Monodispersed Glucose-Responsive Microgels Operating at Physiological Salinity

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Monodispersed poly(*N*-isopropylacrylamide) submicrometric microgels modified with a phenylboronic acid (PBA) derivative have been synthesized by precipitation polymerization. Particles with a well-controlled size and adjustable composition were obtained. These particles were found to be glucose responsive at a pH close to the pK_a of the PBA derivative, with a swelling degree proportional to the concentration of glucose. In addition, the response to glucose was found to strongly depend on the initial state of the microgel, which depended itself on the initial temperature and the functionalization degree of the particle. This result explained the fundamental difference in the behavior of PBA-poor particles and rich ones in the presence of electrolyte. Interestingly, the latter exhibited a high swelling ratio in the presence of glucose at physiological electrolyte concentration. These particles may serve as building blocks for the design of colorimetric sensors based on the light diffraction of colloidal crystals.

Introduction

Stimulus-responsive polymer gels have attracted much interest over the past decades due to their potentiality to design "self-regulated" systems often referred as "smart materials". Various stimuli such as heat, pH, electric field, or light may be used to induce a drastic change in the physical properties of the gel. Of particular interest for the biomedical field is the use of a chemical stimulus, i.e., when the gel responds to a concentration change of a specific molecule in the environment, exactly like biofeedback systems. Such systems appear as promising candidates for sensing, drug delivery, and possibly new biomaterials. One of the most famous target molecules in this field is glucose, owing to the widely spread disease of diabetes and the great demand for lightening the treatment. The design of new sensors for noninvasive glucose monitoring or self-delivering systems aimed to decrease the frequency of insulin injections appears as a great challenge.

To design a chemical-responsive gel, two criteria must be satisfied: first, the gel has to be functionalized with a receptor which has the ability to recognize the target molecule, and second, the complexation between the receptor and the target should induce a physical modification of the gel which will originate its swelling or shrinking based on theoretical considerations as previously developed by Tanaka.[1] The equilibrium volume of a hydrogel is determined by three contributions: the free energy of mixing, which is controlled by the affinity between the polymer chain and the solvent, the hydrogel network elastic restoring force, which depends on the cross-linking density, and the osmotic pressure exerted by the mobile ion concentration inside and outside the gel. Any modification of one of these parameters upon target complexation may affect the gel volume, the last term being the most influential.[2]

Several examples of glucose-responsive gels have already been reported using natural receptors such as the enzyme glucose oxidase (GOD)[3−7] or lectin concanavalin A (Con A)[8,9] as well as synthetic ones such as phenylboronic acid (PBA).[10−13] Although less specific, the latter presents the advantage of greater reliability and long-term storability over possible protein denaturation. Such a receptor presents the characteristics to induce gel swelling upon glucose complexation. Indeed, PBA derivatives exist in both charged—and also hydrophilic—and uncharged—and relatively hydrophobic—states in aqueous solution (Figure 1). However, upon glucose addition, only the charged state forms a stable complex with glucose through reversible covalent bonding,[14] whereas the neutral form is highly susceptible to hydrolysis.[15] This results in a shift in the equilibrium which increases the fraction of charged entities and therefore induces swelling when those entities are linked to a gel polymer chain. On the basis of this concept, gels with on−off regulation of insulin release have been developed by Kataoka et al., initially operating at a pH of 9[13] and more recently improved to operate at physiological pH conditions by modifying the chemical structure of the receptor with an electron-drawing group in the phenyl ring.[16,17] Monolithic gels were investigated with both capillary and submillimetric bead shapes,[18] but both of them displayed very slow response time, on the order of several hours. It is well-known that the size and the porosity of a gel strongly affect the response time, which is ruled by a diffusion process and polymer chain relaxation.[19] An improvement was obtained by Braun et al., who designed glucose-responsive macroporous gels by templating with a colloidal crystal.[12] Our strategy focused on a reduction of the

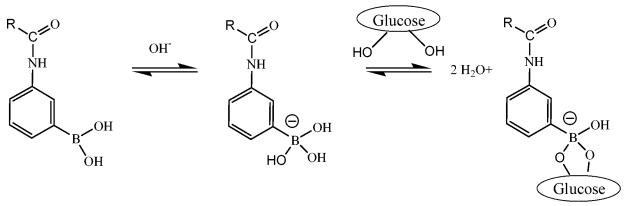

Figure 1. Representation of the complexation between the alkylamidophenylboronic acid and glucose in aqueous solution.

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gel dimensions, which would not only improve swelling or deswelling kinetics but also allow new applications such as in vivo sensing or delivery. Therefore, our work was aimed to design new submicrometric glucose-sensitive gel particles, also referred to as microgels. It should be noted that some thermosensitive particles bearing PBA groups on the surface have already been synthesized, but no swelling upon glucose recognition was reported.[20]

In this paper, we report on the synthesis of thermoresponsive poly(*N*-isopropylacrylamide) (p-NIPAM) microgels bearing a PBA derivative. A series of monodispersed submicrometric gel particles with various PBA ratios were synthesized through a batch process. Their swelling behavior was investigated versus temperature and glucose concentration under various pH and salinity conditions by photon correlation spectroscopy. First, we show that the presence of the PBA derivative influences the volume phase transition temperature, due to the increased hydrophobicity of the particles, and we also demonstrate that the swelling response to glucose recognition is ruled by an increase in the charge density of the polymer. We show evidence that the glucose sensitivity depends not only on the PBA density but also on the initial state of the microgel. Such a dependence could explain that the glucose sensitivity is found to even be enhanced in the presence of salt for PBA-rich particles. This last unexpected result is encouraging for application in a physiological medium and opens a route for the design of new sensors based on microgel colloid crystals.

Experimental Section

Materials. All the reagents were purchased from Sigma-Aldrich unless otherwise noted. *N*-Isopropylacrylamide (NIPAM) was recrystallized from hexane (ICS) and dried under vacuum prior to use. *N*,*N*′-Methylenebis(acrylamide) (BIS), acrylic acid, sodium dodecyl sulfate (SDS), and ammonium persulfate (APS) were used as received. 3-Aminophenylboronic acid hemisulfate and *N*-[3-(dimethylamino)-propyl]-*N*′-ethylcarbodiimide hydrochloride (EDCI) were purchased from Acros Organics and used immediately after opening. Milli-Q water was used for all synthesis reactions, purification, and solution preparation.

PBA Derivative Monomer Synthesis. The synthesis of 3-acrylamidophenylboronic acid (APBA) was achieved by adapting the procedure previously described by Kitano and co-workers.[21] Briefly, 0.93 g (5 mmol) of 3-aminophenylboronic acid was dissolved in 15 mL of water. The pH of the solution was adjusted to 4.3 using HCl, the solution was immersed in an ice bath, and 1.15 g of EDCI (6 mmol) was added. A solution containing 0.413 mL of acrylic acid (6 mmol) in 5 mL of water was prepared separately, and the pH was adjusted to 4.3. The two solutions were mixed together under an inert atmosphere while being maintained in a water bath (at 16 °C) and allowed to stir overnight at room temperature. After filtration, the product was extracted with ethyl ether, which was further removed under vacuum. The obtained crude oil was then mixed with water and the resulting mixture stirred in an ice bath, leading to precipitation of a white solid, which was then collected by filtration. A 0.26 mg portion of a white solid was obtained. Yield: 20%. ^{1}H NMR (APBA) (400.13 MHz, DMSO): δ 5.75 (1H, CH_2═CH−), 6.25 (1H, CH_2═CH−), 6.3 (1H, CH$_2$═CH−), 7.25 (1H, phenyl), 7.5 (1H, phenyl), 7.8 (1H, phenyl), 7.85 (1H, phenyl), 8.0 (2H, −B(OH)$_2$), 10.1 (1H, NH).

Microgel Synthesis. The microgels were elaborated by an aqueous free radical precipitation polymerization classically employed for the synthesis of thermoresponsive microgels and especially p-NIPAM microgels.[22] The incorporation of the PBA derivative was performed by copolymerization with APBA. Polymerization was performed in a 200 mL three-neck round-bottom flask, equipped with a magnetic stir bar, a reflux condenser, a thermometer, and an argon inlet. The initial total monomer concentration was held constant at 70 mM, and the comonomer ratio ((97.5 − *X*):2.5:*X* NIPAM:BIS:APBA) was varied according to the desired receptor concentration. NIPAM, BIS, and surfactant (1 mM) were dissolved in 48 mL of water. APBA was dissolved in 1 mL of methanol and the resulting solution added to the previous solution. The whole solution was filtered through a 0.2 μm membrane filter to remove the remaining particulate matter. It was then heated to 70 °C and thoroughly degassed with argon for at least 1 h prior to initiation. Free radical polymerization was then initiated with APS (0.15 mmol) dissolved in 1 mL of water and degassed for 5 min. The success of initiation was indicated by the occurrence of turbidity. The stirring solution was allowed to react for a period of 6 h under argon. Following synthesis, the microgels were purified by dialysis (dialysis membrane, MCWO 10000, Orange Scientific) against water (two changes per day for two weeks at 5 °C).

Characterizations. *Particle Size and Distribution.* Particle sizes in their dry state and the uniformity of the distribution were measured by electron microscopy, both in scanning mode (SEM) using a JEOL JSM-5200 microscope, operating at 25 kV, and in transmission mode (TEM) (JEOL 2000FX) at an accelerating voltage of 200 kV.

For in situ measurements, particle sizes and polydispersities were measured by photon correlation spectroscopy (PCS). Most of the measurements were performed using a Zetasizer 3000 HS (Malvern Instruments, U.K.) operating with a HeNe laser at 90°. A second instrument operating in backscattering mode (NanoS100, Malvern Instruments) was used any time it was required to perform the measurement directly on a concentrated suspension. The hydrodynamic diameters were calculated from diffusion coefficients using the Stokes−Einstein equation. All correlogram analyses were performed with the manufacturer-supplied software. The polydispersity index (PDI) is given by the cumulant analysis method. Both material characterization and the swelling behavior upon glucose recognition were investigated by this technique. To do so, 10 μL of the initial particle suspension was dispersed in 1 mL of a freshly prepared glucose-buffered solution (Tris, 2 mM), the pH of which was adjusted using a 0.1 N NaOH or 0.1 N HCl solution. Before each data collection, the sample was allowed to equilibrate for 10 min at the proper temperature. Each data point reported is an average of 3 separate size measurements, which themselves consist of 14 measurements with an approximately 15 s integration time. Only one determination was carried out when a temperature program was recorded. The sample was allowed to equilibrate for 10 min between each temperature.

Electrophoretic Mobility Measurements. Experiments were carried out using the Zetasizer 3000 HS (from Malvern Instruments) at the proper temperature. Latex electrophoretic mobilities were obtained after dilution of the microgel suspension in the buffered solution and after the mixture was allowed to equilibrate for 10 min. Each value results from at least three determinations.

Elemental Analysis. The boron content in dilute suspensions of microgels was determined by atomic emission spectroscopy equipped with a He plasma and inductive coupling (ICPAES; Varian Liberty 220). The molar percentage of boron was calculated knowing the dried mass of polymer in the solution.

Results and Discussion

Microgel Synthesis. The synthesis of p-NIPAM microgels was adapted to incorporate the PBA derivative. The usual strategy based on a precipitation polymerization is to start with a homogeneous monomer aqueous solution and then initiate the polymerization in temperature conditions where the polymer has a low affinity for the solvent.[22] This process is known to give latex dispersions with a narrow particle size distribution, owing to the homogeneous nucleation mechanism. Therefore, after initiation by a sulfate radical, the growing oligomer reaches a critical chain length, after which it collapses to become a "precursor particle". Those nuclei aggregate until they reach

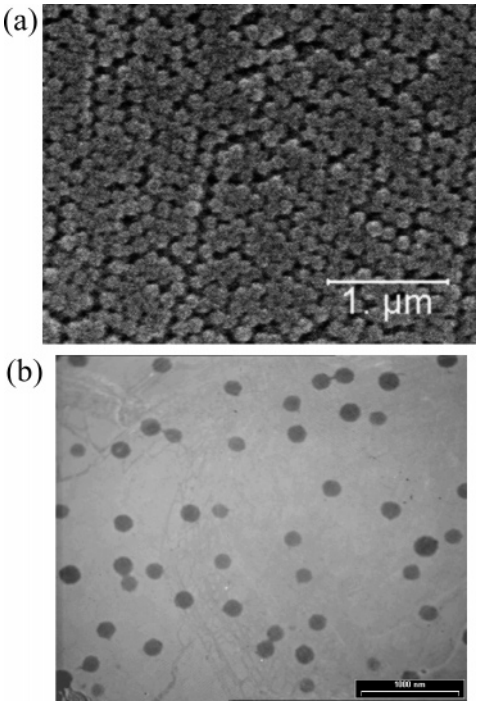

Figure 2. SEM (a) and TEM (b) pictures of APBA-functionalized microgels (15 mol %).

colloidal stability.[23] Colloidal stability is ruled by the balance between interparticular interactions: attractive forces are due to Van der Waals interactions, and repulsive forces arise from electrostatic interactions between charged spheres. In the case of microgels, the surface charge is brought by the sulfate groups resulting from the initiator. In the presence of an anionic surfactant, the particle surface charge density is enhanced and colloidal stability is reached for smaller particles. The addition of an anionic surfactant such as SDS is therefore a way to control the size of the particles.[24]

To introduce PBA functions in a random manner within the p-NIPAM microgels, we tried to adapt the previous polymerization procedure by carrying out a copolymerization with APBA under the same conditions. Therefore, the comonomer solution had to be homogeneous; i.e., APBA had to be solubilized in water. However, APBA was poorly water soluble at a pH of 7. Our first trial consisted in raising the pH of the solution, which favors the charged form of APBA. The polymerization proceeded as expected; it turned turbid a few minutes after initiation. However, the obtained particles presented a very large polydispersity. Another procedure was therefore applied, which consisted in preparing a stock solution of APBA in methanol (1 mL) and adding it to the aqueous solution of NIPAM and BIS. In this case, even after heating, the solution remained clear. It turned turbid a few minutes after initiation, and the polymerization proceeded for 6 h. In this case, we obtained relatively monodispersed particles, as measured by PCS (Figure 2). The calculated PDI is no more than 0.1, indicating that particles are formed during a short nucleation step, as previously reported for alkylacrylamide precipitation polymerization.

This procedure was therefore applied to several ratios of APBA ranging from 0 to 20 mol %. To investigate whether the APBA content influenced the final microgel diameter, we compare the particle diameters in the collapsed state, which is their state during the synthesis. As we will show later in this paper, the microgels exhibited different temperature-responsive behaviors, but all of them are in the collapsed state at 40 °C.

Table 1. Chemical Composition and Particle Size of the Various APBA-Modified Microgels

gel	[APBA] in the recipe (mol %)	[APBA] in the microgel[a] (mol %)	[BIS] in the recipe (mol %)	particle diam in the collapsed state, $d_{40°C}$[b] (nm)
a	5	4	2.5	185
b	10	13.4	2.5	165
c	10	12.4	5	180
d	15	17	2.5	170
e	20	24	2.5	165

[a] Obtained by elemental analysis. [b] Hydrodynamic diameter obtained by PCS.

As listed in Table 1, the particle diameters are all approximately 170 nm. This result seems to indicate that the presence of APBA does not affect the polymerization conditions and that the final diameter is mainly controlled by the amount of surfactant.

The various microgels were also analyzed with respect to APBA incorporation. The purified lattices were analyzed by elemental analysis, which gives the overall amount of copolymerized APBA in the whole particles. It was found that the overall amount increased with increasing APBA in the recipe (Table 1). In rich APBA synthesis, the resulting lattices were richer than the feed ratio of comonomer. This seems to indicate that APBA has a faster polymerization rate than NIPAM. Thus, in addition to the particles, NIPAM polymerization would lead to water-soluble polymers. The presence of water-soluble polymers is typical of heterogeneous polymerization and is already well-known when the consumption of the cross-linker is rapid, which is the case for the system BIS/NIPAM.[24]

The particle characterization was finally completed by electron microscopy. As those measurements were performed in the dry state, no conclusion was drawn from the measured particle sizes. Scanning electron microscopy allowed us to check the narrow size distribution of the microgels after they were deposited onto a glass substrate and subsequently dried (Figure 2a). Transmission electron microscopy was used to investigate the internal structure of the microgels. Contrary to the observation made by Hazot et al.,[20] our particles looked homogeneous and no core−shell structure could be evidenced (Figure 2b).

Volume Phase Transition Temperature (VPTT) of the Microgels. Among many other alkylacrylamide derivatives, p-NIPAM appears as a standard in the class of thermoresponsive materials: it undergoes a reversible transition from a random coil to a desolvated globular state at 32 °C, its characteristic lower critical solution temperature (LCST), due to the disruption of hydrogen bonds at high temperature, causing water to act as a poor solvent for the polymer chain.[25] When this polymer is cross-linked into a covalent gel, the gel exhibits a volume phase transition from a swollen state to a collapsed state at or near the LCST, which is called the VPTT. The width of this transition depends on the cross-linking degree.[26] To investigate the effect of APBA incorporation on the volume phase transition, the diameter of the particles was measured as a function of temperature from 10 to 40 °C (Figure 3). All the modified p-NIPAM microgels exhibit a temperature-induced phase transition. The incorporation of APBA results in a decrease of the VPTT compared to that of the native p-NIPAM particles. This effect is even more pronounced as the ratio of APBA increases. As discussed above, the volume phase transition is the consequence of lowering the affinity between water and the polymer chain through hydrogen bonding. The decrease in the VPTT indicates that the hydrophilic/hydrophobic balance of the polymer has been tuned: the polymer has a lower affinity for

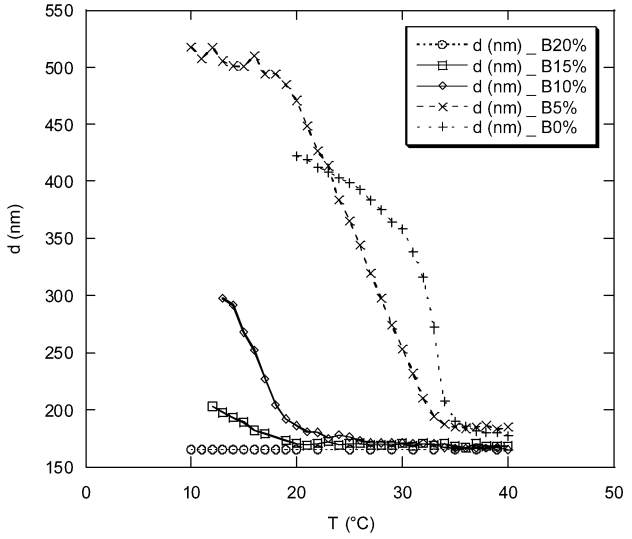

Figure 3. Diameter of p-NIPAM-*co*-APBA microgels with different APBA concentrations (pure p-NIPAM and 5, 10, 15, and 20 mol %) as a function of temperature in Tris (2 mM) at pH 8.5.

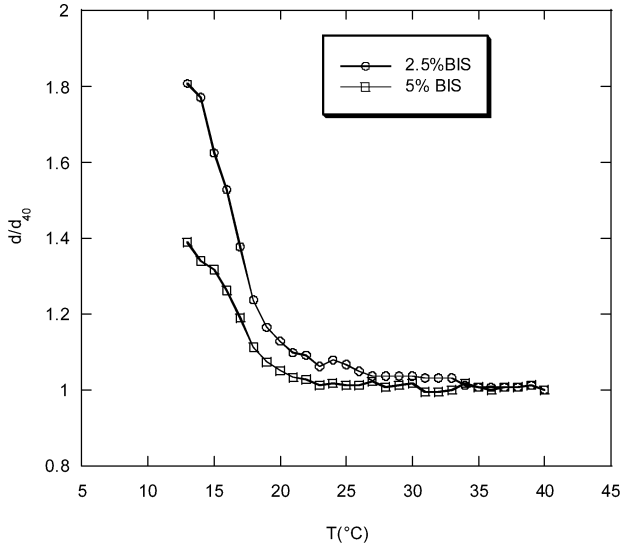

Figure 4. Swelling ratios of p-NIPAM-*co*-APBA (10 mol %) microgels with different BIS concentrations (2.5 and 5 mol %) as a function of temperature in Tris (2 mM) at pH 8.5.

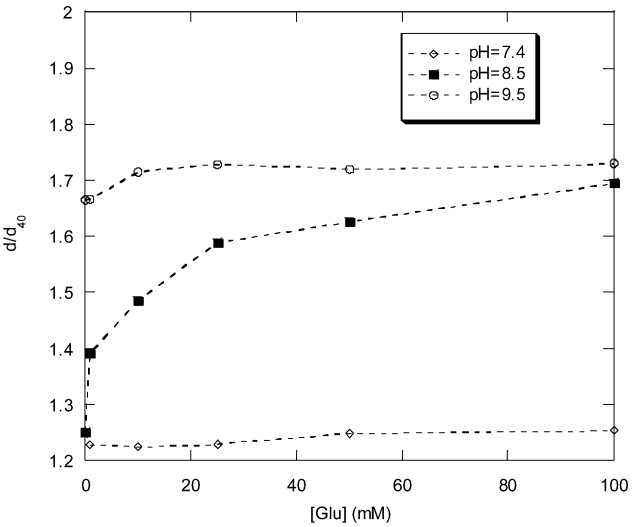

Figure 5. Swelling ratios of p-NIPAM-*co*-APBA (10 mol %) as a function of the glucose concentration in Tris (2 mM) at different pH values (7.5, 8.5, 9.5).

the solvent and an increased tendency to hydrophobic aggregation. This result is a clear indication that APBA plays the role of a hydrophobic monomer, as already observed by Kataoka et al.[13] This result was not trivial as this series of experiments was carried out at pH 8.5, where the phenylboronic acid is supposed to be present in both its neutral and its charged forms ($pK_a = 8.2$[13]). The presence of charged segments should have conferred some hydrophilicity to the polymer backbone. We conclude that the introduction of APBA into the polymer backbone strongly affects the hydrophobicity of the polymer. This is also confirmed by the decrease in the swelling ratio as the APBA content increases, which shows that the polymer chain does not expand in the solvent.

We also investigate the influence of the cross-linking density on the volume phase transition at a constant APBA ratio. For better clarity, we plot the swelling ratio versus temperature. Here, the swelling ratio is defined as the ratio between the diameter at the proper temperature and the diameter in the collapsed state, i.e., at 40 °C since all the microgels were found to be in the collapsed state at this temperature. As shown in Figure 4, the VPTT remains the same, but increasing the cross-linking density strongly affects the swelling ratio. The extent of gel swelling is limited by the presence of cross-links.

This last experiment also provides an indirect proof that the cross-linking density increases by increasing the feed concentration of BIS during the synthesis. We are therefore able to produce monodispersed microgels, with a size controlled by the polymerization conditions and especially the amount of surfactant and a well-defined chemical composition, directly related to the feed concentration during the synthesis.

Microgel Swelling in the Presence of Glucose. The swelling behavior upon glucose exposure was investigated. The hydrodynamic diameter of PBA-modified microgels was again measured after immersion in a medium containing glucose. A first set of experiments was carried out at room temperature at several glucose concentrations and different pH conditions. Figure 5 shows that PBA-modified particles were sensitive to the presence of glucose at pH 8.5, i.e., at pH conditions close to the pK_a of APBA. The absence of a response at a pH of 7.4 can be explained by the absence of any complexation at this pH (the complex between the phenylboronic acid and glucose is unstable). At a pH of 9.5, glucose complexation occurs, but does not induce any gel volume change. The gel is also more swollen in the absence of glucose at this pH than at pH 8.5. All these observations tend to indicate that the mechanism of swelling is governed by the Donnan potential. When the boronic acid derivative is already in its charged form when uncomplexed (pH 9.5), glucose complexation does not induce any charge density increase and the swelling state is constant. At pH 8.5, the complexation induces a shift in the charge equilibrium (the pK_a is lowered) which causes counterion osmotic pressure and gel swelling. Interestingly, the charge density is proportional to the glucose concentration, so the microgel diameter increases when the glucose concentration increases. This result is in perfect agreement with previously reported monolithic gels based on the same mechanism.[10]

To complete the understanding of the swelling mechanism, electrophoretic mobility measurements were carried out as a function of pH. These measurements aimed to seek the surface charge of the particle, although the relation between surface charge and electrophoretic mobility is far from simple due to the possibility that electroosmotic flow occurs within the particle when an external field is applied.[27,28] Figure 6 shows that the microgels exhibit two types of behavior versus pH. In both cases,

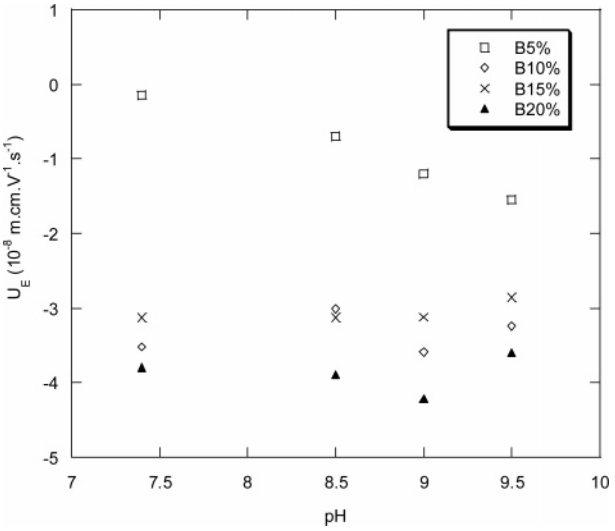

Figure 6. Electrophoretic mobility as a function of pH for APBA-modified microgels (5, 10, 15, and 20 mol %) in Tris (2 mM) at 25 °C.

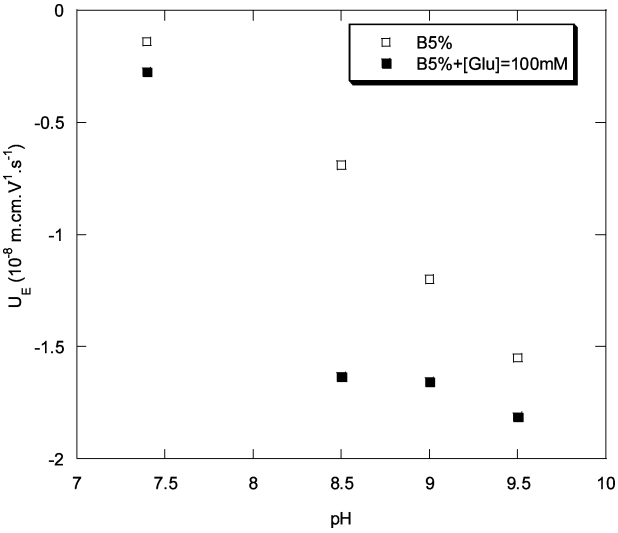

Figure 7. Electrophoretic mobility as a function of the glucose concentration for 5 mol % APBA-modified microgels in Tris (2 mM) at 25 °C.

the mobility presents a negative value due at least to the presence of sulfate groups (originating from APS) and also to the presence of boronate groups. This last contribution is clearly identified for PBA-poor microgels, which exhibit pH dependence. When the pH was raised from 7.5 to 9.5, the absolute value of the mobility increased from 0.41×10^{-8} to 1.5×10^{-8} m cm V^{-1} s^{-1}, indicating that the surface charge strongly increases in this area. This can be easily explained by the equilibrium between the charged and the uncharged forms of boronic acid. However, this behavior was not observed any more for PBA-rich microgels ([APBA] higher than 10 mol %) as the mobility remains almost constant versus pH. Here, the softness of the microgels can explain the difference of the behavior. Indeed, PBA-rich microgels were in a collapsed state at 25 °C, whereas PBA-poor microgels were in a swollen state. Therefore, it seems that the two types of electrophoretic behavior are mostly related to the swollen state. When particles are in a collapsed state, it is expected that the surface charge density is higher than in the swollen state. Therefore, the mobility should be higher. Such an increase has already been observed for p-NIPAM microgels, when the temperature is raised above the LCST.[29] We assess that the very high charge density in the case of collapsed particles hides any variation arising from the hydroxylation of the boronic group, but does not exclude the presence of APBA near the surface.

The electrophoretic behavior was then investigated versus the glucose concentration at pH 8.5 (Figure 7). It was found that the mobility increased (absolute value) when the glucose concentration increased. This shows that the surface charge density also increases when glucose increases, which is in agreement with glucose complexation inducing a shift in the equilibrium between charged and uncharged forms of the boronic group. Therefore, this is another indication that the swelling mechanism is related to the modification of the charge density and counterion osmotic pressure.

Importance of the Initial State: Influence of the APBA Content on the Response to Glucose. The influence of the density of receptors on the sensitivity to glucose was investigated, first by measuring the evolution of the particle diameter as a function of the glucose concentration for the different particles. It is expected that the swelling response will increase with increasing receptor density. However, during the course of these experiments, we noticed that, at a constant glucose concentration, the reached swollen state was also strongly dependent on the initial temperature of the suspension. This was especially true for PBA-rich microgels. This dependence is illustrated in Figure 8a, which shows the evolution of the diameter versus temperature for rich APBA particles ($x = 15\%$) in the presence of 100 mM glucose, starting the temperature scan at two different temperatures: 10 and 20 °C. It can be clearly evidenced that the swelling is much higher when the particles are initially at low temperature. It was verified that the difference was not due to any kinetic effect. This behavior could be related to the temperature itself or to the initial state of the microgel before glucose addition. Indeed, it should be noted that the microgels are fully collapsed at 20 °C without glucose, whereas they are slightly swollen at 10 °C. The same experiment was carried out on a PBA-poor microgel ($x = 5\%$) (Figure 8b). The transition temperature of this microgel is higher than that for the gel with $x = 15\%$; therefore, the initial state of the gel is swollen at both initial temperatures. We observed in this case that the gel response to glucose did not depend any more on the initial temperature. We conclude that the microgel response is mainly governed by the initial swollen state of the gel and not directly related to the amount of receptors.

A first possible explanation is that glucose cannot diffuse inside the particle when it is initially collapsed. In this case, only a small part of the receptors can link to the target molecule. A slight swelling is necessary to allow permeation and molecular diffusion in the inside of the particle. In such conditions, all the receptors can be reached, leading to a much higher swelling degree (Figure 9).

Another parameter is also related to the initial swollen state of the particle: the local receptor concentration depends on the swelling degree. We estimate that, for $x = 15\%$, the local receptor concentration decreases from 0.5 mol L^{-1} in the collapsed state to 0.025 mol L^{-1} in the swollen state. The complexation equilibrium is ruled by the ratio between host and guest molecules. At constant guest concentration, the complex form will be favored when the host concentration is lower. Therefore, the swollen state of the microgel will displace the equilibrium toward the complex state, which would help the particle to swell further in the presence of glucose.

Swelling in the Presence of an Electrolyte. As discussed above, the mechanism for swelling upon glucose recognition seems to be related to an electrostatic effect. Such a statement

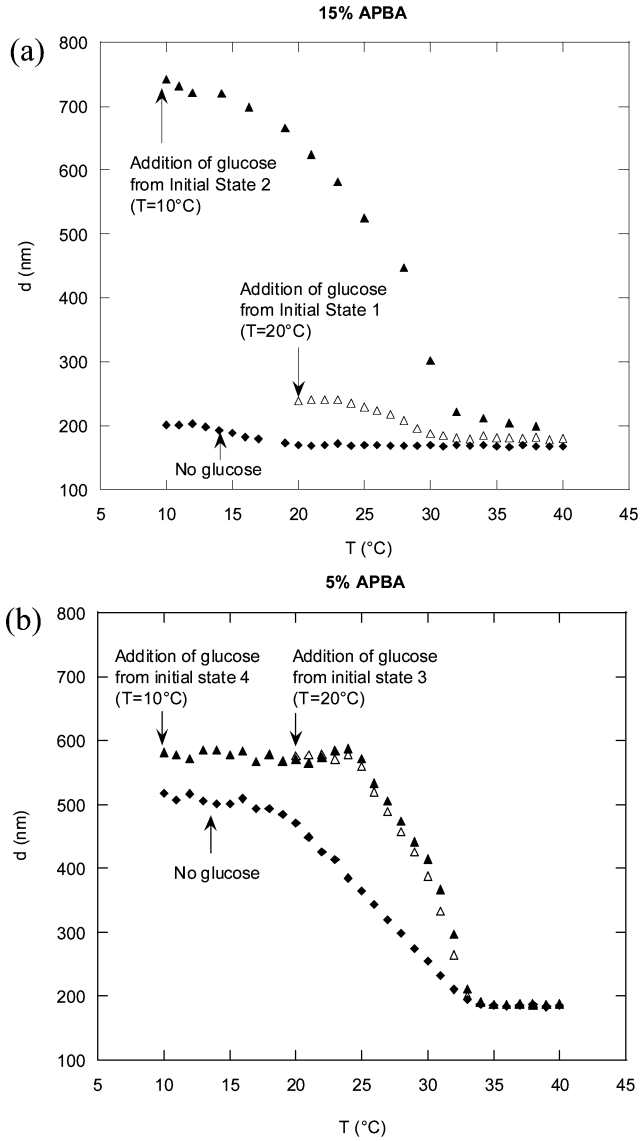

Figure 8. Diameter versus temperature in the absence of glucose (full tilted squares) and in the presence of 100 mM glucose (in Tris (2 mM) at pH 8.5), starting from 10 °C (full triangles) and 20 °C (open triangles): (a) for 15% APBA particles; (b) for 5% APBA particles.

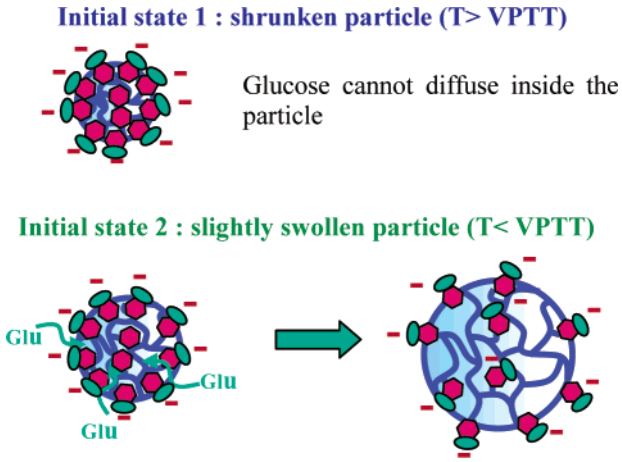

Figure 9. Schematic representation of the microgel swelling upon glucose addition, showing the importance of the initial state.

would discourage anyone to use these microgel systems in a physiological medium where the ionic strength is around 0.15 mol L^{-1}, meaning that all electrostatic effects are screened.

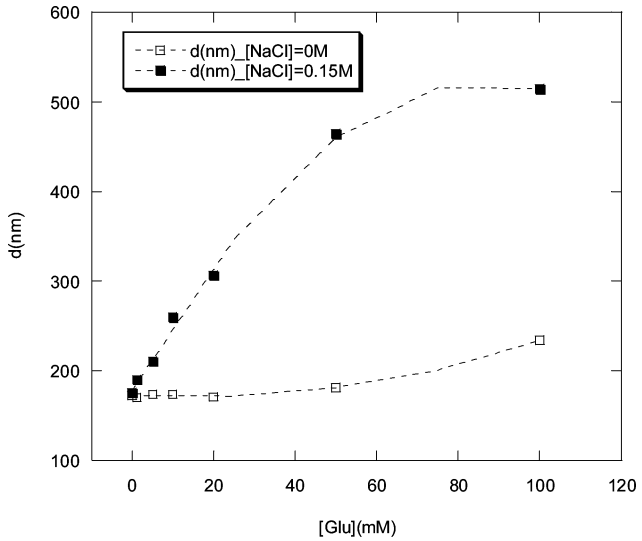

Figure 10. Diameter versus glucose concentration for 15% APBA microgels (in Tris (2 mM), pH 8.5, $T = 25$ °C): in the absence of salt (open squares) and in the presence of [NaCl] = 150 mM (full squares).

However, glucose complexation with phenylboronic acid also modifies the hydrophilicity of the polymer chain, which means that the interaction parameter between the polymer chain and the solvent will increase. It is thus expected that the VPTT of the microgels increases in the presence of glucose, as already observed by Kataoka et al.[13]

The swelling response to glucose of our microgels in the presence of salt was investigated. The results obtained for a 15% APBA-substituted microgel are shown in Figure 10. Surprisingly, the swelling ratio was higher in the presence of salt than with a low ionic strength.

Again, we notice that the two initial states (without glucose, with and without salt) of the microgels are different. The microgels seem to be slightly swollen in the presence of salt, which could explain that they have more ability to further swell in the presence of glucose. A confirmation of this behavior is obtained by measuring their evolution versus temperature in different glucose and salt concentrations (Figure 11a). The presence of salt allows a slight swelling of the microgels. It should be noted that no data are plotted above 27 °C because the particles display aggregation above this temperature. Indeed, in the presence of salt and when they are in the collapsed state, the colloidal stability is not achieved: the particles tend to attract each other due to van der Waals forces, and no electrostatic repulsion helps them to disperse when salt is present.

When glucose is added to the suspension, the particles swell more in the presence of salt than in the absence of salt. They remain swollen over a wide temperature range because the phenylboronic acid−glucose complex gives them a hydrophilic character which shifts the VPTT to higher temperatures (around 40 °C). It should be mentioned that the swelling behavior in the presence of glucose and in the presence of salt did not seem to depend on the initial temperature as seen at low electrolyte concentration. This might be explained by the fact that this dependence only appeared when the particle was initially highly collapsed, which was not the case in the presence of salt.

Again the behavior of PBA-poor microgels can be distinguished from that of PBA-rich ones. It can be observed in Figure 11b that the microgels bearing 5% APBA are less swollen in the presence of salt than without. On this graph, aggregation in the presence of salt occurs above 30 °C, when the particles are sufficiently collapsed to generate van der Waals attraction. The

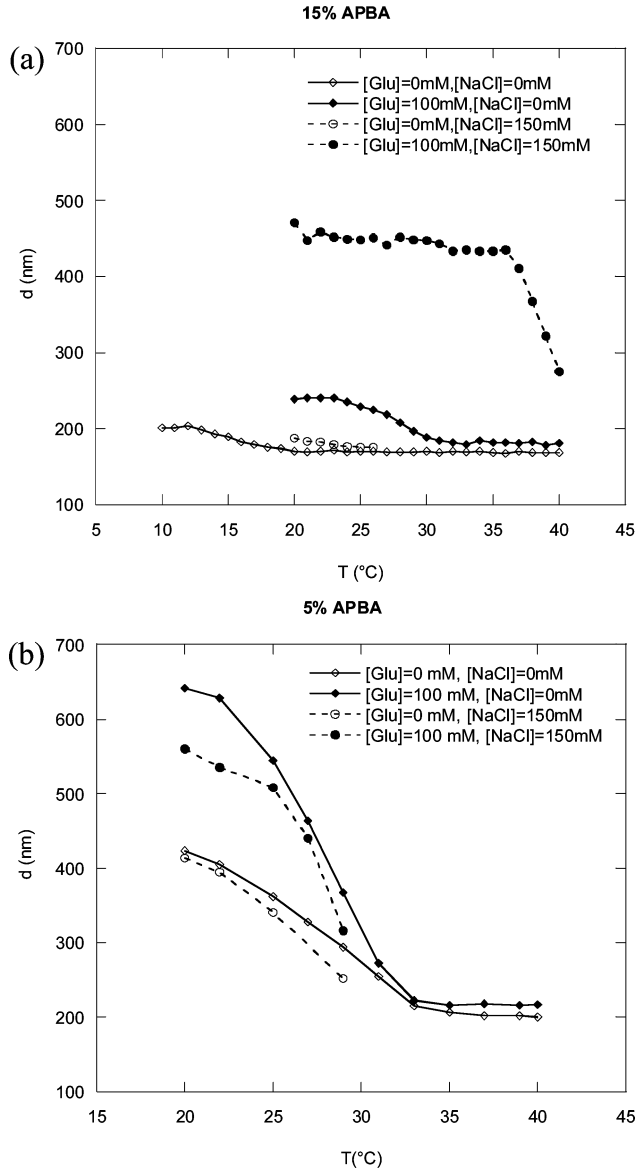

Figure 11. Diameter versus temperature for (a) 15% APBA particles and (b) 5% APBA (in Tris (2 mM), pH 8.5, starting from 20 °C) in the absence of salt (tilted squares), without glucose (open tilted squares) and with glucose (100 mM) (full tilted squares), and in the presence of [NaCl] = 150 mM (circles), without glucose (open circles) and with glucose (100 mM) (full circles).

whole behavior is in perfect agreement with the classical behavior of charged microgels. The presence of an electrolyte will decrease the swelling of a weak polyelectrolyte gel due to the Debye screening effect. PBA-poor microgels behave as hydrophilic particles, and their swelling is mainly governed by the increase in the charged density upon glucose complexation. This is consistent with Asher's results,[10] who probably worked with poorly modified hydrogel films. It is worth noting that these authors overcame the problem by modifying the cross-linking density using the formation of a supramolecular bisbidentate glucose−boronic acid complex.[11] However, their hydrogels shrink instead of swell upon glucose recognition, which excludes any application like insulin delivery.

Contrary to PBA-poor microgels, rich ones present an enhancement of glucose sensitivity in the presence of salt. Their initial hydrophobic character seems to explain that swelling upon glucose complexation is based on a mechanism different from that of PBA-poor particles. Indeed, their swelling seems to be

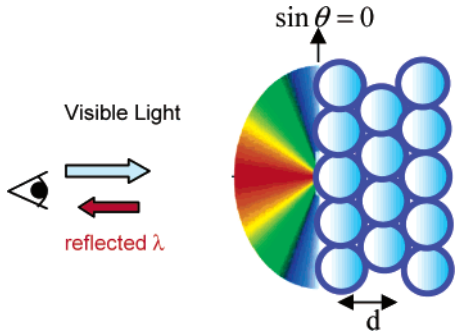

Figure 12. Schematic representation of a colloidal crystal composed of monodispersed microgels. Light diffraction results from Bragg's law: $m\lambda = 2nd \sin\theta$, where m is the diffraction order, n the refractive index of the surrounding medium, d the interparticle distance, and θ the observation angle.

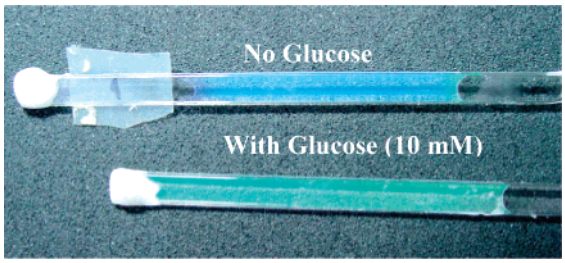

Figure 13. Photograph of the rectangular capillaries filled with the colloidal crystals composed of 5% APBA microgels without glucose and in the presence of glucose (10 mM) in Tris (2 mM), pH 8.5.

mainly due to the change of hydrophilicity upon glucose complexation, which tunes the polymer−solvent interaction parameter. This principle should not be affected by the presence of an electrolyte. When combined with an initial favorable state (slight particle swelling in the presence of salt), this leads to the enhancement of swelling upon glucose complexation in the presence of salt at physiological concentration. This last result is encouraging for application purposes such as glucose sensing or insulin delivery. Moreover, PBA-rich particles would be recommended to increase glucose sensitivity: the addition of 100 mM glucose may induce a variation of 15 times (compared to 2.5 for PBA-poor microgels).

Application to Glucose Sensing. The previously described microgels are monodispersed particles. When their volume fraction is increased above a critical volume fraction, they reach a dense phase and assemble into ordered colloidal crystals, which have the ability to diffract light according to Bragg's law.[30] When the size of the particles is correctly chosen, visible light is diffracted and the colloidal crystals appear colored (Figure 12). The diffracted color depends on the interparticle distance, which itself depends on the particle size. Therefore, in the case of microgels, the color depends on their swollen state.

To obtain colloidal crystals, we increase the volume fraction of a suspension of 5% APBA particles by centrifuging the particles at a speed of 13000 rpm for 1 h. A colored bottom phase was observed. The supernatant water was removed. The viscous bottom phase, containing the highly concentrated particles, was heated above the volume phase transition temperature, yielding a turbid liquid suspension which was sucked by capillarity into a 200 μm thick rectangular glass capillary. The liquid was allowed to cool. At room temperature, below the VPTT, the particles swelled again and turned back into the blue-colored compact state corresponding to a colloidal crystal. The same experiment was performed with adding glucose to

the suspension before centrifugation. The microgel-surrounding medium contained 10 mM glucose. The resulting colloidal crystal appeared green, indicating an increase in the particle diameter (Figure 13). Therefore, we show that the color displayed by the colloidal crystal is directly linked to the glucose concentration. A colorimetric glucose sensor can be built from the assembly of the previously described monodispersed microgels.

Conclusion

Monodispersed p-NIPAM submicrometric microgels modified with APBA were synthesized by precipitation polymerization. A well-defined size was obtained through the polymerization conditions by controlling the amount of surfactant, and a good control over their chemical composition was also achieved through the feed concentration. Such particles displayed both thermoresponsive properties and glucose sensitivity. The VPTT was found to depend on the amount of PBA derivative incorporated into the particle. The response to glucose was proportional to the glucose concentration at pH conditions close to the pK_a of the PBA derivative. These submicrometric particles were found to behave like previously developed monolithic hydrogels with similar chemical composition.

An original result was found through the dependence of the glucose response on the initial state of the microgel. It was shown, in particular, that both the initial temperature and the ratio of APBA in the particle affected the swelling ratio of the particle upon glucose addition. More generally, highly collapsed hydrophobic microgels could not swell fully in the presence of glucose, probably due to the impossibility for the molecule to penetrate inside the microgel. A slight initial swelling was necessary to enhance the microgel swelling capability upon glucose recognition. This result might be of importance as it could be a general feature for other functionalized microgels aimed at swelling upon molecular recognition.

At physiological salinity (I = 0.15 M), the PBA-rich microgels were slightly swollen in their initial state and their response to glucose was found to be enhanced. Such particles exhibited a high sensitivity to glucose in conditions close to physiological ones. We demonstrate that they may be used as building blocks for the design of new colorimetric sensors. Improvements to this system will be turned in the direction of lowering the operating pH and increasing the volume phase transition temperature to design new sensors or particles responding at physiological conditions and possibly at body temperature.

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References and Notes

(1) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820.
(2) Hisotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392.
(3) Ishihara, K.; Kobayashi, M.; Ishimaru, N.; Shinohara, I. *Polym. J.* **1984**, *16*, 625.
(4) Ishihara, K.; Matsui, K. *J. Polym. Sci., Polym. Lett. Ed.* **1986**, *24*, 413
(5) Albin, G. W.; Horbett, T. A.; Ratner, B. D. *J. Controlled Release* **1985**, *2*, 153.
(6) Albin, G. W.; Horbett, T. A.; Miller, S. R.; Ricker, N. L. *J. Controlled Release* **1987**, *6*, 267.
(7) Hassan, C. M.; Doyle, F. J.; Peppas, N. A. *Macromolecules* **1997**, *30*, 6166.
(8) Miyata, T.; Jikihara, A.; Nakamae, K.; Hoffman, A. S. *Macromol. Chem. Phys.* **1996**, *197*, 1135.
(9) Miyata, T.; Jikihara, A.; Nakamae, K.; Uragami, T.; Hoffman, A. S.; Kinomura, K.; Okumura, M. *Advanced Biomaterials in Biomedical Engineering and Drug Delivery Systems*; Springer: Tokyo, 1996; pp 237−238.
(10) Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 3322.
(11) Alexeev, V. L.; Sharma, A. C.; Goponenko, A. V.; Das, S.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N.; Asher, S. A. *Anal. Chem.* **2003**, *75*, 2316.
(12) Lee, Y.J.; Pruzinsky, S.A.; Braun, P.V. *Langmuir* **2004**, *20*, 3096−3106.
(13) Kataoka, K.; Miyaski, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *37*, 12694.
(14) Boeseken, J. *Adv. Carbohydr. Chem.* **1949**, *47*, 189. Aronoff, S.; Chen, T.; Cheveldayoff, M. *Carbohydr. Res.* **1975**, *40*, 299.
(15) Lorand, J. P.; Edwards, J. D. *J. Org. Chem.* **1959**, *24*, 769.
(16) Matsumoto, A.; Ikeda, S.; Harada, A.; Kataoka, K. *Biomacromolecules* **2003**, *4*, 1410.
(17) Matsumoto, A.; Yoshida, R.; A., Kataoka, K. *Biomacromolecules* **2004**, *5*, 1038.
(18) Matsumoto, A.; Kurata, T.; Shiino, D.; Kataoka, K. *Biomacromolecules* **2004**, *4*, 1502.
(19) Tanaka, T.; Fillmore, D. J. *J. Chem. Phys.* **1979**, *70*, 1214.
(20) Hazot, P.; Delair, T.; Ellaissari, A.; Chapel, J. P.; Pichot, C. *Colloid Polym. Sci.* **2002**, *280*, 637.
(21) Kitano, S.; Koyama, Y.; Kataoka, K.; Okano, T., Sakurai, Y. *J. Controlled Release* **1992**, *19*, 161.
(22) Pelton, R. H.; Chibante, P. *Colloids Surf.* **1986**, *20*, 247.
(23) Pelton, R. *Adv. Colloid Interface Sci.* **2000**, *85*, 1.
(24) Wu, X.; Pelton, R. H.; Hamielec, A. E.; Woods, D. R.; McPhee, W. *Colloid Polym. Sci.* **1994**, *272*, 467.
(25) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(26) McPhee, W.; Tam, K. C.; Pelton, R. *J. Colloid Interface Sci.* **1993**, *156*, 24.
(27) Oshima, H. *J. Colloid Interf. Sci.* **1994**, *163*, 474.
(28) Rasmusson, M.; Vincent, B.; Marston, N. *Colloid Polym. Sci.* **2000**, *278*, 253.
(29) Hoare, T.; Pelton, R. *Polymer* **2005**, *46*, 1139.
(30) Lyon, L. A.; Debord, J. D.; Debord, S. B.; Jones, C. D.; McGrath, J. G.; Serpe, M. J. *J. Phys. Chem. B* **2004**, *108*, 19099.

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