Thermoresponsive Properties of Sugar Sensitive Copolymer of N-isopropylacrylamide and 3-(Acrylamido) phenylboronic Acid

Koichiro Shiomori[1,2], Alexander E. Ivanov[1], Igor Yu. Galaev[1], Yoshinobu Kawano[2] Bo Mattiasson*[1]

[1]Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

Fax: 46-46-222-4713; E-mail: bo.mattiasson@biotek.lu.se

[2]Department of Applied Chemistry, Miyazaki University, Miyazaki 889-2192, Japan

Summary

The copolymer of N-isopropylacrylamide and 3-(acrylamido) phenylboronic acid (82:18, $M_n = 47000$ g/mol) was prepared by free radical polymerization. The copolymer showed typical thermal precipitation behavior in aqueous solutions, its precipitation temperature (T_P) being increased from 23 ˚C to 32 ˚C by increasing pH from 6.5 to 9.7, due to ionization of the phenylboronate units. The pK_a was evaluated as 8.9±0.1 from the effect of pH on T_P. At pH>9, i.e. in the anionic form of the copolymer, T_P was affected by very low concentration of glucose (5.6 μM, ΔT_P = 1-1.5 ˚C), due to the complex formation with a high binding constant. At higher concentration of polyols (560 μM, pH>8) the increase of T_P was maximal for the copolymer complexes with fructose (7-10 ˚C) and decreased in the order: fructose>glucose mannitol>pentaerythritol>galactose>Tris >glycerol. Di- and oligosaccharides (lactose, sucrose and dextran) caused a slight increase of T_P at pH 7.5-8.7 while no effect was observed at pH>9. Isothermal dissolution of the copolymer suspension in water (27 ˚C, pH 8.5) was possible in the presence of fructose or mannitol but required higher concentrations (1.4-3.6x10^3 μM) as compared to those which enabled the shift of T_P in the soluble copolymer. The dissolution rate increased with fructose concentrations.

Introduction

Stimuli-responsive polymers, which undergo strong conformational change and phase transition when a factor in the environment, such as pH, temperature, light, ionic strength and so on, is slightly changed, have been paid much attention in recent time.[30] Stimuli-responsive polymers are studied for the development of biomimetic actuators, immobilized biocatalysts, drug delivery system, thermoresponsive surfaces and for bioseparation.[1,3,5,6,17,21-28] The structure and behavior of stimuli-responsive polymer in aqueous solution is also quite interesting in connection with biopolymers playing an important role in life, such as proteins, enzymes, nucleic acid, and polysaccharides.[2,3] One of intensively studied stimuli-responsive polymers is poly-N-isopropylacrylamide and its derivative. Its thermal precipitation temperature (T_P) in aqueous solution is 32 °C.[29]

By incorporation of molecular recognition ligands into either backbone or side-chain of stimuli-responsive polymers, the applications for chemical sensors and affinity separation of biomolecules by the molecular recognition between ligand of polymer and target molecules have been described.[4] Affinity precipitation[1,5,17,23-27] and affinity aqueous two-phase partitioning[5] are quite powerful methods for affinity separation of proteins. These methods are based on complex formation of the affinity ligand of the polymer with the target protein, phase transition of the polymer triggered by stimuli, and finally desorption of the purified protein from the polymer.

Phenylboronic acid is a well known ligand employed in chromatographic studies of sugars and glycoproteins.[7,15,16] These chromatographic applications have been usually based on the complex formation between diol groups of the target molecules and boronic acid groups of the support material.[7,16] Based on the same interaction, many studies have been done with the boronic acid-functionalized

thermoresponsive copolymers employed for glucose sensing,[8,9,10,19] drug delivery system[20] and affinity separation.[11,18,28] At the same time, interaction of the boronate-containing copolymers with sugars and polyols of varied nature was not studied in a comparative way although different reactivities of these compounds with immobilized phenylboronic acid have been demonstrated.[7] Binding of sugars and polyols to the phenylboronic ligands was studied qualitatively, with no attempt to estimate the equilibrium constants and propose binding mechanisms. In the present paper we attempt to fill the above gaps and evaluate sensitivity of T_P of the copolymers at low concentration of various interacting solutes. Isothermal dissolution of thermally precipitated copolymers promoted by the complex formation was also studied.

Experimental Part

Materials

3-Aminophenylboronic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acryloyl chloride was purchased from Fluka Chemie AG (Buchs, Switzerland). N-Isopropylacrylamide (NIPAAM) was obtained from Eastman Kodak Co. (New York, USA). 2,2 -Azobis(2-metylpropionitrile) (AIBN) was purchased from Acros Organics (Geel, Belgium). 1,4-Dioxane, diethylether, sodium hydroxide, sodium chloride, hydrochloric acid, fructose, glucose, sucrose, raffinose, pentaerythritol and polyvinyl alcohol (M_r 30,000-70,000; hydrolysis degree 87-90 %) were analytical grade reagents from Sigma Chemical Co. (St. Louis, MO, USA). Dextran (M_r 10,000) was purchased from Pharmacia (Uppsala, Sweden). Galactose and tris(hydroxymethyl)aminomethane (Tris) were obtained from Merck (Darmstadt, Germany).

Synthesis of N-acryloyl-3-aminophenylboronic acid (AAPBA)

3-Aminophenylboronic acid (20 mmol) was dissolved in 40 ml 2M NaOH (80 mmol) and cooled in an ice bath. Acryloyl chloride (3.2 ml, 40 mmol) was added dropwise to the solution at intensive magnetic stirring for 15 min. Hydrochloric acid (2 M) was added dropwise to the reaction mixture to adjust pH to ca. 1. The precipitate of the product was separated by filtration on a sintered glass filter (Scott, Duran, No. 3) and then washed on the filter by cold distilled water (50 ml). The precipitate was dissolved in 80 ml of distilled water on heating to ca. 60 ˚C. At that time, the residual insolubilities were filtered off. The product was obtained as light violet needles by crystallization of the solution for overnight in a refrigerator. The crystals were filtered off on paper filter (Munktell, No. 3), washed with cold distilled water and dried under vacuum in a desiccator over dry calcium chloride. The yield of N-acryloyl-3-aminophenylboronic acid (AAPBA) was 35%. The structure of AAPBA was confirmed by the 500MHz ^{1}H-NMR (Bruker DRX500) spectrum. ^{1}H-NMR (DMSO-d6): δ = 5.73 (dd, 1H, vinyl CH), 6.24, 6.45 (dd, 1H each, vinyl CH_2), 7.28, 7.50, 7.82, 7.88 (dd, d, d, s, 1H each, ArH), 8.02 (s, 2H, $B(OH)_2$), 10.07 (s, 1H, NH).

Copolymerization of N-acryloyl-3-aminophenylboronic acid (AAPBA) with N-isopropylacrylamide (NIPAAM)

NIPAAM (2.14 g, 19 mmol), AAPBA (0.19 g, 1 mmol) and AIBN (20 mg, 0.1 mmol) were dissolved in 20 ml of 1,4-dioxane. The reaction mixture was placed in a two-necked flask with cooling condenser. Free radical polymerization was started by heating the reaction mixture to 70 ˚C under nitrogen bubbling and carried out for 6 h. The obtained solution having high viscosity was added dropwise to 150 ml of diethyl ether for precipitation of the copolymer and its separation from the unreacted monomers. The

precipitate was collected by filtration with paper filter, washed with diethyl ether, and dried in air and under vacuum. The precipitation is tinged with pale rose red. The yield of the copolymer was 67 %.

Characterization of (NIPAAM-AAPBA)-copolymer

^{1}H-NMR spectrum of the copolymer was recorded with a 500 MHz Bruker DRX500 spectrometer using DMSO-d6 as a solvent. Two parallel samples were used for the determination of phenylboronate group content. Size-exclusion HPLC of the copolymer was performed on a Shodex GPC KF-804L column using a detection at 210 nm. The column was eluted with tetrahydrofran at 0.5 ml/min. The column was calibrated with standard polystyrenes. All analyses of HPLC were performed at ambient temperature. A size-exclusion chromatogram of the copolymer showed a wide molecular weight distribution with $M_n = 4.7 \times 10^4$ g/mol, $M_w = 18.3 \times 10^4$ g/mol and peak molecular weight $M_p = 22.4 \times 10^4$ g/mol.

Thermoresponsive properties of polymers

Polymers were dissolved at 1.0 mg/ml in 0.1 M NaCl aqueous solution either without or with sugar at given concentration. Optical transmittance of the polymer aqueous solutions at various temperatures was measured at 500 nm using a spectrophotometer (Ultrospec 1000, Pharmacia Biotech). The pH of (NIPAAM-AAPBA)-copolymer solution was changed by adding a small amount of NaOH aqueous solution. The precipitation temperature (T_P) was defined as the temperature at 50% of optical transmittance.

Isothermal dissolution of the precipitated (NIPAAM-AAPBA)-copolymer by the addition

of sugars

(NIPAAM-AAPBA)-copolymer was dissolved in 0.1 M glycine buffer at 0.25 mg/ml. The polymer solution was stored at a temperature slightly above T_P. After 20 min., a small amount of sugar solution was added to the solution. The absorbance change of the solution was measured at fixed intervals. The ratio of absorbances prior to and after the addition of sugar was defined as the residual ratio of the copolymer, *X*.

Results and Discussion

Characterization of (NIPAAM-AAPBA)-copolymer

^{1}H-NMR spectrum of (NIPAAM-AAPBA)-copolymer is shown in **Figure 1**. Assignment of protons of the copolymer was done according to references[3,17] and ^{1}H-NMR spectrum of AAPBA. The content of phenylboronic acid in the copolymer was 18 mol-%, which was determined from the peak integration ratio between protons of phenylboronic acid moiety (6.8-8.0 ppm) and methine proton of NIPAAM (3.8 ppm). This phenylboronic acid content is larger than the molar ratio of AAPBA to NIPAAM used for the polymerization. When (NIPAAM-AAPBA)-copolymer was synthesized at different molar ratio of AAPBA to NIPAAM, phenylboronic group content was also larger than that in the starting reaction medium probably due to somewhat higher reactivity of AAPBA than that of NIPAAM.

The number- and weight-average molecular mass (M_n and M_w) of the (NIPAAM-AAPBA)-copolymer were 47,000 g/mol and 183,000 g/mol, respectively, and M_w/M_n was 3.9, as determined from a SEC chromatogram. The molecular mass at peak-top of SEC (M_p) was 224,000 g/mol.

Thermal precipitation property of (NIPAAM-AAPBA)-copolymer

(NIPAAM-AAPBA)-copolymer exhibits a typical thermal precipitation behavior (**Figure 2**). The optical transmittance of the copolymer solution sharply decreased at a specific temperature, which increased with an increase in pH. The increase would be due to the ionization of phenylboronate group from an uncharged trigonal form, which is hydrophobic, to a charged boronate anion, which is a hydrophilic (**Figure 3**). The thermal precipitation of poly(NIPAAM-AAPBA) at pH 9.7, where almost all phenylboronate groups would be in a form of charged boronic anion, was almost the same as that of poly-NIPAAM. This precipitation behavior of poly(NIPAAM-AAPBA) is considered as follows; the introduction of non-charged hydrophobic phenylboronate group into the polymer chain of poly-NIPAAM causes the decrease of the phase transition temperature; the ionization of phenylboronic group by increasing pH counterweights hydrophobicity, hence charged poly(NIPAAM-AAPBA) precipitated at nearly the same temperature as that of poly-NIPAAM.

The effect of pH on T_P is illustrated in **Figure 4**. Large change of T_P was observed between pH 8.8 and 9.1 indicating that the *pK*a of phenylboronate group of poly(NIPAAM-AAPBA) happens to be around this pH region. This *pK*a value suggested from Figure 4 is almost the same as those of phenylboronate group reported in literatures: phenylboronic acid for 8.92,[12] 3-(propionamido) phenylboronic acid for 8.6,[8] m-aminophenylboronic acid bounded to aminoethyl polyacrylamide beads for 9.2.[13]

Effect of sugars on the thermal precipitation of poly(NIPAAM-AAPBA)

The thermal precipitation of poly(NIPAAM-AAPBA) was measured in the presence of glucose. The thermal precipitation at pH 6.4-6.5, where almost all of boronic acid

groups would be in the uncharged form, was unaffected by glucose (**Figure 5a**). This indicates that the uncharged form of boronic acid does not interact with glucose.

The thermal precipitation of the copolymer at pH 9.3-9.5, where boronic acid group would be in the charged form, was shifted to higher temperatures by the addition of extremely small amount of glucose, 5.6 μM (1.0×10^{-3} mg/ml) (**Figure 5b**). The thermal precipitation temperature increased with an increase in the glucose concentration. Probably, the increase in T_P is caused by an increase in hydrophilicity of the copolymer due to the formation of the complex between phenylboronic acid group of the copolymer and glucose (**Figure 6**).

The effect of glucose addition at such very low concentrations on the thermal precipitation of poly(NIPAAM-AAPBA) would suggest a large association equilibrium constant between charged phenylboronate anion and glucose. Let us roughly evaluate its value. At pH 9.3-9.5 and 31.5 ˚C almost all NIPAAM-AAPBA copolymer is precipitated. In the presence of 11 μM glucose under the same conditions the copolymer is almost completely dissolved (see Figure 5b, white triangle). This means that most of the polymeric chains of the sample were bound to at least one molecule of glucose and acquired higher hydrophilicity. Note that concentration of glucose in this experiment was ca. 130-fold lower than that of boronate groups (1.42 mM, 1 mg-copolymer/ml) that obtained by a simple calculation using phenylboronate content in the copolymer. On the other hand, the number of phenylboronate groups per copolymer molecule can be calculated as ca. 70 for the chains of M_n = 47,000 g/mol, and 320 for the chains of M_P = 224,000 g/mol. Apparently that some shorter chains can bind one or no molecules of glucose while some longer ones 2-3 or more molecules, the equilibrium concentration of non-bound glucose [G] being much lower than the starting 11 μM. The ratio between equilibrium concentration of phenylboronate-glucose complex [BG] and

free phenylboronate $[B^-]$ is, therefore, higher than 1/130. Presuming that $[BG]/[B^-] >$ 1/130 and $[G] << 1.1 \times 10^{-5}$ M, one can conclude that equilibrium association constant K_{ass} = $[BG]/([B^-][G])$ should be much higher than 700 M^{-1} and thus significantly higher than association constant of glucose with monomeric phenylboronic acid: 110 M^{-1}.[14] Glucose, mannitol and pentaerythritol are the only polyols known to form the 1:2 type complex with phenylboronic acid in diluted solution.[14] Hence, chelating of glucose with two pendant phenylboronate groups might be a reason for the high binding affinity.

At glucose concentrations higher than 5.6 mM (1 mg/ml), which is of the same order of concentration as that of phenylboronate groups of the copolymer in the solution, the thermal precipitation started at around 34 ˚C and the transmittance of the copolymer solution gently decreased with growing temperature. The thermoprecipitation profiles at high glucose concentrations were unaffected by the glucose concentration indicating that phenylboronate groups in the copolymer chain are saturated with glucose.

The effect of pH on T_P of poly(NIPAAM-AAPBA) at various glucose concentrations is shown in Figure 4. At pH higher than 7.5, the T_P became higher than that in the absence of glucose. The observed increase in T_P (ΔT_P) grow up with growth of the glucose concentration. The phenylboronate groups in (NIPAAM-AAPBA)-copolymer chains interacted readily with glucose in solution, resulting in decreased apparent pKa with increasing glucose concentration as the chemical equilibrium shifts to right side (Figure 6). ΔT_P at 560 µM (0.1 mg/ml) glucose was about 4-5 ˚C at pH higher than 8. Kataoka et al. have reported that the precipitation temperature of the same type of copolymer with 15 mol-% of phenylboronic acid content and 9×10^4 of molecular weight increases approximately 15 ˚C at pH 7.4 and 0.1 wt.-%, but at high glucose concentration (16.7 mg/ml or 93 mM).[8] Aoki et al. have reported that the

10

phase transition temperature of the same type copolymer containing 2.42 mol-% of phenylboronate group slightly increase from 18.1 °C to 18.7 °C due to interaction with glucose at glucose concentration of 10 mg/ml (56 mM), copolymer concentration of 1 wt.-%, and pH 7.4.[9] The phenylboronic acid group content of (NIPAAM-AAPBA)-copolymer synthesized in this work was 18 mol-%, which is relatively large value compared with that of the polymer containing phenylboronate group previously reported.[8,9] The high sensitivity for glucose and large increase in T_P of poly(NIPAAM-AAPBA) at low glucose concentration is considered to be due to a high content of phenylboronate group in the copolymer and appropriate pH of the medium.

The effect of the addition of sugars and polyol compounds on T_P of poly(NIPAAM-AAPBA) was examined (**Figure 7 and 8**). The increase in T_P caused by the addition of fructose was about 7-10 °C at pH higher than 8. The effect of mannitol on T_P was almost the same as that of glucose. In the presence of galactose, though the small increase in T_P, 1-3 °C, was observed at pH7.5-8.8. The order of the increase in T_P is the following: fructose > glucose   mannitol > galactose. This order except glucose seems to reflect the association constant for a complex between monomeric phenyl boronic acid and sugar (fructose>mannitol>>galactose>glucose).[14] Glucose has an ability to form a 1:2 complex with phenylboronic acid. Therefore, glucose might show a specific high affinity with phenylboronates in poly(NIPAAM-AAPBA) due to multi-point interaction like chelating of glucose with two pendant phenylboronates in the polymer chain as mentioned above.

A small increase in T_P was observed in the presence of glycerol and oligosaccharides at pH 7.5-8.7 while no effect was observed at pH>9 (Figure 7b). The affinity of these additives towards phenylboronate groups would be relatively low compared with monosaccharides used.

11

The presence of pentaerythritol showed a slightly larger increase in the precipitation temperature of the copolymer (Figure 8) as compared to that observed for tris(hydroxymethyl)aminomethane (Tris). The latter effect was observed only at high concentrations. It is known that pentaerythritol and Tris have high affinity towards phenylboronate groups due to the formation of tridentate complex involving all three hydroxyl groups of the boronate anion (**Figure 9**).[7] There is, however, a difference in the effect of pentaerythritol and Tris on the thermoprecipitation of poly(NIPAAM-AAPBA). The hydrophilicity of the complex formed by tridentate interactions might affect the thermoprecipitation behavior of the copolymer. The complex with Tris might show lower hydrophilicity than that with pentaerythritol, because amino group of Tris would not dissociate at pH 9.3 (pK_a = 8.09). Glycerol did not affect the thermoprecipitation of the copolymer even at high concentration. Although glycerol has three hydroxyl groups, the tridentate interaction of glycerol with the boronate anion is impossible due to the short carbon chain.[7] From the above results, the effect of sugars and polyols on the increase in T_P of poly(NIPAAM-AAPBA) is concluded as follows: fructose > glucose ≈ mannitol > pentaerythritol > galactose > Tris > glycerol

Dextran did not affect also the thermoprecipitation of the copolymer even at high concentration. C_1-OH in sugar molecule has been reported to be important for complex formation with the boronate anion.[7,31] C_1 position of monosaccharide units in dextran, and also oligosaccharides, is occupied by the next saccharide molecule. Hence, the interaction of the copolymer with dextran and oligosaccharides is hindered. On the other hand, in the presence of PVA, thermoprecipitation temperature decreased slightly. At higher PVA concentrations (5.6 mM of monomer units), an insoluble gel was formed. The gel formation from PVA and poly-NIPAAM containing phenylboronic acid has been earlier reported.[10,19] PVA can interact with the boronate anions due to its flexible

structure, which provides conformations suitable for binding of hydroxyl groups to the boronate anion.[7]

Isothermal dissolution of the precipitated (NIPAAM-AAPBA)-copolymer induced by the addition of sugars

Owing to reversibility of the thermal precipitation one may expect that a suspension of (NIPAAM-AAPBA)-copolymer would dissolve in aqueous solutions at temperatures higher than T_P for the copolymer when complexing sugars are added. It follows from Figure 7 that T_P of the glucose-copolymer complex is ca. 32 °C at glucose concentration of 560 µM and pH 8.5. The fructose-copolymer complex exhibits T_P of ca. 36 °C in the same conditions. At temperatures lower than T_P and sugar concentrations higher than 560 µM the complexes of the copolymer and sugars might be soluble in aqueous solutions. The effect of fructose concentration on the dissolution of the copolymer at pH 8.5 and 27 °C is shown in **Figure 10**. The dissolution rate increased with an increase in the fructose concentration and was quite sensitive to the fructose concentration. When the fructose concentration was low (1.4 mM and 2.2 mM), the precipitates were not dissolved completely after 1 hr. On the other hand, at concentration of 3.6 mM the copolymer might be dissolved in a few minutes. Obviously, a higher fructose concentration is required for dissolution of the precipitates as compared to that needed to prevent the copolymer precipitation at given temperature and pH. The dissolution rate quickly increased in the above range of fructose concentrations, probably due to its competitive interaction with boronate ligands involved in hydrophobic interaction with the dehydrated segments of poly-NIPAAM. To compete for binding to boronate a higher concentration of sugar is needed, as compared to that enabling the shift of T_P in the soluble copolymer.

The effect of the addition of various kinds of sugar on the dissolution of the copolymer at sugar concentration of 2.9 mM, pH 8.5 and 27 °C is shown in **Figure 11**. When no sugar was added, the apparent turbidity was increasing with time probably due to the aging of precipitate and formation of larger copolymer particles. The addition of lactose or sucrose had no effect on the development of turbidity. The addition of glucose or galactose inhibited the apparent increase in turbidity while the addition of mannitol dissolved the precipitate. The dissolution rate in case of mannitol was, however, slower than that for fructose. It is clear that the difference in the dissolution property of sugars reflects the difference in the association equilibrium constant of sugar with phenylboronic acid. Fructose and mannitol have very large association constant and were capable of dissolving the precipitates. However glucose and galactose, which have moderate association constant, interact with the phenylboronic acid group and affect the precipitate aging, neither of the sugars could dissolve the precipitates.

Conclusions

It was found that thermoprecipitation temperature (T_P) of (NIPAAM-AAPBA)-copolymers markedly increases (by 1-1.5 °C) in the presence of very small concentration of glucose: 5.6 µM or 1 µg/ml, at pH>8, due to specific complex formation with high association equilibrium constant ($\gg 700$ M^{-1}). Its value is much higher than the known association constant of glucose with monomeric phenylboronate: 110 M^{-1}. The difference can be ascribed to bidentate binding of glucose to the pendant boronates of the copolymer. In 560 µM glucose the shift of T_P to higher temperatures becomes 4-5 °C. Even larger increase of T_P was observed in the presence of fructose, which has higher affinity towards phenylboronic acid. Such inhibition of thermoprecipitation of the copolymer depends on both the affinity to the boronate anion

14

and the hydrophilicty of the complex. The isothermal dissolution of the thermo-precipitated copolymer was achieved by the addition of fructose and mannitol.

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Figure captions

Figure 1. ^{1}H-NMR spectrum (500 MHz) of the (NIPAAM-AAPBA)-copolymer. Solvent: DMSO-$d6$.

Figure 2. Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) and poly-NIPAAM at various pH values.

Figure 3. Equilibrium of phenylboronic acid group between an uncharged trigonal form and a charged boronate anion.

Figure 4. Effect of pH on T_P of poly(NIPAAM-AAPBA) in the absence and the presence of glucose.

Figure 5. Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) in the absence and presence of glucose. a) pH 6.4-6.5, and b) at pH 9.3-9.5

Figure 6. Equilibrium of between a charged phenylboronate anion and sugar in aqueous media.

Figure 7. Effect of pH on T_P of poly(NIPAAM-AAPBA) in the presence of various sugars and polyol compounds. a) monosaccharides, and b) glycerol and oligosaccharides.

Figure 8. Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) at pH 9.3 in the absence and the presence of various sugars and polyol compounds. a) polyol compounds, and b) glucose, fructose, dextran and PVA.

Figure 9. Tridentate interaction of boronate with pentaerythritol and Tris.

Figure 10. Effect of fructose concentration on the dissolution of the poly(NIPAAM-AAPBA) suspension.

Figure 11. Effect of different sugars on the dissolution of the poly(NIPAAM-AAPBA) suspension.
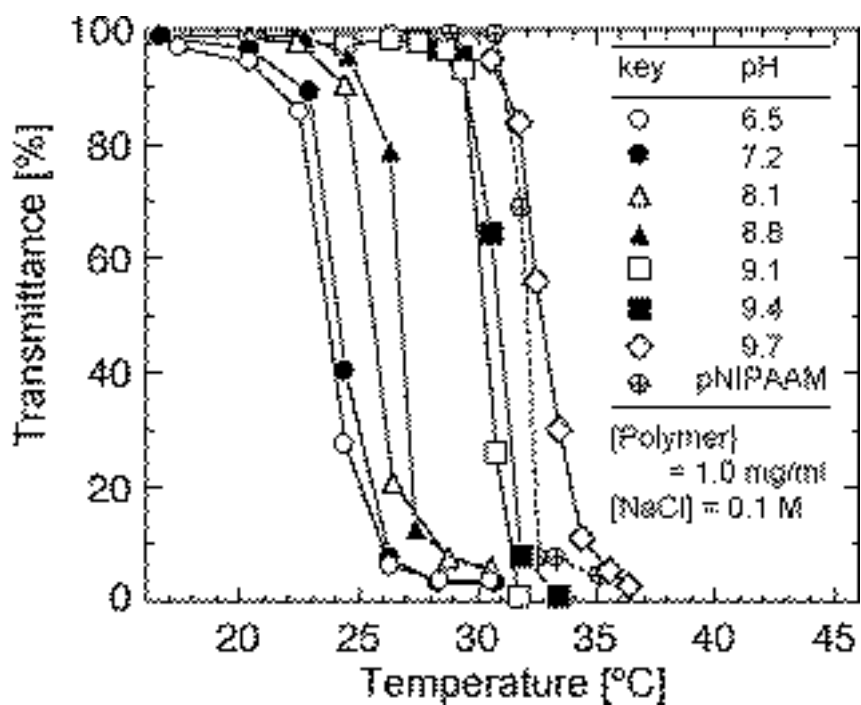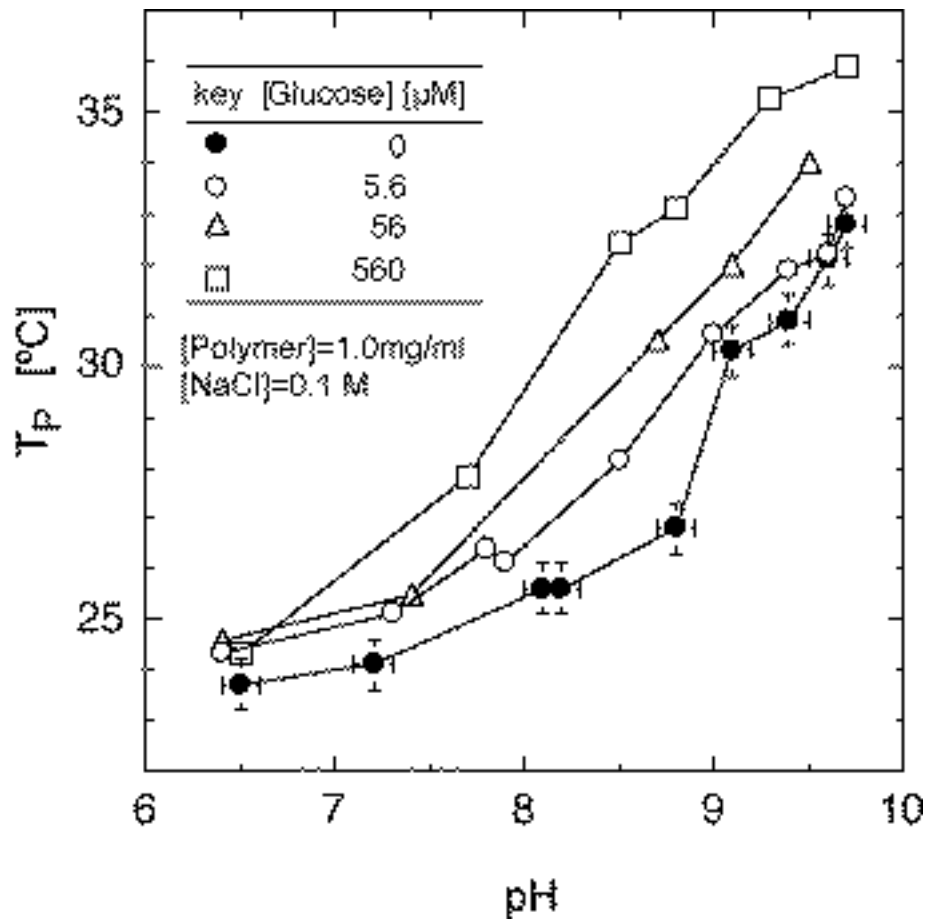
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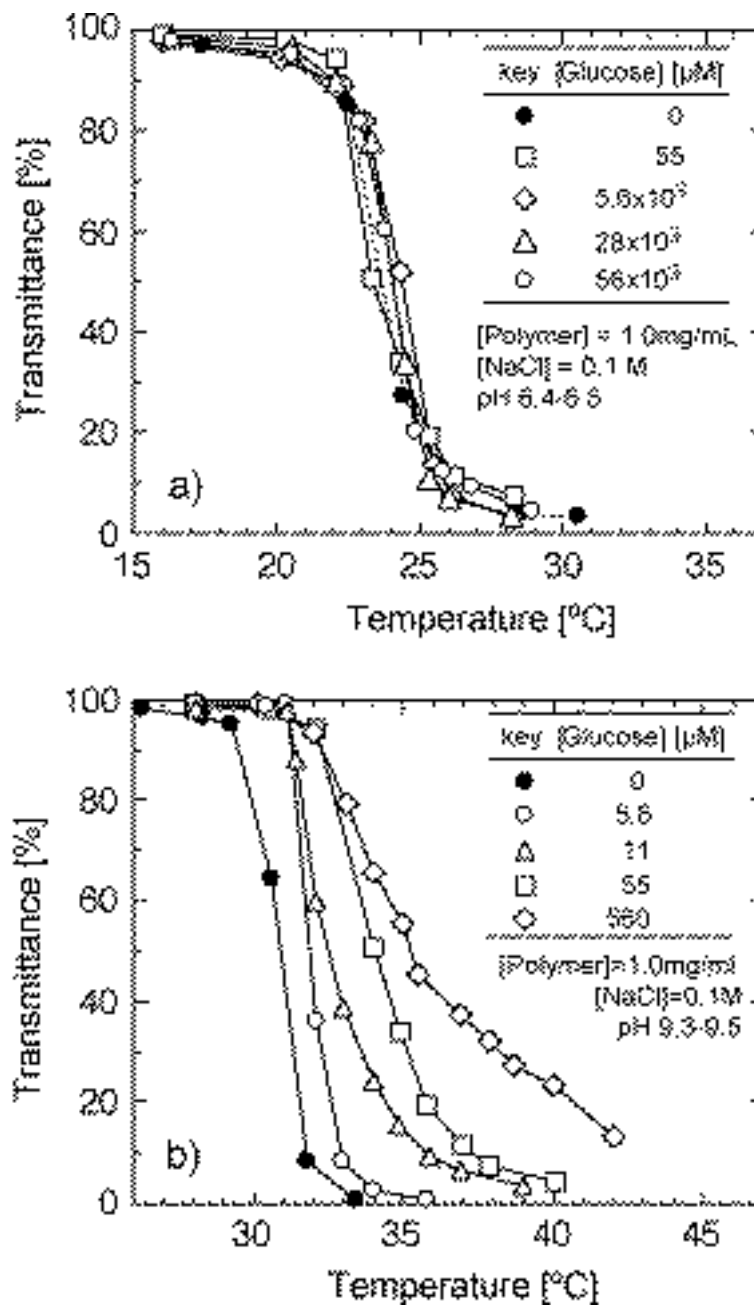
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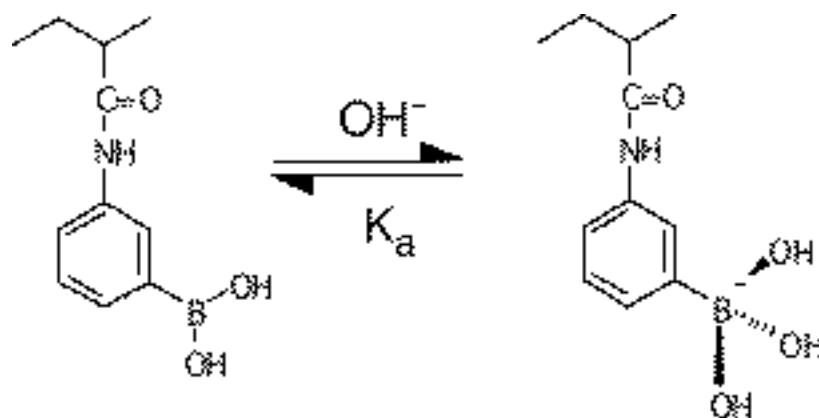
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Figure 3.   Equilibrium of phenylboronic acid group between an uncharged trigonal

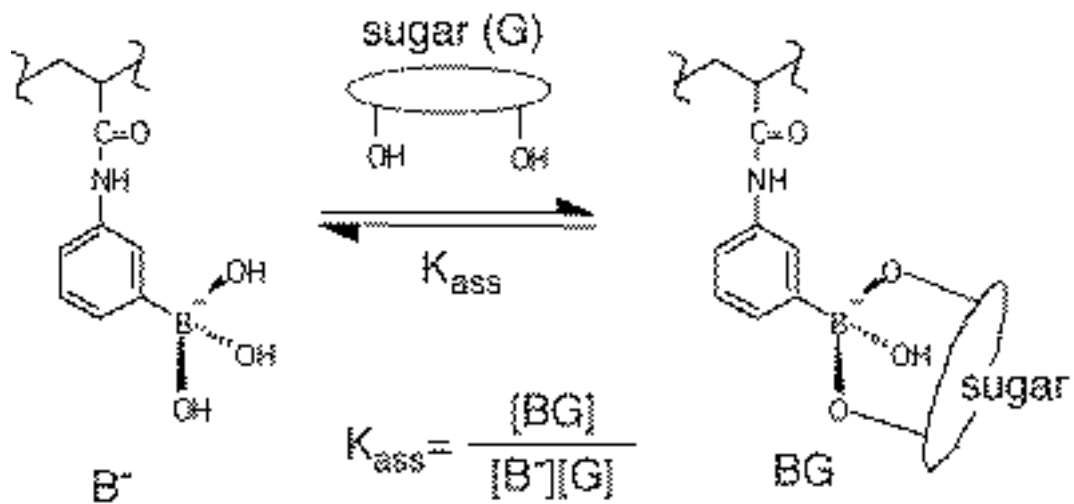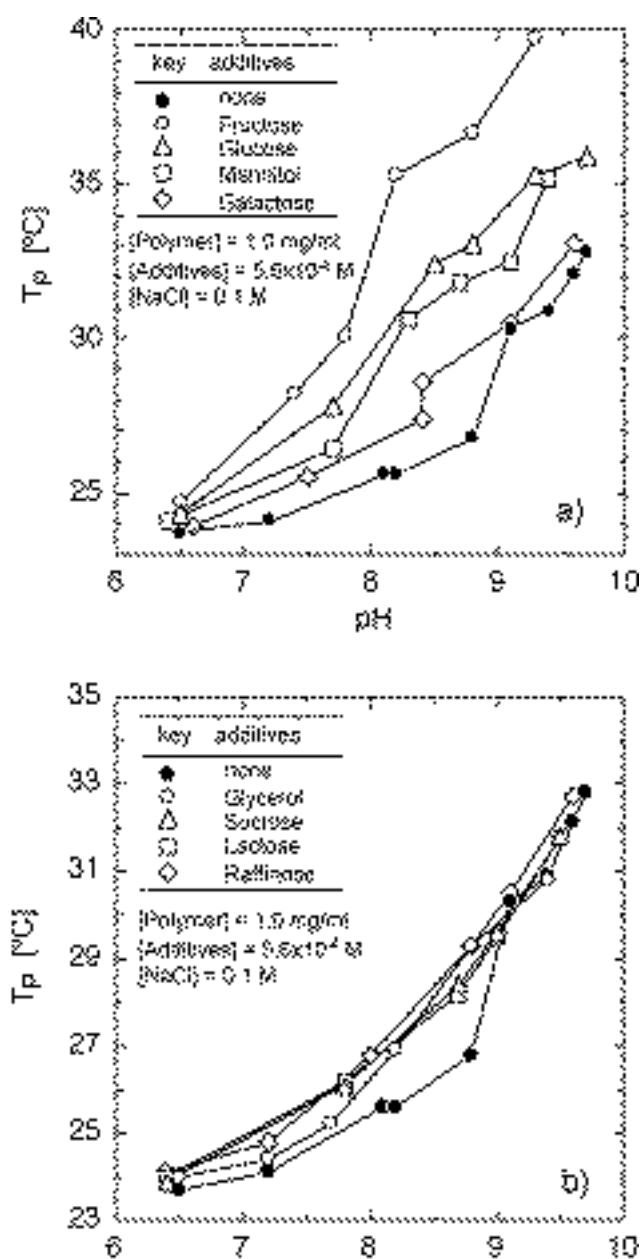form and a charged boronate anion.

K. Shiomori et al.

Figure 4. Effect of pH on T_P of poly(NIPAAM-AAPBA) in the absence and the presence of glucose.

K. Shiomori et al.

Figure 5. Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) in the absence and presence of glucose. a) pH 6.4-6.5, and b) at pH 9.3-9.5

K. Shiomori et al.

$$K_{ass} = \frac{[BG]}{[B^-][G]}$$

**Figure 6**.   Equilibrium of between a charged phenylboronate anion and sugar in aqueous media.

K. Shiomori et al.

Figure 7. Effect of pH on T_P of poly(NIPAAM-AAPBA) in the presence of various sugars and polyol compounds. a) monosaccharides, and b) glycerol and oligosaccharides.
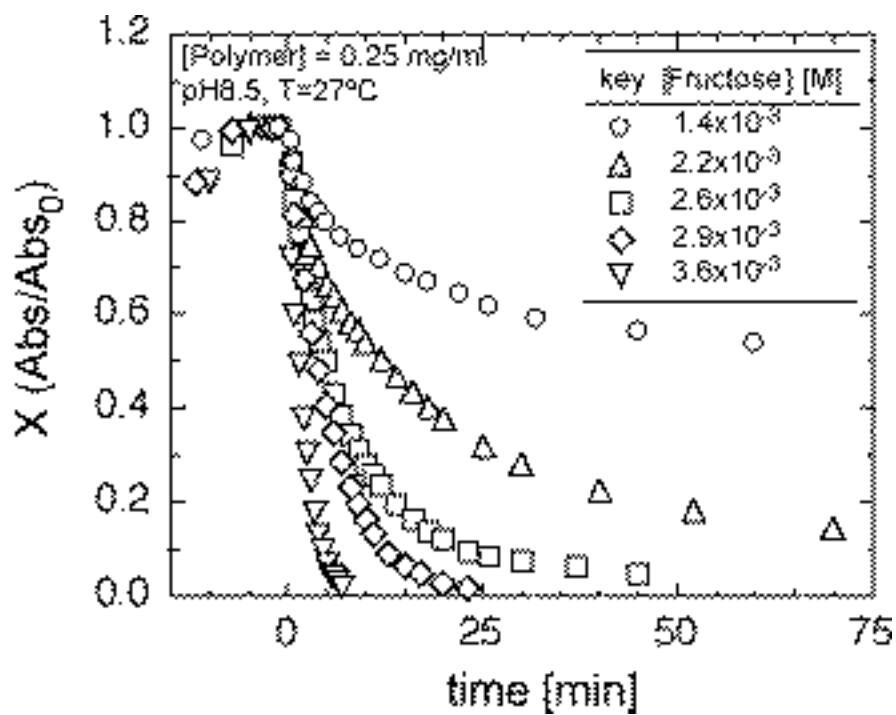
K. Shiomori et al.
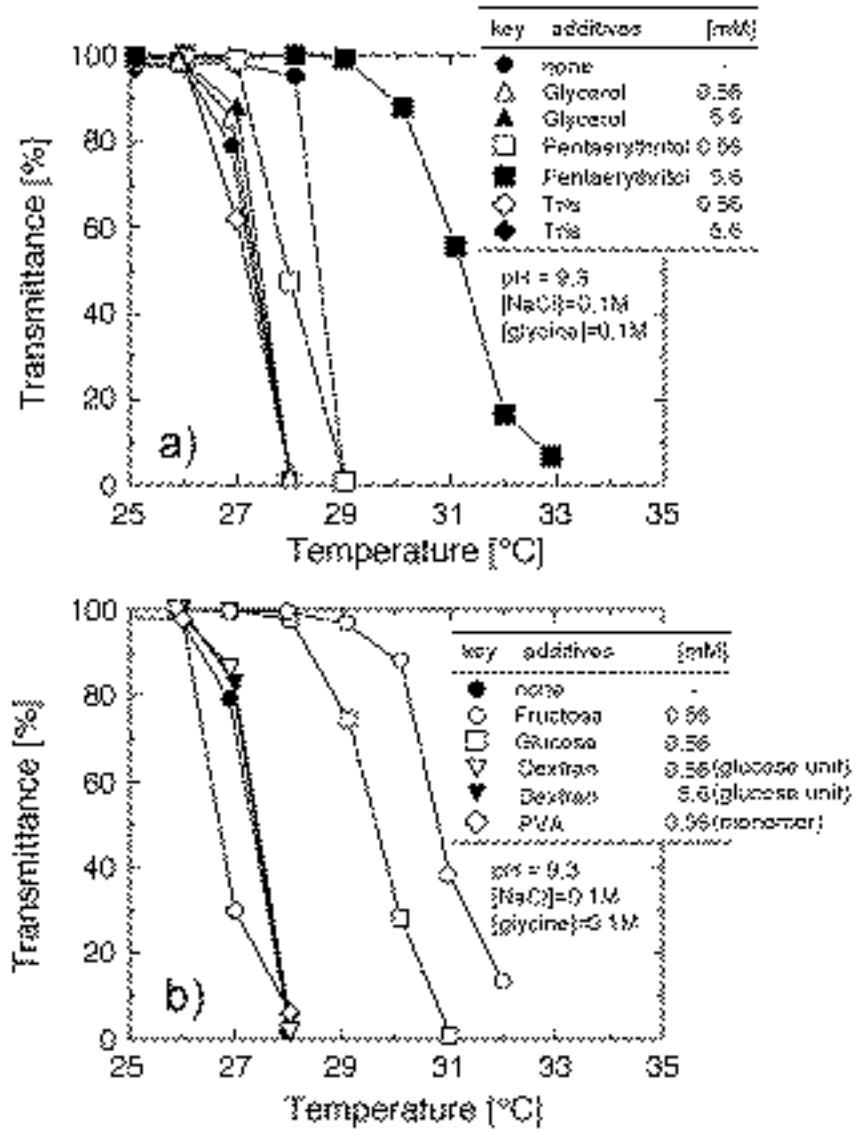
Figure 8. Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) at pH 9.3 in the absence and the presence of various sugars and polyol compounds. a) polyol compounds, and b) glucose, fructose, dextran and PVA.
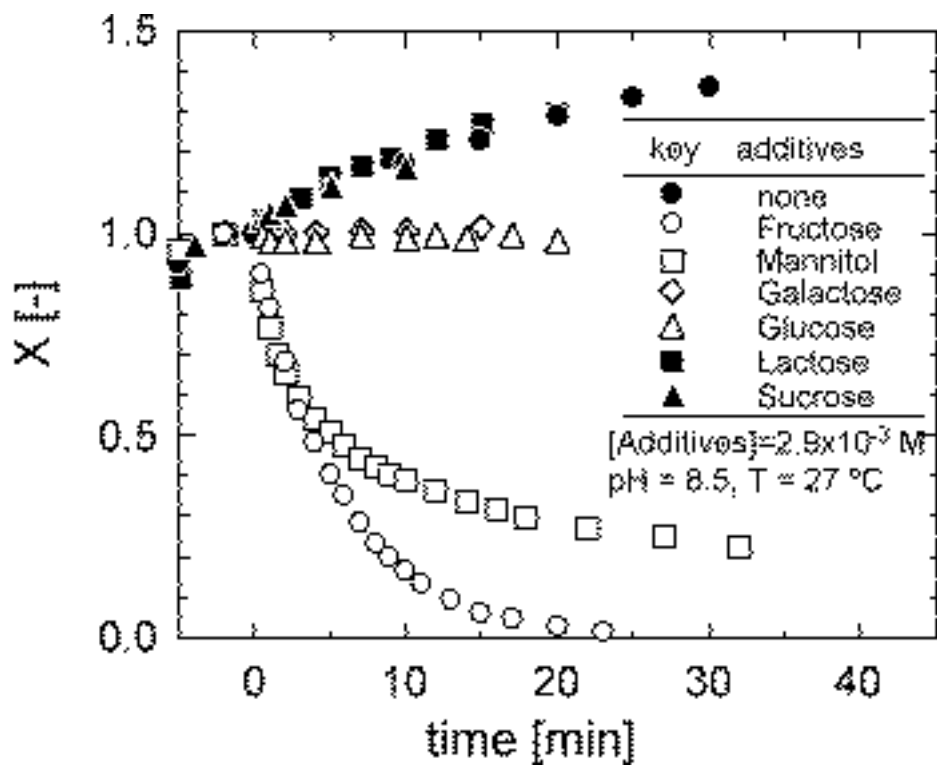
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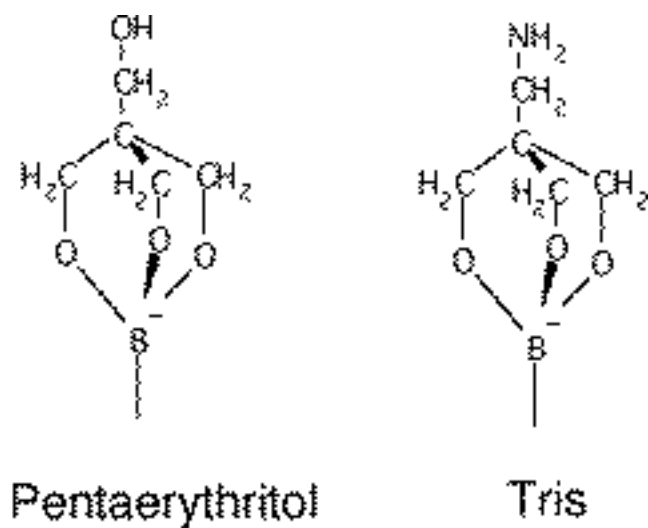
Figure 9.   Tridentate interaction of boronate with pentaerythritol and Tris.

K. Shiomori et al.

Figure 10. Effect of fructose concentration on the dissolution of the poly(NIPAAM-

AAPBA) suspension.

K. Shiomori et al.

28

Figure 11. Effect of different sugars on the dissolution of the poly(NIPAAM-AAPBA) suspension.

K. Shiomori et al.