

State of Water in Chitosan–PVA Hydrogel

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ABSTRACT: The bound water fraction (X_{BW}) of a newly developed pH-sensitive, biodegradable chitosan-polyvinyl alcohol (PVA) hydrogel crosslinked with glutaraldehyde (GA) was investigated as a function of the chitosan/PVA molar ratio, GA concentration (C_{GA}), and ionization state. Differential scanning calorimetry (DSC) was used to determine the X_{BW} of the initial hydrogel, and of the hydrogel equilibrated in pH 3 and pH 7 buffers. Changes in X_{BW} during swelling and shrinking of hydrogel were also investigated. In the initial state of hydrogel, X_{BW} increased with increasing PVA concentration (C_{PVA}), without being signifi-

cantly affected by C_{GA} . In the buffer-equilibrated hydrogels, X_{BW} decreased with increasing C_{PVA} and decreasing C_{GA} . The amount of bound water based on dry mass (C_{BW}) was substantially higher when the hydrogel was in the ionized (swollen) state compared to its unionized counterpart. This may be due to the association of a large quantity of water molecules with $-\text{NH}_3^+$ groups of chitosan when the gel swelled in the acidic environment. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 101: 3227–3232, 2006

Key words: chitosan; PVA; hydrogel; bound water; DSC

INTRODUCTION

Hydrophilic polymer networks imbibed with a large amount of water or other biological fluids are known as hydrogels.¹ They could simulate the function of natural gels in living organisms such as swelling, but they do not dissolve in water. The strong interest in hydrogels is because these polymeric materials can trap large amounts of water and solutes, and form a solid-like gel structure. In particular, the relatively high water content and soft, rubbery consistency gives hydrogels a strong resemblance to many soft living tissues. In addition, hydrogels are highly biocompatible with minimal mechanical (friction) irritation to surrounding cells and tissues, which make them ideal for artificial tissues and organ implants.²

pH-sensitive hydrogels change their volume upon subtle changes of pH in the surrounding medium, because the characteristic of a pH-sensitive hydrogel is the crosslinked polyanions or polycations. It has a high density of pendant groups with either electronegative or electropositive charges. These charged groups are the weak or strong acidic groups such as carboxylic acids and sulfonic acids, or weak or strong basic groups such as primary amines and quaternary ammonium salts. Depending on the specific ionizable groups present in hydrogels, they could either ionize

or deionize in response to pH changes in the surrounding medium, and as a result, they absorb water (swell) or discharge water (shrink, contract), correspondingly. A substantial volume or mass change can significantly affect physical properties of the gel matrix.³

Although hydrogels undergo substantial volume or mass change due to the change in total water content, the state of water in the hydrated polymeric networks is more crucial because it governs the interaction between the polymer and other substances, which is vital when the hydrogel is used as bioactive carriers. The state of water in the hydrogel can also influence the biocompatibility when the hydrogel is used as an implant material. It determines the reactivity and bioavailability when used as an encapsulation material for enzyme or cell immobilization.⁴

According to its molecular nature, the water in hydrogels can exist in the following categories:

- category *a* polarized around charged ionic groups, such as $-\text{NH}^+$ and $-\text{COO}^-$ etc.,
- category *b* oriented around hydrogen bonding groups or other dipoles,
- category *c* structured in “ice-cage” conformation surrounding the hydrophobic groups, and
- category *d* imbibed in the capillary pores as bulk (free) water.⁵

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These categories of water present in hydrogels are illustrated in Figure 1. Attempts have been made to separate the total gel water into many categories using

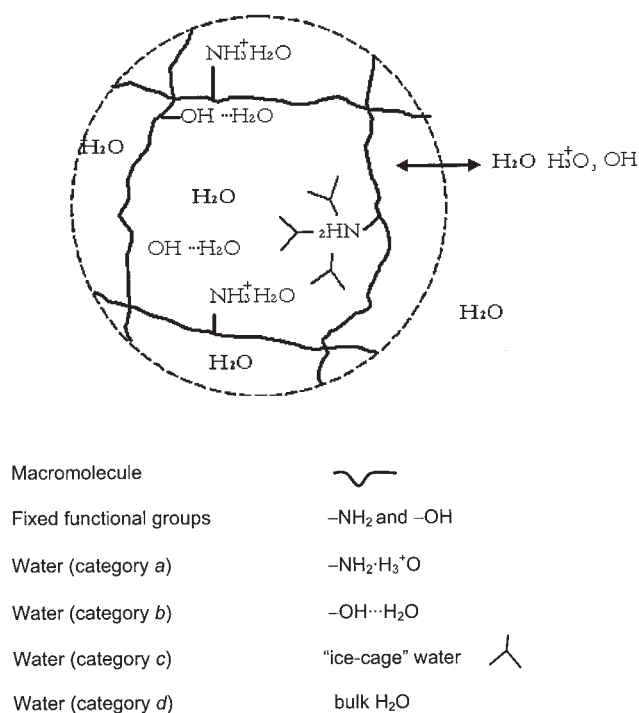


Figure 1 The different water states present in the PVA-chitosan hydrogel.

nuclear magnetic resonance (NMR) technique,^{6–8} by which gel water was classified as “free water” (category *d*), “bound water” (categories *a* and *b*), and the remaining water, called intermediate water (category *c* or others). In general, as the total water content of the hydrogel increases, the free water fraction increases and bound water fraction decreases. The intermediate water fraction undergoes only small changes with the changing gel water content.⁹ Similar conclusions were drawn concerning the state of water in poly 2-hydroxyethyl methacrylate (P-HEMA) based hydrogel.¹⁰ In addition to NMR,¹¹ other techniques like differential scanning calorimetry (DSC) and wide-angle X-ray diffraction are also commonly employed to study the water states of hydrogels.^{10,12–15}

A pH-sensitive chitosan–polyvinyl alcohol (PVA) hydrogel crosslinked with glutaraldehyde has been developed.^{16,17} This hydrogel exhibits some promising attributes such as high swelling and shrinking ratio, high sensitivity to pH change, and possibility of biodegradable (chitosan and PVA are both biodegradable). Its structural properties have been reported previously.¹⁷ To take full advantage of this new hydrogel, it is essential to characterize its basic properties. The goal of this investigation was to better understand the state of water present in the chitosan–PVA hydrogel. The specific objectives were to investigate: (1) change in bound water content during swelling of the hydrogel, (2) effect of hydrogel composition and crosslinker concentration on the bound water content, (3) effect of

ionization state of the hydrogel (when swollen in pH 3 buffer or shrunk in pH 7 buffer) on the bound water content.

MATERIALS AND METHODS

Hydrogel formation

Chitosan and PVA mixture solution (chitosan:PVA molar ratios of 1 : 0, 1 : 5, and 1 : 10) was prepared as described in our previous publication (Wang et al., 2004). Various amounts of the crosslinker, glutaraldehyde (GA), was slowly added into the mixture under constant stirring to obtain final GA concentrations (C_{GA}) of 33.3, 83.3, and 166.5 μM in the pregel solution. The well-mixed pregel solution was immediately discharged from a burette into a 15-mL centrifuge tube containing oleic acid to help the formation of gel beads. The diameter and shape of burette tip controlled the size of final beads. About 15 to 20 beads were prepared in one tube. The tubes were tightly screw-covered and horizontally placed on a rotator (7637-01, VWR Scientific, West Chester, PA) and rotated at 12 rpm for 1 h to 8 h depending on C_{GA} and PVA concentration (C_{PVA}) for solidifying the gel structure. Gel beads were harvested after filtering the oleic acid and kept in an airtight container in a refrigerator until experiment. Surface oleic acid was removed by rinsing the beads with petroleum ether for a short time just before using.

The diameter of the hydrogel beads was measured in two perpendicular positions using a digital micrometer (Mitutoyo CD-6" BS, Mitutoyo Corp., Japan). The mass of bead was recorded by an analytical balance (AG245 Toledo, Mettler, Worthington, OH). The average diameter and mass of gel beads (based on five beads) were 4.68 ± 0.16 mm and 53.54 ± 8.03 mg, respectively.

Determining hydrogel total water content

The total water (TW) content of the hydrogel was determined gravimetrically by oven drying. At a specific time the hydrogel beads were taken from the buffer solution and surface dried carefully using a filter paper. The total wet mass of the gel beads (M_{TOTAL}) was measured by the balance. Then the gel beads were dried in an oven set at 105°C for 4 h. The dry gel mass (M_{DRY}) was measured after the gel beads were cooled in a desiccator. The total water content of the hydrogel (X_{TW}) was calculated as:

$$X_{TW} = 1 - \frac{M_{DRY}}{M_{TOTAL}} \quad (1)$$

Determining the free and bound water contents

A differential scanning calorimeter, DSC (2920 Model, TA Instruments, New Castle, DE) was used to

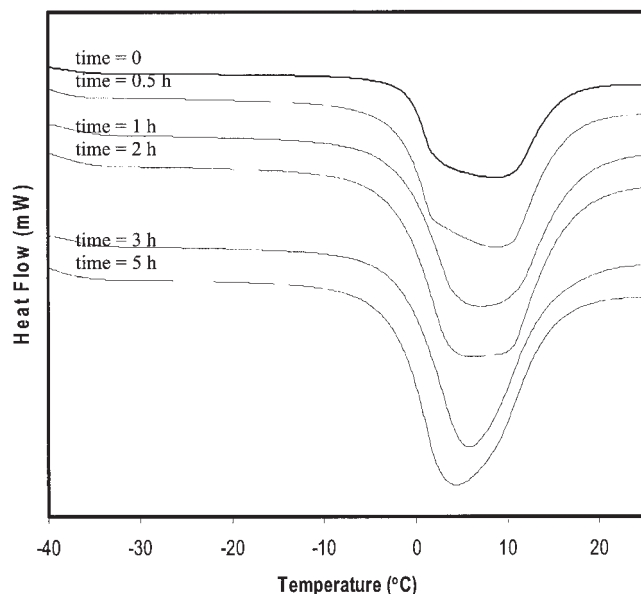


Figure 2 DSC endothermic profiles of hydrogel (chitosan:PVA molar ratio = 1 : 10, glutaraldehyde concentration = 33.3 μM) swelling in pH 3 buffer solution at time 0, 0.5, 1, 2, 3, and 5 h.

monitor the bound water of the hydrogel during swelling or shrinking. 3 to 10-mg sample of hydrogel was transferred into a DSC aluminum hermetic pan and sealed tightly to prevent water loss during DSC scanning. The pan was first cooled to 210 K, and then heated to 303 K at a rate of 10 K/min. The phase transition of water in the hydrogel during heating was recorded as the endothermic peak, which was later integrated using commercial software (Universal Analysis 2.3, TA Instruments, New Castle, DE). Fraction of the freezable (mostly category *d*) water and unfreezable (mostly categories *a* and *b*) water in the samples was determined using the following equation, assuming that the heat of fusion of free water in the hydrogel is the same as that for ice.^{12–14}

$$X_{\text{BW}} = X_{\text{TW}} - \left(\frac{Q_{\text{endo}}}{Q_f} \right) \quad (2)$$

where, X_{TW} is the total water fraction in hydrogel; X_{BW} is the bound water fraction in hydrogel; Q_{endo} is the heat of fusion for freezable water in hydrogel as obtained from the DSC-thermogram (J/g). Q_f is the heat of fusion of pure water (=333 J/g).

Eighteen beads with similar dimensions were selected and immersed in 100-mL pH 3 (0.1M sodium acetate–acetic acid) buffer for swelling or in pH 7 (0.1M sodium phosphate) buffer for shrinking at room temperature ($\sim 25^\circ\text{C}$). The beads were periodically removed from the buffer solutions and surface dried with a filter paper and a small portion of bead was sampled to determine X_{BW} using the DSC as described

above. Similarly, X_{BW} of the hydrogel beads at different pH was determined after they were equilibrated in various pH buffers ranged from pH 2 to 11. The hydrogel beads were prepared with a different C_{GA} and C_{PVA} combination and soaked in the pH 3 or 7 buffer until equilibrium. The X_{BW} of its initial (unswollen), swollen, and shrunken beads was also determined using the DSC as described previously.

RESULTS AND DISCUSSION

Bound water changes during swelling and shrinking

A series of DSC scanning profiles of hydrogel swelling in pH 3 buffer for the initial 5 h was recorded and showed in Figure 2. The gradual expansion of endothermic peak indicated the increasing of free water fraction of hydrogel up to 3 h of swelling. Changes in X_{BW} in the hydrogel during soaking in pH 3 and 7 buffers are shown in Figure 3. Possible interaction effects due to the presence of water concentration profiles within the preequilibrium hydrogel samples were considered negligible. Initially, the X_{BW} increased in both buffer solutions. It may be because of $-\text{NH}_2 \rightarrow -\text{NH}_3^+$ protonization due to its acidic characteristics when the hydrogel first comes in contact with the water molecules in the buffer. The formation of $-\text{NH}_3^+$ leads to swelling of the hydrogel during this initial moment regardless of the surrounding buffer pH and $-\text{NH}_3^+$ formed tends to tie up water and attract counter ions. As a result, there is more bound water molecules consequently (X_{BW} , categories

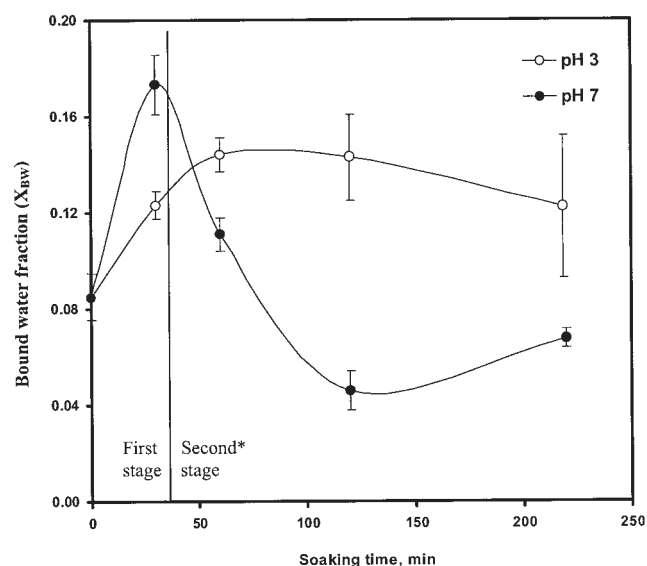


Figure 3 Bound water fraction in the hydrogel (chitosan:PVA molar ratio = 1 : 10, glutaraldehyde concentration = 33.3 μM) during swelling in pH 3 buffer and contracting in pH 7 buffer. *The stage when hydrogel started contraction in pH 7 buffer.

a and *b*), as shown by the first ~30 min in both pH 3 and 7 buffers. The X_{BW} decreased slightly with time as swelling continued in the pH 3 buffer. This change is not statistically significant. This may be explained by simultaneous increase in free water fraction (X_{FW}) when total water fraction (X_{TW}) increased. In other words, the increase of total water at this stage (second stage in Fig. 3 of pH 3 swelling) is solely caused by the increase of free water in the hydrogel. The water imbibed later tended to stay relatively far from the charged groups, which are already surrounded by water molecules absorbed earlier. For this reason, this part of water would more likely to be the free water (category *d*) when determined using DSC. The higher amount X_{BW} of hydrogel in pH 7 buffer (first stage) may due to its lower X_{TW} compared to its pH 3 buffer counterpart.

As the hydrogel continues soaked in pH 7 buffer, the excessive pH 7 buffer slowly neutralized the acidic environment of the hydrogel bead. Therefore, the transformation of $-\text{NH}_3^+ \rightarrow -\text{NH}_2$ took place. When hydrogel lost its charged groups, so did its ability to attract water molecules. Consequently, the X_{TW} decreased. This was confirmed by soaking the hydrogel during the second stage in pH 7 buffer (Fig. 3). The slight increase in X_{BW} at end of the second stage while soaked in pH 7 buffer was shown in Figure 3. It could not be verified true at this moment. One possibility is that this might cause by a faster decrease of X_{TW} than its X_{FW} due to the dramatic loss of total gel mass at that time.

Bound water content after equilibrium at different pHs

Bound water concentrations (C_{BW} , mg/mg dry hydrogel) at various pHs are shown in Figure 4. In the acidic medium, most available amino groups were protonated, and might couple with CH_3COO^- (Ac^-). As a consequence, they were more likely to associate with water molecules compared to their electroneutral counterparts, therefore, it attracted more counterions and water molecules, and formed more hydration layers around the polymer chains. The observed higher C_{BW} in the acidic region (pH <6) supports this hypothesis (Fig. 4). Although charged amino groups were deionized when buffer pH is higher than pK_a of the chitosan (≈ 6.3), the attractive forces (driving force) for the counterions decrease as well as the ability to associate or "bind" with water molecules. The hydration layers also diminish correspondingly, which is indicated by the lower C_{BW} value in Figure 4 when buffer pH passed from the acidic to neutral and further to alkali region.¹⁰

The hydrogel had the maximum C_{BW} in pH ~3 medium (Fig. 4). The bound water for a polymer network is mainly associated with (1) the ionic groups such as $-\text{NH}_3^+$ present in the network (category *a*);

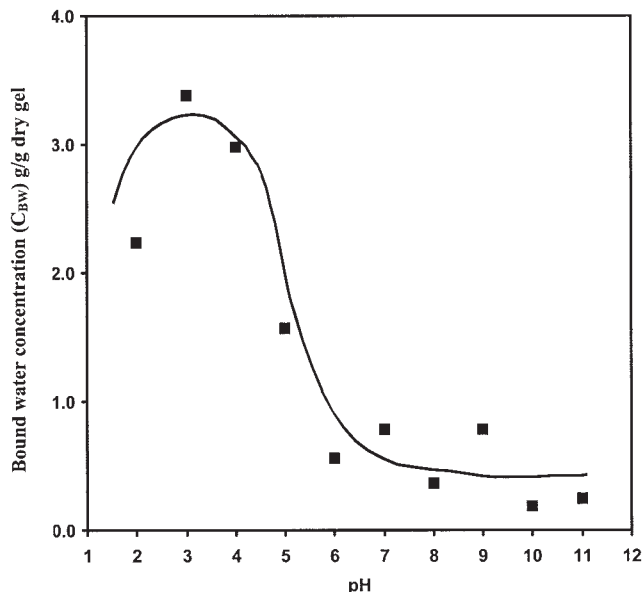


Figure 4 Bound water concentration in the chitosan-PVA hydrogel at different pHs (chitosan : PVA molar ratio = 1 : 10, glutaraldehyde concentration = 33.3 μM).

(2) total hydrophilic groups such as hydrogen bonding formation groups, like $-\text{OH}$ (category *b*). As hydrogel approaching pH 3, the effective H^+ concentration seemed to reach the optimum number because of a sodium acetate and acetic acid (NaAc-HAc) buffer system. At this pH, the ionization of $-\text{NH}_2$ was maximized, which attracted most counterions and water molecules as a result, that is, formed maximum amount of BW as shown in Figure 4. If the pH goes too low, the high concentration of H^+ will actually impair the swelling, that is, decrease X_{TW} . On the other hand, if pH goes too high, it may not fully ionize all the $-\text{NH}_2$. Certainly, when pH is greater than the pK_a of chitosan, there is very little ionization of $-\text{NH}_2$, which substantially decreases C_{BW} as shown in neutral and alkali regions of Figure 4.

Bound water as a function of gel composition

The X_{BW} of initial hydrogels with different C_{PVA} and C_{GA} are listed in Table I. The X_{BW} and C_{BW} of the pH 3 and pH 7 buffer-equilibrated hydrogels with different C_{PVA} and C_{GA} are listed in Table II.

The X_{BW} of the initial hydrogel increased with increasing PVA concentration (C_{PVA}). This was because of two reasons: (1) the initial gel is a "closed" system, which has limited water content; (2) any addition of hydrophilic groups into this system inevitably lowers the total water content, and more importantly, lowers the free water content due to category *b* association with water molecules. As a hydrophilic polymer, PVA contains high density of hydrophilic groups, $-\text{OH}$, which could easily form hydrogen bonds between the

TABLE I
Initial Bound Water Fraction (X_{BW}) and Bound Water Concentration (C_{BW}) in Different Chitosan-PVA Hydrogels at Different Glutaraldehyde Concentration (C_{GA})

Chitosan/PVA molar ratio	C_{GA} (μM)	Initial $X_{BW} \times 100$	Initial C_{BW} (g/g)
1/0	33.3	$8.88 \pm 0.28^*$	8.7 ± 0.3
1/0	83.2	7.54 ± 0.58	7.4 ± 0.6
1/0	166.5	8.25 ± 0.32	8.1 ± 0.3
1/5	33.3	9.93 ± 0.41	4.5 ± 0.2
1/5	83.2	8.47 ± 0.67	3.8 ± 0.2
1/5	166.5	8.55 ± 0.35	3.9 ± 0.1
1/10	33.3	16.5 ± 0.28	4.8 ± 0.1
1/10	83.2	16.1 ± 0.62	4.7 ± 0.2
1/10	166.5	15.5 ± 0.44	4.5 ± 0.1

^a Standard deviation was calculated based on three replicates.

limited free water molecules.¹⁸ As water associated with hydroxyl groups of PVA polymer chains, there is less free water available in this system, that is, higher X_{BW} . This occurs substantially at higher C_{PVA} , which is believed to facilitate the formation of hydrogen bonds.¹⁹ A similar conclusion has been drawn by studying the water-binding ability of hydrophilic and hydrophobic groups of chemically modified cellulose. The hydrophilic hydroxypropylcellulose favored a relatively large amount of water-binding capacity compared to methylcellulose.²⁰ The increasing C_{GA} investigated here does not seem to have a substantial effect on X_{BW} (Table I).

For the hydrogel equilibrated in pH 3 and pH 7 buffers, the C_{BW} was higher in pH 3 buffer than in pH 7, which is consistent with our findings that the charged $-\text{NH}_3^+$ groups are responsible for the formation of bound water in the hydrogel (Table II).

For the hydrogel equilibrated in pH 3 or pH 7 buffer solutions, the C_{BW} increased with increasing C_{GA} (Fig. 5). As we know, glutaraldehyde reacted with two $-\text{NH}_2$ groups either on the same chitosan molecule or between two different molecules to form a "loop" or a "bridge" structure, respectively. It was reasonable to postulate that as more crosslinkers are used in the hydrogel, the higher degree of localization or immo-

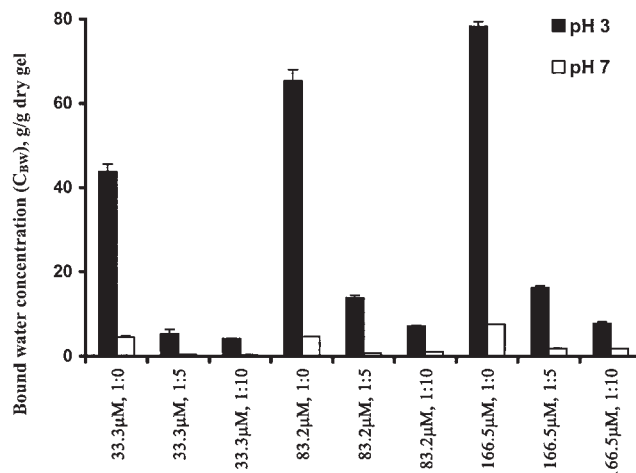


Figure 5 Bound water concentration in the chitosan-PVA hydrogel at different pHs, chitosan-PVA molar ratios (1 : 0, 1 : 5, 1 : 10) and crosslinker concentrations (33.3, 83.2, 166.5 μM).

bilization of water molecules in a unit of gel matrix. As a result, it might change the properties of the localized water and result in an increase of C_{BW} . This result was also true in hydrogels of different chitosan-PVA molar ratios (Fig. 5).

The effect of C_{PVA} on equilibrium X_{BW} was somewhat "complex." It was not like the previous initial hydrogel, a "closed" system (without water and ion exchanges with external buffer medium, the $-\text{NH}_3^+$ and $-\text{OH}$ groups competed to associate with water molecules) in which increased C_{PVA} increased the X_{BW} . The buffer-equilibrated hydrogel is an "open" system, which could freely exchange for water and ion molecules with an external buffer solution and has an excessive supply of water molecules for binding and association. After a long period of exchanging water and ions with the external buffer solution, the hydrogels underwent significant volume or mass changes, the X_{BW} actually decreased with increasing C_{PVA} . This indicated a decrease in the water binding ability of the hydrogel, which could be caused by interfering the interaction between $-\text{NH}_3^+$ and water molecules due

TABLE II
Bound Water Fraction $\times 100$ ($X_{BW} \times 100$) and Bound Water Concentration (C_{BW} , g/g Dry Polymer) in the Chitosan-PVA Hydrogels Equilibrated in pH 3 and pH 7 Buffers at Different Glutaraldehyde Concentration (C_{GA})

C_{GA} (μM)	Chitosan/PVA molar ratio											
	1/0		1/5		1/10		1/0		1/5		1/10	
	pH3	pH7	pH3	pH7	pH3	pH7	pH3	pH7	pH3	pH7	pH3	pH7
	X_{BW}	C_{BW}	X_{BW}	C_{BW}	X_{BW}	C_{BW}	X_{BW}	C_{BW}	X_{BW}	C_{BW}	X_{BW}	C_{BW}
33.3	14.9	43.8	16.6	4.5	3.6	5.4	4.4	0.4	5.4	4.8	3.2	0.3
83.2	22.3	65.4	17.2	4.6	9.3	13.9	7.0	0.7	8.1	7.3	10.0	0.8
166.5	26.7	78.3	28.2	7.6	10.8	16.2	17.9	1.9	8.7	7.9	18.1	1.8

to the competition of a high concentration of —OH groups present in the hydrogel. Also, introduction of PVA may create an unstable, inhomogeneous crosslinkage and less localization for water molecules. The significantly low C_{BW} was caused by averaging down the effect by the introduced PVA content (mass), because of the weaker water-binding ability of the —OH group compared to the —NH₃⁺ group due to its fewer polar moieties.

CONCLUSIONS

The bound water fraction (X_{BW}) of the chitosan–PVA hydrogel depended on the composition and ionization state of the hydrogel. For an initial “closed” hydrogel system, the X_{BW} increased with increasing PVA concentration (C_{PVA}). The effect of the added crosslinker glutaraldehyde concentration (C_{GA}) was minimal. For an “open” equilibrated hydrogel, the X_{BW} decreased with increasing C_{PVA} and decreasing C_{GA} . The bound water concentration (C_{BW}) reached the maximum value when the hydrogel was swollen in pH 3 buffer. The significant difference in C_{BW} at pH 3 and 7 was caused by the ionized —NH₃⁺ groups at pH 3, which prompted the formation of bound water.

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