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# A comparative study on two phenylboronic acid based glucose-sensitive hydrogels

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## 1. ABSTRACT

Two phenylboronic acid based glucose-sensitive hydrogels, A•PBA-DMAPMA-EGDMA and A•PBA-PEG, were initially prepared by free-radical polymerization. Swelling properties of the gels were studied by determining the diameter changes in different buffer solutions, with or without glucose or fructose. The hydrogels were designed as "valves" to control the flow of glucose solutions. The results showed that gel A•PBA-DMAPMA-EGDMA was sensitive to pH and glucose, but not to fructose. It shrunk in weak basic solution and the addition of glucose made it shrink more. In this gel PBA moiety and glucose is supposed to form a 1:2 bis-bidentate complex. Hydrogel A•PBA-PEG was sensitive to pH, glucose and fructose, all of which made it swell in weak basic solution. A 1:1 complex is believed to form between PBA and glucose/fructose in this gel. All the stimuli-responses are reversible and the glucose-responses occurred in the range of the physiological/pathological glucose level. Both A•PBA-DMAPMA-EGDMA and A•PBA-PEG exhibited sufficient volume change to the alteration of glucose concentration and could be employed as a "valve" to control liquid flow in weak basic solution.

## 2. INTRODUCTION

Environment sensitive hydrogels are intelligent materials which undergo volume changes or sol-gel transition in response to the change of some of the surrounding stimuli. The stimulus-sensitive hydrogels have both sensor and effector functions, and can be widely used in the design of triggers, switches, sensors, mechanochemical actuators, specialized separation systems and bioreactors (1). The widely investigated stimulus-sensitive hydrogels include pH-sensitive hydrogel, temperature-sensitive hydrogel and glucose-sensitive hydrogel. In addition, enzyme-sensitive hydrogels, antigen-sensitive hydrogels, and nucleic acid base-sensitive hydrogels were also reported.

In pharmaceutics, the environment responsive materials are most commonly used in self-regulated drug delivery system. Glucose-sensitive hydrogel is the basis of the dynamic blood glucose sensor and insulin self-regulated DDS. There are mainly three kinds of glucose-sensitive hydrogels: 1) glucose oxidase (GOD)-loaded pH-sensitive hydrogels (2-4); 2) concanavalin A-containing hydrogels (5-8); 3) phenylboronic acid (PBA) based hydrogels (9-14).

GOD-pH sensitive hydrogels have been used in glucose sensors both *in vitro* and *in vivo*. However, the accuracy and sensitivity for distinguishing normal blood glucose from hyperglycemia still needs to be improved, and the stability and depletion of GOD are still problems to be overcome (15, 16).

PBA-based hydrogel is promising because it does not contain any bio-macromolecules and thereby will not lose any glucose-sensitivity because of the instability or depletion of bio-molecules. PBA and its derivatives combine with diols or polyols to form a complex. The complex between PBA and a polyol compound can be dissociated in the presence of competing polyol compound, which is able to form a stronger complex (1). This is one way to use PBA-polyol based hydrogel in glucose controlled DDS. Zhu Y and Zheng L (17) reported the preparation of a glucose-sensitive hydrogel with nanoparticles. Free glucose competed with gluconated insulin to cause the release of gluconated insulin from the hydrogel nanoparticles, as the surrounding glucose concentration increased. Similar studies were also reported by Shiino D (18). We think the use of direct binding of PBA and gluconized insulin has some shortcomings. These include the limiting of the drug loading and proper ways to eliminate hydrogel carriers from the body after drug release.

Another mechanism of PBA-based glucose-sensitive hydrogel comes from the influence of glucose on PBA equilibrium between its uncharged and charged form ($PBA^-$). The addition of glucose (or other proper diols or polyols) at a pH close to the $pk_a$ of the phenylboronate causes a shift towards an increase of $PBA^-$, which in turn increases the solubility or swelling state of the material in aqueous solution (1, 19, 20). The volume change caused by the change of swelling state can be used as a "valve" to control drug release.

As the complex between uncharged PBA and glucose is unstable and the $pK_a$ of PBA is reported as 8.8, systems in which PBA alone (*i.e.*, PBA with no electron-withdrawing substituents) is grafted to the polymer backbone are, in practicality, useful only at pH>8, above physiological pH (14). Neighboring amine groups are considered to be able to lower the $pK_a$ of PBA, because the interaction between the boron atom and the nitrogen atom increases the Lewis acidity of PBA. Therefore, incorporation of an amine group to PBA hydrogels has been widely accepted in the construction of glucose-responsive materials at physiological pH (14, 18, 21-22).

Poly(ethylene glycol) (PEG) is a widely used biomaterial because of its non-toxic, water-soluble, protein-adsorption-resistance and cell-adhesion-resistance, and immune "stealth" properties (23). N.A. Peppas' group has incorporated PEG in GOD-containing hydrogels to retard the degradation of the enzyme (24, 25). Alexeev VL (26) incorporated small amounts of PEG to PBA-acrylamide (AA)-bisAA gel and found a huge difference between gels with or without PEG.

In this study, we developed an amine-contained PBA-based glucose-sensitive hydrogel A•PBA-DMAPMA-EGDMA. The swelling-deswelling properties of the hydrogel in response to the change of glucose concentration were studied, and the gel was employed as a "valve" to control the liquid flow. Poly(ethylene glycol) was first used as the main composition of hydrogel backbone to stabilize the gel to a surrounding Lewis acid and to improve the bio-compatibility of the gel. The swelling properties of this A•PBA-PEG gel were also evaluated.

## 3. MATERIALS AND METHODS

### 3.1. Reagents and instruments

Acrylic acid, 3-aminophenylboronic acid hemisulfate ($PBA^+$), N-[3-(Dimethylamino)propyl]-methylacrylamide (DMAPMA) and Ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich; Polyethylene Glycol 3000, N,N,N,N'-tetramethylethylenediamine (TEMED), Dichlorodimethylsilane and Triethylamine were purchased from Fluka. Acryloyl Chloride was from Alfa Aesar; ammonium persulfate (APS) was from Sigma; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was from TCI. Glucose was obtained from Sinopharm Chemical Reagent Beijing Co Ltd and fructose from ACROS. The XSZ2H Biological Microscope from ChongQing Optical & Electrical Instrument Co Ltd and the S-4500 Scanning Electric Microscope from Hitachi were employed in this study.

### 3.2. Synthesis of acrylaminophenylboronic acid (A•PBA)

A•PBA was synthesized in a similar method as Lee and coworkers reported (27). In a round bottom flask, acrylic acid (30mmol) was dissolved in water and the pH of the solution was adjusted to 4.8 by adding NaOH solution, the total volume of this solution was about 30ml. In another container, $PBA^+$ (25mmol) was suspended in 20 ml water and the suspension was adjusted to pH 4.8 with about 20 ml 1M NaOH aqueous solution. The flask containing acrylic acid was stirred in an ice bath, and after temperature equilibration, an EDC solution (30mmol in 15 ml, pH 4.8) was added into the flask. This mixture was stirred in an ice bath under $N_2$ purge for half an hour before $PBA^+$ suspension was added dropwise into it. The reaction was allowed to continue for 1 h in an ice bath and under $N_2$ purge. The mixture was then transferred to a separation funnel and 100ml of dry diethyl ether was used to extract A•PBA. The product was recrystallized in water and A•PBA crystal was collected and vacuum dried. The yield ranged from 45% to 55% for different batches. $^1$H-NMR spectroscopy in $CD_3OD$ showed chemical shifts (Delta value) at 5.8 (1H, $CH_2$=CH-), 6.4 (2H, $CH_2$=CH-), and 7.3-7.9 (4H, phenyl).

### 3.3. Synthesis of poly(ethylene glycol) diacrylate (PEG•$A_2$)

PEG•$A_2$ was synthesized in a procedure similar to that reported by Cruise GM (28). Three grams of dry PEG 3000 was dissolved in 30 ml toluene at 38°C. Triethylamine, in four times molar excess (based on PEG diol end groups), was added to PEG solution.

Methylacryloyl chloride, in four times molar excess (based on PEG diol end groups), was added dropwise to the above solution. The mixture was stirred overnight at 38 °C under argon. The insoluble triethylamine salts formed were removed by filtration and the filtrate was precipitated by adding 50 ml cold ethyl ether (4°C). The PEG diamethylacrylate precipitate was collected by filtration, redissolved in 10 ml of toluene, and reprecipitated with cold ethyl ether twice more. The polymer was collected and vacuum dried. The yield was about 60%~70%.

NMR was used to analyze the methylacrylation rate of PEG by comparing the ratio of the integration from the PEG backbone (3.25~3.65 ppm) and the acrylate peaks (5.9~6.3 ppm). The calculation used the following formula: Methylacrylation rate = n × [(integral of vinyl hydrogen)/4]/ [(integral of PEG backbone)/4] × 100%, where n is the polymerization degree of the polymer. The typical methylacrylation rate we obtained was around 70%~80%.

### 3.4. Preparation of hydrogel A•PBA-DMAPMA-EGDMA and hydrogel A•PBA-PEG

A•PBA and DMAPMA were dissolved in 1 milliliter of methanol-water (1:1) at a molar ratio of 1:4. Crosslinking agent EGDMA and accelerator TEMED were added in small amounts (less than 0.01% of the backbone monomers in molar ratio). A 10% Ammonium persulfate (APS) solution was added and the solution was put into a glass tube (i.d. 0.97mm) overnight to form columnar hydrogel A•PBA-DMAPMA-EGDMA.

Hydrogel A•PBA-PEG was prepared in a similar manner with A•PBA and PEG•$A_2$ being dissolved in 1 milliliter of methanol-water (1:1) at a molar ratio of 2:1. A 10% APS solution was used as an initiator to obtain the crosslinking hydrogel network.

### 3.5. The pH- and glucose/fructose-sensitivity of the hydrogel

The hydrogel was balanced in a pH 7.4 phosphate buffer solution (PBS) before the experiment and then exposed to a series of stimuli (glucose, fructose and pH) in solution. Swelling rate, an indicator of the change of the hydrogel diameter, was used to quantitatively depict the change of swelling state of the hydrogel. The swelling rate is calculated as $D_i/D_0$*100%, where $D_i$ is the diameter of the gel in PBS containing a series concentration of glucose or fructose (or the diameter in PBS with different pH other than pH 7.4), whereas $D_0$ is the gel diameter in pH 7.4 PBS.

The swelling state of the gel was recorded as the concentration of glucose or fructose changed from 2.5 mmol/L to 50 mmol/L, particularly at 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, and 50 mmol/L of the monoses, or *vice versa*. The experiment was conducted in a series of PBS buffers with pH varying from pH 4.5 to pH 10.4. Carbonate buffer solution (CBS) was also used to conduct a contrast study in evaluating the environment-sensitivity of the gel.

### 3.6. Morphology study of the hydrogels

Dry hydrogel cylinders (about 0.5 mm in diameter) were balanced in PBS (pH 7.4) and then put into different solutions. After swelling to steady state, the gels were moved into freezing vials, and frozen in liquid nitrogen for 20 min before being shifted into a freeze dryer. Then, the gels were freeze dried with its original three-dimensional network.

The samples included hydrogel A•PBA-DMAPMA-EGDMA balanced in: 1) injectable water, 2) PBS (pH 7.4), 3) PBS (pH 10.4), 4) PBS with 10 mmol/L glucose (pH 10.4), as well as hydrogel A•PBA-PEG in PBS (pH 7.4). All the samples were plated until covered with a film of 5 nm thickness. Their surface characteristics were observed under SEM.

### 3.7. Response rate of the hydrogel to the change of glucose concentration

A piece of columned hydrogel (about 1.5 mm in diameter) was fixed in a tube, which is a little wider than the well-swelled hydrogel. Phosphate buffer solution flew through the tube with a fixed static pressure difference as the driving force. The hydrogel was immersed in the flowing PBS for about 2 days until fully swelled and balanced. The flow rate was recorded as the amount of the solution (g) that flew out in 30 seconds, and was monitored every other 30 sec during the experiment. The change of the flow rate was employed to indicate the change of the swelling state of the hydrogel, *i.e.*, the gel acted as a "valve" to control the liquid flow, according to its swell or shrink. The "steady state rate" of the liquid was determined for the solution with or without 5 mmol/L or 10 mmol/L glucose, and, after the gel was well balanced in it, to indicate when the environmental condition (or the stimuli) could be changed for the existing setup. All the solutions used in this experiment had the same phosphate ion concentration and were adjusted to pH 10.

The experiment started after the gel was well balanced in PBS without glucose. The flow rate was determined and written down. Then the solution was changed to PBS with 5mmol/L glucose, PBS with 10mmol/L glucose, PBS with 5mmol/L glucose and PBS without glucose, subsequently, once the fluid reached its steady flow rate in these respective solutions. Three cycles were conducted and recorded.

## 4. RESULTS

### 4.1. The pH- and glucose/fructose-sensitivity of hydrogel A•PBA-DMAPMA-EGDMA

The swelling profiles of this gel in solutions with different glucose concentration or pH were shown in Figure 1 and Figure 2. The data of the influence of glucose on the pH sensitivity of gel A•PBA-DMAPMA-EGDMA were also shown in Table 1. Gel in pH 7.4 PBS was regarded as the initial state to calculate the swelling rate as pH/glucose concentration changed and the swelling rate-pH/glucose concentration profile was drawn. Hydrogel A•PBA-DMAPMA-EGDMA is sensitive to the acidity of solution.

**Table 1.** Swelling of gel A•PBA-DMAPMA-EGDMA in PBS with varying pH and varying glucose concentration

| | Glucose Conc (mmol/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **2.5** | **5** | **7.5** | **10** |
| pH 4.5 | 128.37±5.49 | 132.46±0.51 | 133.68±1.33 | 135.32±0.06 | 136.32±0.49 |
| pH 5.4 | 123.05±2.50 | 125.81±2.49 | 127.43±3.01 | 127.04±3.44 | 127.04±3.44 |
| pH 6.4 | 113.06±0.11 | 113.06±0.11 | 113.06±0.11 | 113.06±0.11 | 113.06±0.11 |
| pH 7.4 | 100±0 | 97.24±0.79 | 96.84±0.02 | 97.24±0.79 | 96.84±0.02 |
| pH 8.4 | 99.60±0.79 | 96.06±0.86 | 94.48±0.84 | 94.48±0.84 | 94.10±2.29 |
| pH 9.4 | 98.36±0.03 | 93.04±1.55 | 92.63±1.58 | 92.63±1.58 | 91.81±1.23 |
| pH 10.4 | 87.61±0.96 | 70.95±1.20 | 65.13±0.71 | 64.74±1.28 | 64.36±1.34 |

n=4

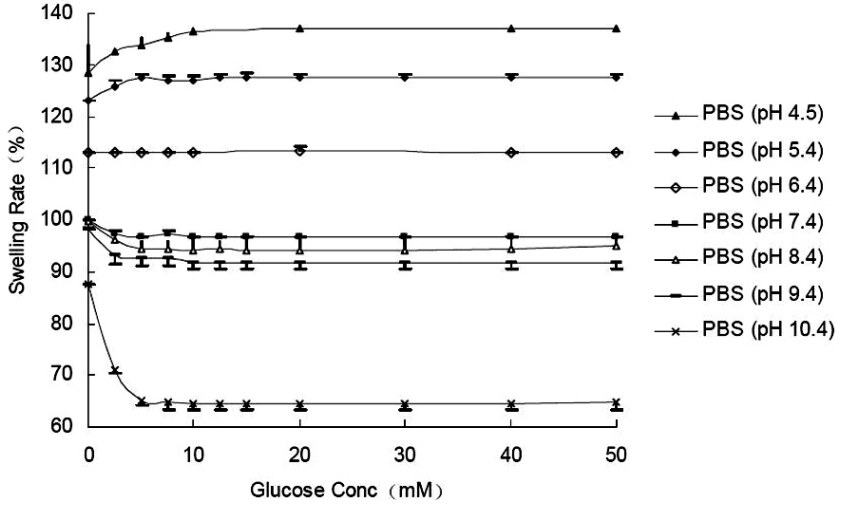


**Figure 1.** Swelling of gel A•PBA-DMAPMA-EGDMA in glucose solution of varying pH (n=4).

It swelled with the decrease of pH from 10.4 to 4.5, but from pH 9.4 to 7.4, there were no detectable changes.

This hydrogel is also responsive to glucose alteration, though the situation is somewhat complicated. At pH 6.4, the swelling state of the gel was not affected by the addition of glucose. At a lower pH, the gel swelled a little as glucose concentration increased, and the swelling caused by glucose after the gel balanced was about 4% at pH 5.4 and 8% at pH 4.5. However, the gel shrank on glucose addition at a pH above 7.4. The shrinkage of the gel became more obvious with the increase of glucose concentration, as well as solution pH, and a quite significant deswelling caused by glucose (up to 23%) was seen at pH 10.4. The absolute swelling rate of gels of different batches in the same solution were somewhat different, but the overall swelling characteristics reocurred.

The hydrogel is stable to fructose under the experimental condition, which means that the addition of fructose or the increase of fructose until 50mmol/L did not bring any change to the swelling state of the gel (data not shown).

The swelling of the gel in weak acidic glucose solution mainly resulted from the acidity of the solution, whereas the swelling in glucose solution above pH 7.4 was mainly caused by glucose. All the swelling rate change occurred within 0 to 20 mmol/L glucose. These data suggested that the binding sites of the gel to glucose were saturated below 20 mmol/L.

This glucose-sensitivity of the gel is reversible, which is important to a glucose "valve". After the gel balanced in 50mmol/L glucose-containing buffer, it swelled gradually as the glucose concentration decreased to 0, just along the shrinking route.

### 4.2. The pH- and glucose/fructose-sensitivity of hydrogel A•PBA-PEG

In PBS, hydrogel A•PBA-PEG did not make obvious changes until the pH was above 9.4, but in glucose or fructose solution, it began to swell when pH was up to 8.4, even at 7.4 in fructose. The results indicated that the gel is stable in weak acidic to neutral solution. The swelling profiles of the gel were illustrated in Figure 3 and Figure 4. Obviously, the binding of $HPO_4^{2-}$ to PBA lowered the apparent $pK_a$ of PBA in PBS, rather than in NaCl-NaOH-HCl solution ($Na^+$ 0.154 mol/L) (Figure 4).

Under the same condition, the gel swelled a little more in fructose solution than in glucose solution. This conformed to the report that the binding constants between

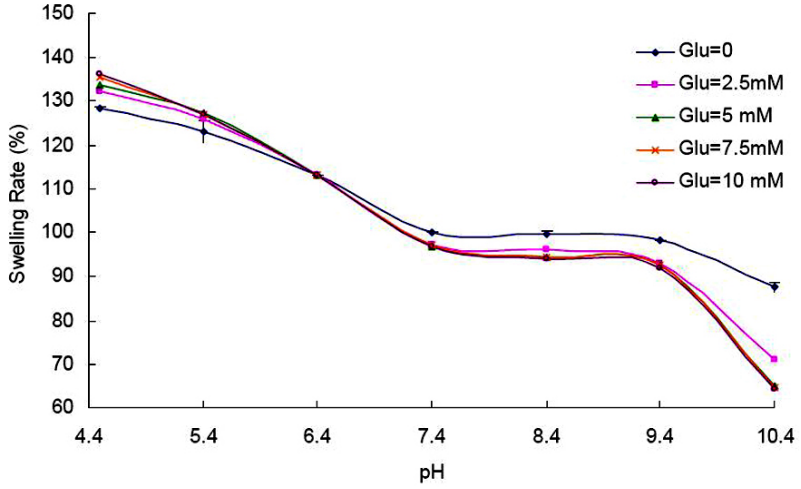


**Figure 2.** The influence of glucose on the pH sensitivity of gel A•PBA-DMAPMA-EGDMA in PBS (n=4).

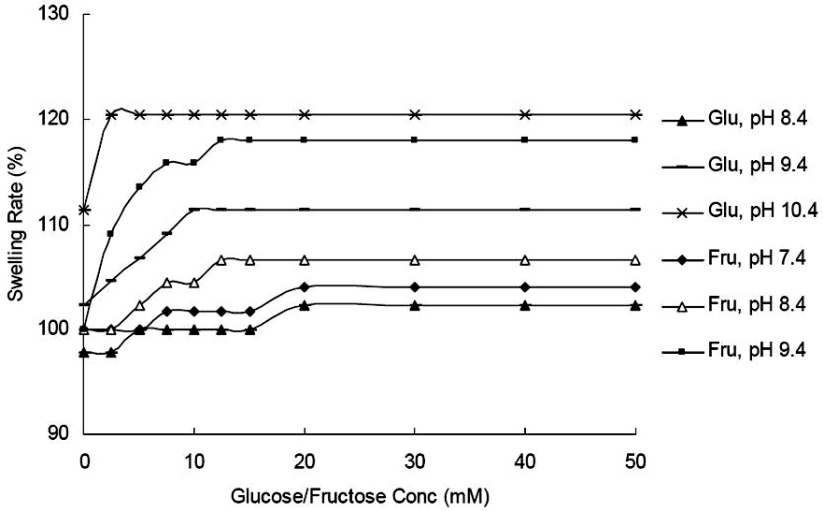


**Figure 3.** Swelling of hydrogel A•PBA-PEG (2:1) in glucose or fructose solution (PBS, n=4).

boronic acid and fructose were larger than that between boronic acid and glucose (20). The swelling of hydrogel A•PBA-PEG in weak basic buffer is due to the ionization of PBA, which resulted from hydrolysis and the binding of saccharides. This binding increases the hydrophilicity of the gel. The glucose-sensitivity and the fructose-sensitivity of the gel are reversible, that is, when the concentration of glucose/fructose decreased, the gels went back to its original swelling state along the accompanying route.

### 4.3. The stability of the hydrogel to buffers

The species of ions in buffer also affect the glucose-sensitivity of the gel, as seen in Figure 5. Balanced in PBS (pH 7.4), Hydrogel A•PBA-DMAPMA-EGDMA shrank slightly after being moved to PBS with pH 9.4, in sharp contrast to a swelling up to a 20% increase in diameter, after moved to CBS with pH 9.4. Its glucose-sensitivity behavior in these two buffers was also different: glucose induced shrink was about 6.5% in PBS, but more than 20% in CBS. The glucose-response behavior in CBS (pH 9.4) was similar to that in PBS when pH was 10.4.

As to gel A•PBA-PEG, the difference of swelling in PBS and in CBS at the same pH was not as much as that of gel A•PBA-DMAPMA-EGDMA. The swelling of A•PBA-PEG in PBS (pH 9.4), CBS (pH 9.4) and PBS (pH 10.4) were nearly parallel to each other. These results suggested that hydrogel A•PBA-PEG would be more stable to the environmental variations, which readily happen in physiological conditions.

### 4.4. Morphology study of the hydrogels

Morphology pictures under a scanning electric microscope (SEM) of the gels balanced in different buffers were shown in Figure 6. The hydrogel is not a solid column under SEM. It was composed of interlaced membranes and chambers enclosed. Obviously, the chambers act as a "reservoir" when the gel swelled in water or buffer

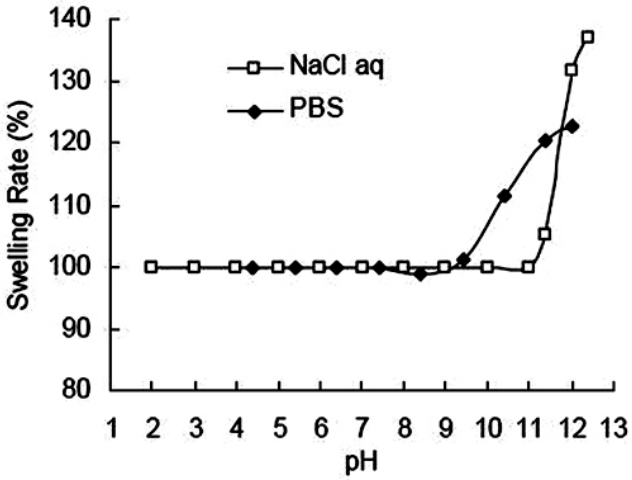


**Figure 4.** The pH sensitivity of hydrogel A•PBA-PEG (2:1) in PBS and in NaCl-NaOH-HCl solution ($Na^+$ 0.154 mol/L) (n=4).

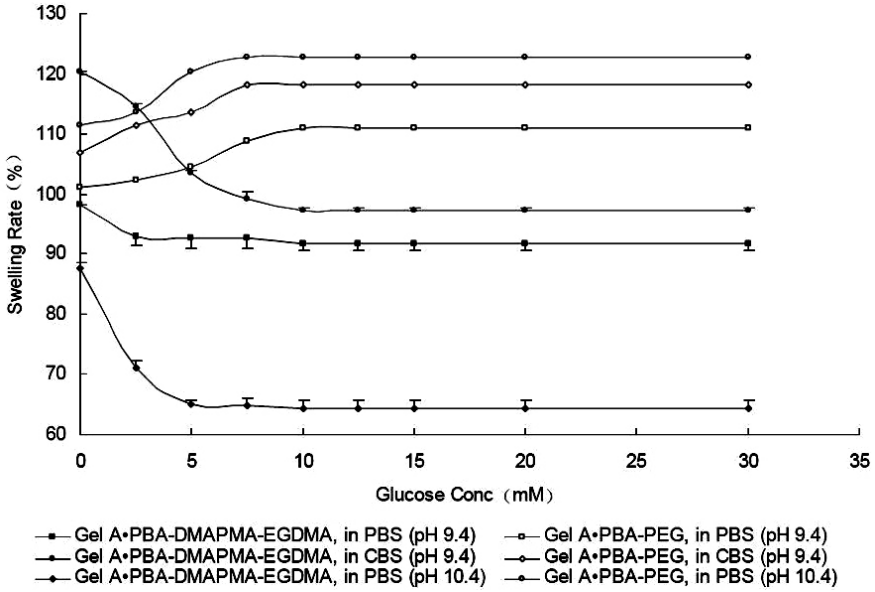


**Figure 5.** Swelling of the hydrogels in glucose solution in different buffers (n=4).

solution. Water contained in the gel was determined by the spread or shrink of the chamber walls. Figure 6(A) showed us flimsy and flexible walls, with membranes stretching out randomly in pure water. In contrast, after the gels were balanced in buffer solution, the chambers arrayed orderly (Figure 6(B)), indicating that the existence of ions in water will inhibit hydrogel swelling. The addition of glucose did not bring much difference to the gel (Figure not shown). In PBS buffer (pH 10.4), the walls became thicker significantly (Figure 6(C)), and the addition of glucose made the walls even denser (Figure 6(D)). These changes conform to the swelling results determined by diameter measurement. The morphology varieties of the gels give explanations to the swelling rate varieties and provide intuitionist evidence to the glucose-sensitivity and pH-sensitivity of the gels.

Morphology of hydrogel A•PBA-PEG was seen in Figure 7. The walls were denser than that of hydrogel A•PBA-DMAPMA-EGDMA.

**4.5. Responses to the variation of glucose concentration**

The response of hydrogel A•PBA-DMAPMA-EGDMA to the variation of glucose concentration was carried out in PBS at pH 10. When the hydrogel was used as a "valve" to control the buffer flow, the flow rate changed according to the change of glucose concentration in buffer. As glucose concentration in the buffer increased from 0 to 5 mmol/L and then to 10 mmol/L glucose, the flow rate increased up to its steady flow rate (which was determined in advance) within 5 to 10 minutes. A reverse process was obtained by replacing the solution with glucose concentrations from high concentration to low

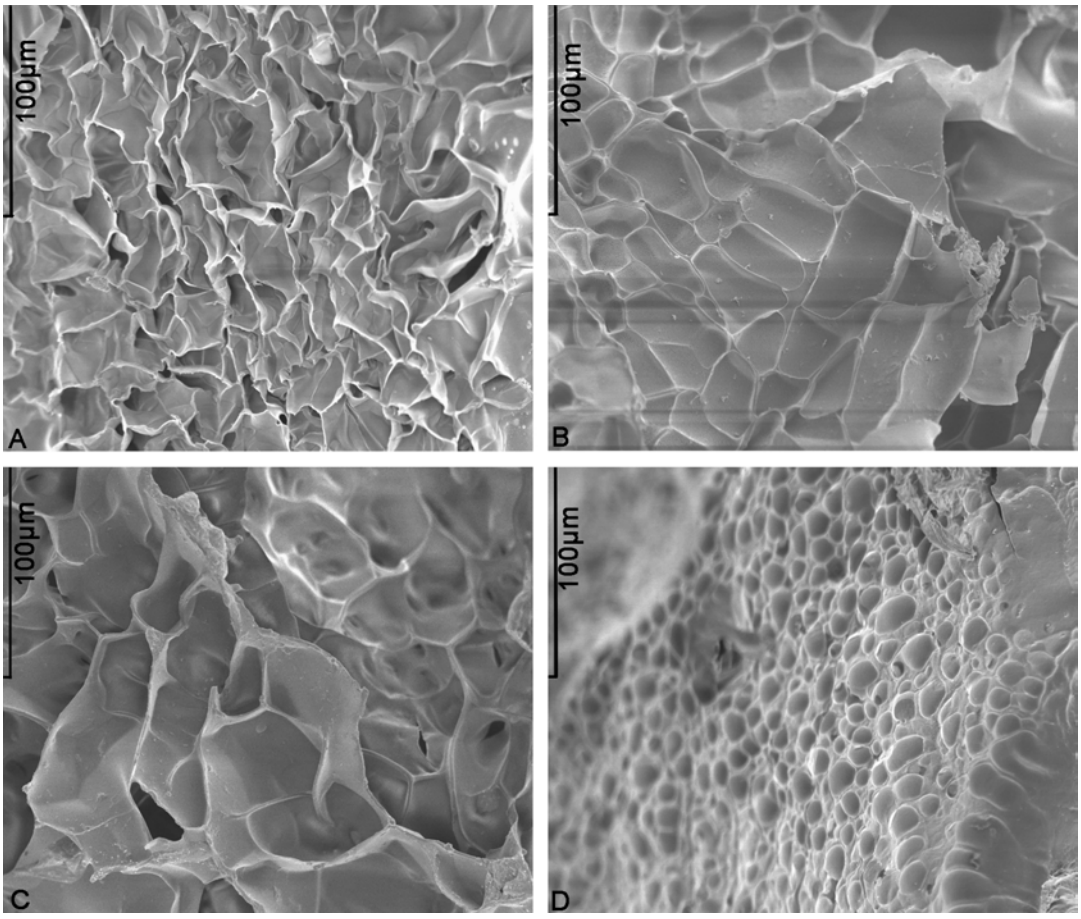


**Figure 6.** Morphology of Gel A•PBA-DMAPMA-EGDMA under SEM in: (A) Injectable water; (B) PBS (pH 7.4); (C) PBS (pH 10.4); (D) PBS (pH 10.4) + 10 mmol/L glucose.

concentration and to PBS without glucose. The time to the steady flow rate for this process was also within 5 to 10 minutes (Figure 8). During the 3 cycles of the experiments, the hydrogel kept its glucose-sensitivity fairly well with a stable response rate.

This study was also carried out with hydrogel A•PBA-PEG (2:1) in PBS (pH 9.4). While hydrogel A•PBA-DMAPMA-EGDMA reacted within 5~10 min on the change of glucose concentration, it took hydrogel A•PBA-PEG (2:1) about 25~30 minutes to finish the response process (data not shown).

## 5. DISCUSSION

Environment sensitive materials have been one of the most concerned areas in recent decades. Among these materials, hydrogel, which is sensitive to physiological and pathological serum glucose at physiological pH, is especially fascinating. The normal fasting plasma glucose (FPG) level is from 3.6~5.8mmol/L, and 2-hour postprandial glucose should be no more than 7.8 mmol/L. A person is diagnosed as diabetes mellitus when FPG is higher than 7.0 mmol/L or 2-hour postprandial glucose is higher than 11.1 mmol/L. The highest blood glucose will not exceed 28 mmol/L if the renal function of a patient with diabetes is normal. So in our study, glucose concentration varied from 0 to 50 mmol/L. The hydrogels we studied are sensitive to the concentration change of glucose under 10 mmol/L, suggesting that these phenylboronic acid contained hydrogels have the potential to be used as plasma glucose probes *in vivo*.

In PBS (pH 7.4) with 0.015 mol/L, 0.030 mol/L and 0.045 mol/L phosphate ions, the diameter of the hydrogel swelled to 3.03±0.12, 2.92±0.23 and 2.86±0.13 times (n=4) of its original diameter, respectively, indicating that slight variation of the buffer concentration did not bring any detectable changes in diameter. The hydrogel swelling changes recorded in the experiments were due to the designed factors alteration in buffer solution.

From the swelling characteristics of the gel in PBS and in CBS, we can deduce that $PO_4^{3-}$ and $CO_3^{2-}$ might combine to the hydrogel in some way and hence alter the hydrophilicity of the gel. The 'medium dependence' equilibrium of PBA was reported in previous work(20,29). Bosch LI and coworkers (20) reported that phenylboronic acid and $PO_4^{3-}$ or citrate$^{3-}$ can form complexes, and the logarithm of derived stepwise constants for Lewis bases to

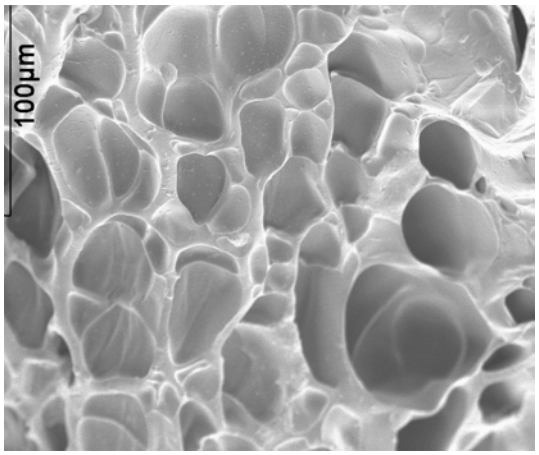


**Figure 7.** Morphology of hydrogel A•PBA-PEG in PBS (pH 7.4) under SEM.

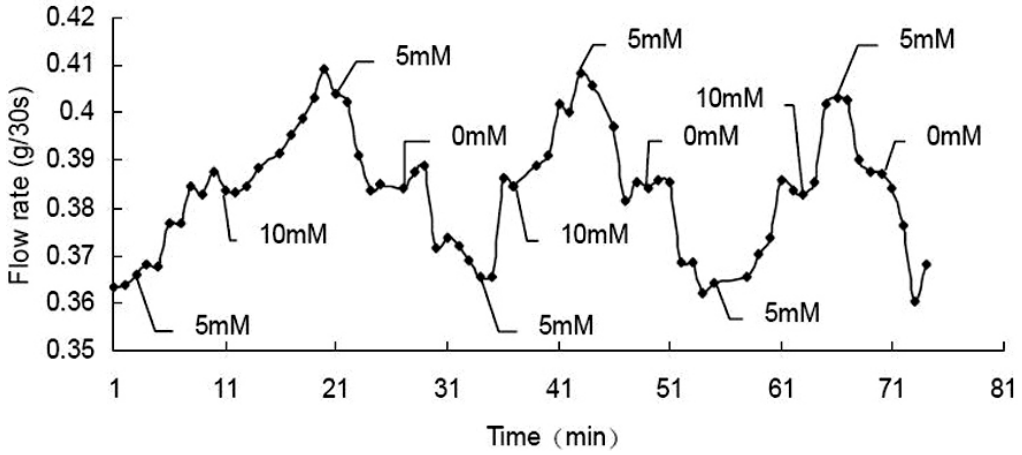


**Figure 8.** The response of hydrogel A•PBA-DMAPMA-EGDMA to glucose in PBS buffer (pH 10).

neutral boronic acids were given. In our study, at pH 9.4, when the buffer PBS and CBS exhibited the same molar concentration (0.015mol/L), the hydrogel absorbed more water in CBS than in PBS. Since phosphate existed mainly in the form of $HPO_4^{2-}$ (more than 99%) in PBS and $HCO_3^-$ accounted for about 90% of the carbonates in CBS when the pH value is 9.4, it can be concluded that the combination constant of $PBA^-$-$HCO_3^-$ is higher than that of $PBA^-$-$HPO_4^{2-}$.

The pH sensitivity of hydrogel A•PBA-DMAPMA-EGDMA is characterized not only by PBA, but also by DMAPMA. In weak acidic solution, the gel swelled because of DMAPMA. Tertiary amine ($R_3N$) in DMAPMA combined with surrounding $H^+$ and increased the hydrophilicity of the gel, which in turn led to hydrogel swelling. Similarly, PBA containing hydrogel should have swelled in weak base solution because of the ionization of PBA. This was the case for hydrogel A•PBA-PEG. However, gel A•PBA-DMAPMA-EGDMA showed an opposite behavior. It shrank in weak basic solution. Two explanations might account for this: firstly, $A•PBA^-$ formed a B-N adduct with $-N(CH_3)_2$ and decreased the hydrophilicity of the gel. Secondly, the interaction of boron and amine within macromolecules made the network shrink.

Similarly, due to the binding between $PBA^-$ and saccharides, there was a shift in the equilibrium towards an increase of charged phenylboronic acid groups (1), the PBA-diol complex formation should cause gel swelling. Again, this was true for hydrogel A•PBA-PEG, which swelled with the addition of both glucose and fructose in weak basic solution. In contrast, hydrogel A•PBA-DMAPMA-EGDMA deswelled with the addition of glucose and remained constant when immersed in fructose solution.

There are papers that reported both swelling (27, 30-34) and contraction (32-34) of 3-PBA-containing hydrogels on glucose addition. Glucose has been reported to combine with PBA to form a 1:1 complex (27, 31-33) or 1:2 bis-bidentate complex(32-39). The 1:1 complex will cause gel swelling, according to the mechanism mentioned above. However, the 1:2 bis-bidentate complex might cause shrinkage of the gel because of the interaction within the network. In our study, we assumed that hydrogel A•PBA-PEG formed a 1:1 complex with both glucose and fructose,

and hydrogel A•PBA-DMAPMA-EGDMA formed a 1:2 bis-bidentate with glucose boronate. However, our present data cannot explain why hydrogel A•PBA-DMAPMA-EGDMA is stable to fructose. Tierney S (34) also found that the incorporation of DMAPAA into A•PBA-containing hydrogel greatly decreased gel sensitivity to fructose. Similar results were reported by Horgan AM (32, 33) when (3-acrylamidopropyl)trimethylammonium chloride (ATMA) was incorporated into a 3-A•PBA-containing hydrogel. Pan X (37, 38) reported that 2-acrylamidophenylboronic acid (APB) formed a 2:1 bis-dentate with glucose, though 3-APB formed a 1:1 complex with a diol compound. Since B-N interaction happened in 2-APB but not in 3-PBA, the results indicated that B-N interaction influenced the complex formation.

In Alexeev VL's study (26), PBA acted as pendants of the gel backbone, but not part of the backbone itself. The incorporation of PEG to hydrogel backbone (2.5 AA per ethylene glycol monomer) converted the glucose-response of gel from swelling (gel PBA-AA-bisAA) to shrinking (gel PBA-AA-PEG-bisAA) at low glucose concentration (below 10 mmol/L), but swelling at high glucose concentration. In contrast, our results showed the glucose-induced swelling of gel A•PBA-PEG. From the point of complex formation view, PBA-AA-PEG formed a 2:1 bis-bidentate with glucose at low glucose concentration but formed a 1:1 complex at high glucose concentration because of competition binding. The molar ratio of PBA to PEG in this study was 2:1, corresponding to PBA:EG monomer (1:34). These sparsely scattered PBA moieties cannot form bis-bidentate with glucose and might have contributed to the relatively slow response rate of the gel. Although this A•PBA-PEG hydrogel is a little more sensitive to fructose than to glucose, considering blood fructose is less than 1% of blood glucose (40), this fructose-sensitivity will not bring a lot of interference in blood glucose detection.

The addition of neighboring amine groups is utilized to increase binding constants of PBA with saccharides at neutral pH. Hydrogel A•PBA-PEG did not show its glucose-sensitivity at a proper concentration range until pH 9.4, and the incorporation of DMAPMA to this gel did not lower the pH at which it started to respond to saccharide (data not shown). On the contrary, DMAPMA lowered the pH value at which hydrogel A•PBA-DMAPMA-EGDMA deswelled. However, the amplitude of this change was very limited and more sensitive detection methods were needed to make use of this response at a neutral pH.

In this study, there are two purposes of introducing PEG as a hydrogel backbone: 1) to increase the *in vivo* compatibility of the hydrogel, and 2) to stabilize the hydrogel to the surrounding ions such as $PO_4^{3-}$ and $CO_3^{2-}$. The results showed that the hydrogel was stabilized to those ions. The somewhat slow response to the change of glucose concentration might be improved by adjusting the molecular weight of PEG used or the ratio of PBA:PEG in the backbone, and this will be our future research work.

## 6. CONCLUSIONS

Glucose-sensitive hydrogel A•PBA-DMAPMA-EGDMA and A•PBA-PEG were prepared and the swelling properties of these two gels in buffer with or without glucose/fructose were studied. Hydrogel A•PBA-DMAPMA-EGDMA was sensitive to pH and glucose but was stable to fructose. It shrank in weak basic PBS solution and the addition of glucose made it shrink more. The complex between glucose and PBA moiety in this gel was supposed to be a 1:2 bis-bidentate complex.

PEG was, for the first time, used as the main composition of the backbone of a PBA-based hydrogel. Hydrogel A•PBA-PEG was sensitive to pH, glucose and fructose. All these stimuli made it swell in neutral to weak basic solution. A 1:1 complex was believed to form between PBA and glucose/fructose.

All the stimuli-responses in the experiments were reversible and the glucose-responses occurred in the range of the physiological/pathological glucose level. Except for the different direction of the response (contraction for gel A•PBA-DMAPMA-EGDMA and swelling for gel A•PBA-PEG), both hydrogels exhibited useable volume change only at a weak basic pH. The structure modifications are still needed in order to get hydrogel, which is glucose-sensitive in physiological/pathological conditions and well tolerated *in vivo*.

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**Abbreviations:** GOD: glucose oxidase, PBA: phenylboronic acid, $PBA^{+}$: 3-aminophenylboronic acid hemisulfate, PEG: poly(ethylene glycol), DMAPMA: N-[3-(dimethylamino)propyl]-methylacrylamide, EGDMA: ethylene glycol dimethacrylate, TEMED: N,N,N,N'-tetramethylethylenediamine, APS: ammonium persulfate, EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, A•PBA: acrylaminophenylboronic acid, PEG•$A_2$: poly(ethylene glycol) diacrylate, EG: ethylene glycol, PBS: phosphate buffer solution, CBS: carbonate buffer solution, Glu: glucose, Fru: fructose, SEM: Scanning electric microscope.

**Key Words:** Glucose-sensitive hydrogel, Phenylboronic acid; Poly(ethylene glycol); Swelling; Self-regulated drug delivery

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