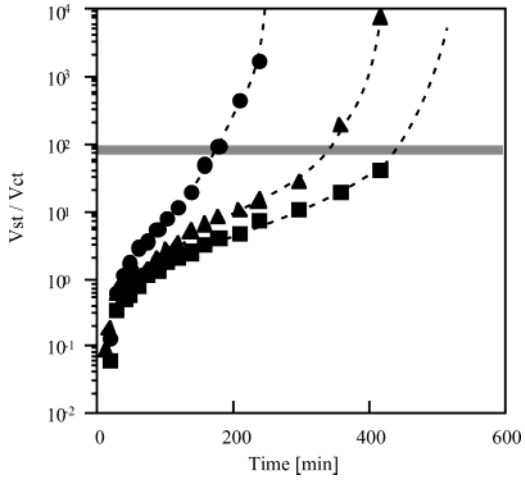

Figure 10. Changes in volume ratio of the swollen layer to the collapsed core in the glucose-induced (concentration change from 0 to 5 g/L) swelling process of an NB gel bead, for varied bead size, as a function of time at 25 °C in a pH 9 CHES buffer saline (I = 0.15). The initial diameters of the gel in the completely collapsed state were (●) 278, (▲) 400, and (■) 556 μm. The corresponding values of R_{max} were 544, 867, and 1189 μm, respectively.

in Figure 9 were all observed to be in the same range as in Figure 8, supporting again that the acceleration phenomenon is induced by a vanished pressure balance between the glassy gel core and the outer swollen layer.

Shrinking Process of NB Gel. During the swelling process of the NB gel, it was demonstrated that an already existing swollen layer and the remaining collapsed phase affect its swelling kinetics. It can also be expected that during the shrinking process of the gel the structural change in the gel caused by the aggregated polymer chains should affect the shrinking kinetics of the gel.

Images of each stimulus-induced shrinking behavior of the gel are shown in Figure 11. Figure 12 shows the shrinking curves as a function of time. With a temperature increase from 15 to 30 °C (Figures 11 and 12a), the initially swollen gel starts shrinking (Figure 11a: A−C). During this early stage of shrinkage, the formation of the collapsed (skin) layer on the surface of the gel is visible as an increasing turbidity from the initially transparent state. This is due to the dehydration of the polymer chains with an increased temperature higher than the LCST and a resultant aggregation of the polymer chains. Thereafter, the gel remained at an apparently constant volume for a certain period of time (C, D), exhibited distorted shapes with partially bubbled phases (E), reshrank with a radical structural change (F−I), and finally the entire phase made a transition to reach the equilibrium shrinking phase (J). The apparently sustained volume of the gel (C, D) can be interpreted as the state with decreased permeability of the water molecules due to the growing thickness of the collapsed layer on the gel's surface.[34] The state of the gel may then go into an unstable region where the two phases can no longer coexist, thus causing radical structural changes.[34]

The same series of steps were observed for the glucose-induced shrinking process (Figures 11 and 12b). In the case with decreased glucose concentration (from 5 to 0 g/L), where the shrinkage of the gel occurs due to the dehydration of the polymer chains caused by dissociation of the complexes between glucose and the phenylborates, a much longer-lasting (as long as 400 min) preservation of a constant volume of the gel were observed, as compared to the case that is induced by a temperature (15 min) change. The difference in the preservation time should be attributed to the different diffusivities between the two stimuli. For the temperature-induced shrinking, heat propagates quickly and uniformly throughout the entire volume of the gel. Although the gel reaches an apparently constant volume when the skin layer has grown to some degree, after a while, the core of the gel should also reach the equilibrium state because of the quick propagation of heat. Consequently, a formation of collapsed phases (phase transition) should soon begin even in the swollen core, inducing drastic structural changes. On the other hand, in the glucose-induced shrinkage, once the collapsed layer grows to a certain thickness, the diffusivity of the glucose molecules through the skin layer should significantly decrease, further delaying the decreasing rate of the glucose concentration in the inner core of the gel. As a result, the fraction change in the charged phenylborates existing in the inner network is significantly suppressed, leading to a gradual aggregation of the network and a less radical structural change. Moreover, the slow fraction change of the charged phenylborates synchronized with the gradual change in the glucose concentration in the gel core may become the rate-determining step for the structural change, enabling the gel to sequentially change the structure and to reshrink for an extended period of time. This long-time maintenance of the quasi-swollen state of the gel accompanied by a skin layer formation on the gel surface may be advantageous for use of the gel as a repetitive on−off drug-releasing device with a prolonged interval.

Conclusion

The NB gel displayed a discontinuous change in the equilibrium swelling volume at each critical glucose concentration for various temperatures in a pH 9 solution (I = 0.15).

During the swelling process of the gel in response to the increased glucose concentration, a "swelling front" was clearly observed. The swelling rate was significantly dependent on the varied terminal glucose concentrations and size of the gel bead. In addition, during the early stage of the glucose-induced swelling process, a constant decrease in the radius of the collapsed phase was observed, indicating that the early-stage process is essentially rate determined by the relaxation process of the polymer chains accompanied by hydration (case II transport). However, during the final stage of the process, an acceleration of the swelling as well as an accelerated disappearance of the collapsed core simultaneously occurred. The observed increasing rate of disappearance of the collapsed phase immediately before equilibrium swelling, which became remarkable with an increased bead size, was reasonably interpreted as a vanished pressure balance between the glassy gel core and the outer swollen layer.

The shrinking process of the gel in response to the decreased glucose concentration can be interpreted by the following steps: (1) the collapsed layer is formed on the gel surface as a consequence of the dissociation of the complexes between glucose and the phenylborates, (2) the thickness of the collapsed layer grows into the gel core, significantly decreasing the permeability of the glucose molecules thus the gel reveals an appar-

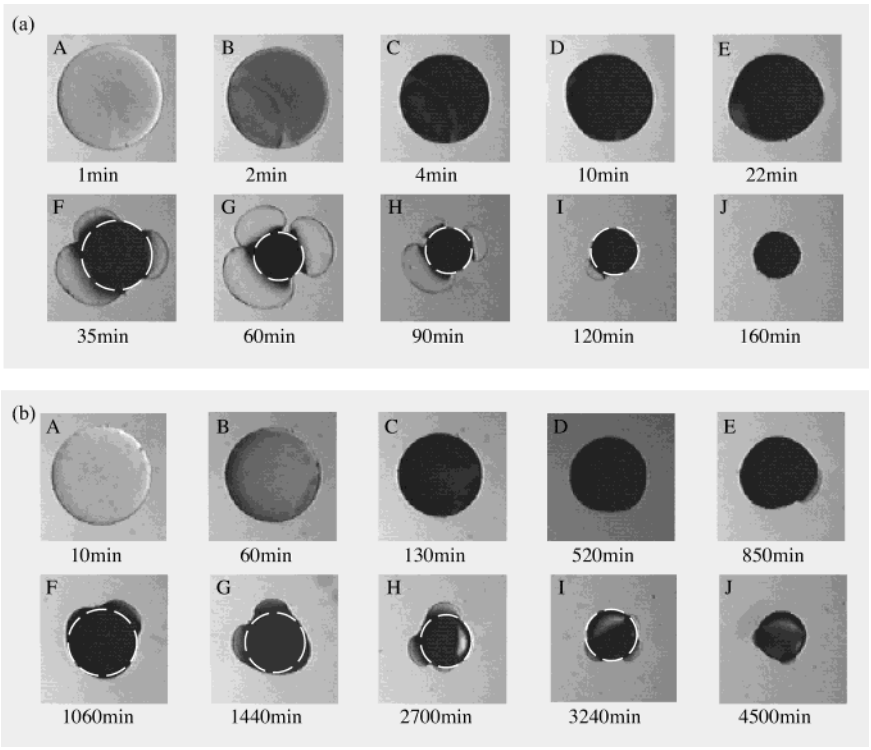

Figure 11. Images of shrinking behavior of an NB gel bead induced by (a) an increased temperature from 15 to 30 °C in a pH 9 CHES buffer saline ($I = 0.15$) in the absence of glucose and (b) a decreased glucose concentration from 5 to 0 g/L at 25 °C in a pH 9 CHES buffer saline. The terminal diameter of the gel in the completely collapsed state was 400 μm.

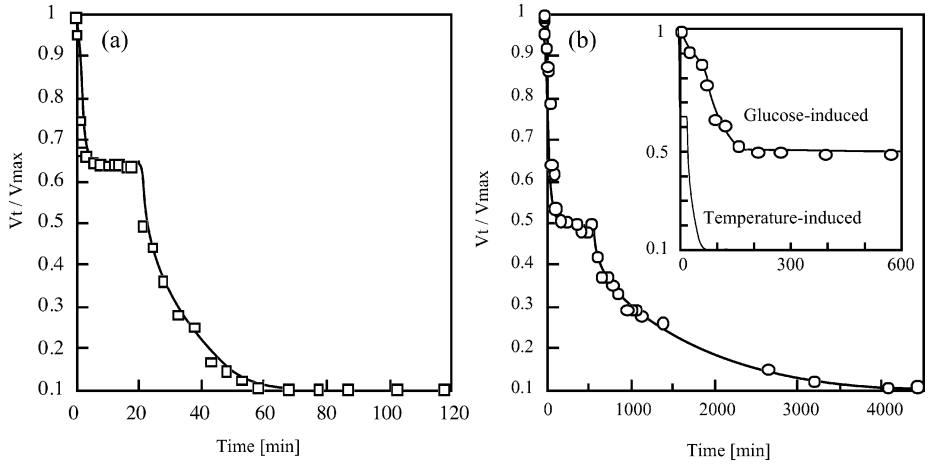

Figure 12. Shrinking curves of an NB gel bead in a pH 9 CHES buffer saline induced by (a) an increased temperature from 15 to 30 °C in the absence of glucose and (b) a decreased glucose concentration from 5 to 0 g/L at 25 °C. The terminal diameter of the gel in the completely collapsed state was 400 μm in both cases. While the gel exhibited a changing distorted (nonspherical) shape, the regions encircled by the dotted line in Figure 11 were evaluated.

ently constant volume, and (3) the gel starts a slow shrinking while exhibiting distorted shapes with partially bubbled phases and finally reaches the completely shrunken state (the overall collapsed phase). The observed, very long time (as long as 400 min) maintenance of the quasi-swollen state of a small (sub-millimeter) gel bead in the glucose-induced shrinking process should become a significant advantage for the use of the gel as a glyco-sensitive drug delivery system achieving on−off regulation of drug release with prolonged time intervals.

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