

ORIGINAL ARTICLE

Facile preparation of transparent poly(vinyl alcohol) hydrogels with uniform microcrystalline structure by hot-pressing without using organic solvents

Tomoyo Sakaguchi¹, Shusaku Nagano², Mitsuo Hara³, Suong-Hyu Hyon⁴, Monika Patel¹ and Kazuaki Matsumura¹

Poly(vinyl alcohol) hydrogels (PVA-Hs) are promising materials for various biomedical applications and have been studied extensively. Low-temperature crystallization is the most popular method used to prepare PVA-Hs with excellent mechanical properties. However, this method uses dimethylsulfoxide (DMSO) as a solvent, which is toxic and difficult to handle. In this study, a novel hot-pressing method was developed for preparing transparent PVA-Hs in order to eliminate the need of DMSO for solubilizing PVA during gelation. Unlike the conventional methods, this method used high initial concentrations of PVA, which made the molding of the gels easy and enhanced their gelation. The hydrogels prepared by hot-pressing showed rapid gelation of the PVA molecules along with an enhanced crystallinity, unlike the hydrogels prepared by freezing and thawing. The efficiency of different solvents (water and DMSO/water mixtures) for the preparation of PVA-Hs by the hot-pressing method was tested. The total amount of crystallites was the same for all the gels irrespective of the solvent used. However, the gels solubilized in only water showed a decrease in the net crystal size. This method not only eliminates the use of DMSO in preparing PVA-Hs but also produces gels with high mechanical properties for future use.

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INTRODUCTION

Hydrogels are three-dimensional cross-linked macromolecular networks having high water holding capability.^{1–3} Their unique properties provide a great opportunity to explore their uses in various dimensions of biological fields.^{4–6}

Since the preparation of crosslinked poly(2-hydroxyethyl methacrylate) hydrogels by Wichterle and Lim,⁷ hydrogels have rapidly made their way from laboratory to market being of primary interest to the field of biomaterials for applications such as wound dressing,^{8,9} drug delivery,^{10,11} agriculture,¹² implants^{13,14} and so on.

Hydrogels can be categorized into various categories based on their chemical nature, stimuli responsiveness, strength and so on. In addition, hydrogels are broadly classified into two types: chemical and physical gels.¹⁵

Chemical gels are covalently crosslinked networks formed by replacing the hydrogen bonds in the main chain by stronger and stable covalent bonds. The commonly used chemical methods for the synthesis of hydrogels include crosslinking with chemical crosslinkers, grafting and radiation in solid and/or aqueous states.^{15,16}

Physical gel networks on the other hand are held together by molecular entanglements and/or secondary forces including ionic, hydrogen bonding or hydrophobic interactions. Recently, physical gels

have gained immense attention because they are relatively easy to produce, exhibit reversible sol–gel transitions and do not use chemical crosslinking agents, which are toxic and need to be extracted or neutralized before using the gels for their intended applications. Moreover, chemical crosslinking agents can also undergo unwanted reactions with bioactive substances present in the hydrogel matrix.^{15,16}

In this context, poly(vinyl alcohol) hydrogels (PVA-Hs), which are physically crosslinked, have several advantages over chemically crosslinked gels, especially for biomaterial applications. Since Bray and Merrill¹⁷ first reported the use of PVA-Hs in artificial cartilages, they have been extensively studied for various biomedical owing to their ease of characterization. Moreover, PVA-Hs exhibit biocompatibility and mechanical, fluid flow and frictional properties similar to those exhibited by articular cartilage.^{1,17–21}

It is well known that aqueous solutions of PVA gradually undergo gelation upon standing at room temperature. This gelation results from the formation of networks, in which the PVA crystallites generated by spinodal decomposition serve as the junction points.²² However, such gels do not exhibit properties required to be used as biomaterials.²³

A new freezing and thawing method was reported by Peppas for preparing PVA-Hs with enhanced properties.²⁴ In this method, an aqueous solution of 2.5–15 wt% PVA is frozen at $-20\text{ }^{\circ}\text{C}$ and is

¹School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa, Japan; ²Nagoya University Venture Business Laboratory, Nagoya University, Nagoya, Japan; ³Graduate School of Engineering, Nagoya University, Nagoya, Japan and ⁴Center for Fiber and Textile Science, Kyoto Institute of Technology, Kyoto, Japan

Correspondence: Professor K Matsumura, School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi 923-1292 Ishikawa, Japan. E-mail: mkazuaki@jaist.ac.jp

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Table 1 Preparation processes of various PVA-hydrogels: LTC; FT; HP-W; and HP-D/W

Process	LTC	FT	HP-W	HP-D/W
Solution condition	DMSO/water = 80/20 (w/w) Initial PVA concentration 10% (w/w)	Only water Initial PVA concentration 10% (w/w)	Only water Initial PVA concentration 50% (w/w)	DMSO/water = 50/50 (w/w) Initial PVA concentration 50% (w/w)
Dissolving method	Stirring (95 °C, <1 h)	Stirring (95 °C, <1 h)	Hot-pressing (95 °C, <30 min, <2–20 MPa)	Hot-pressing (95–130 °C, <30 min, <2–20 MPa)
Gelation	–20 °C, <24 h	–20 °C, <24 h	25 °C, 7 days	25 °C, 3 days
Desolvation	Soak in ethanol for 2 days	Drying under room temperature	Drying under room temperature	Soak in ethanol for 2 days
Drying	Under vacuum (1 day)	Under vacuum (1 day)	Under vacuum (1 day)	Under vacuum (1 day)

Abbreviations: DMSO, dimethylsulfoxide; FT, freezing-and-thawing method; HP-D/W hot-pressing method (DMSO and water); HP-W, hot-pressing method (only water); LTC, low-temperature crystallization method; PVA, poly(vinyl alcohol).

subsequently thawed back to room temperature to facilitate the crystal formation. As the number of freezing/thawing cycles is increased, the number and stability of these crystallites also increases because of the condensation of the PVA solution by the formation of ice.²⁵ Since this pioneering report, the freezing and thawing method has been extensively studied.^{26–29} However, because of the macroscopic phase separation between the concentrated and dilute PVA solutions during the crystallization of ice, the gels prepared by this method are opaque and weak. The other problems associated with this technique include the melting out of the crystallites and over-crystallization with time.³⁰ These complications can significantly affect the long-term performance of the resulting gels and need to be addressed when considering long-term applications.

As a solution to this problem, Hyon and Ikada prepared transparent PVA-Hs by the low-temperature crystallization method and achieved PVA-Hs with high mechanical strength, high water content (WC) and excellent transparency.^{31,32} In this method, PVA was first dissolved in a water/dimethylsulfoxide (DMSO) solvent at a low temperature (–20 °C). This promoted the crystallization and crosslinking of the PVA molecules without causing the spinodal decomposition.³³ However, the use of water/DMSO solvents has safety issues.

In a similar study, Suzuki and co-workers prepared PVA cast gels using only water as the solvent.³⁴ The resulting hydrogels showed good mechanical properties. However, their method required a prolonged drying time, which influenced the physical properties of the gels.

Therefore, in order to address these issues, we report a novel hot-pressing method to prepare PVA-H with uniform microcrystallite structure, which should lead to good mechanical properties by simply utilizing highly concentrated aqueous PVA solutions, thus eliminating the need to use DMSO as the solvent.

MATERIALS AND METHODS

Sample preparation

Freezing and thawing method: PVA-H (FT). A unit of 15 g PVA, with a viscosity-average degree of polymerization of 1700 and a degree of saponification of 98.5 mol% (Japan VAM & POVAL, Osaka, Japan), was dissolved in 135 g H₂O at 95 °C. The resultant solution (10% w/w) was poured between two brass plates with a 3 mm-thick spacer and cooled to –20 °C for 24 h. After thawing at 4 °C, the obtained hydrogels were dried in air for 3 days and in vacuum for 2 days (Supplementary Figure S1). Thus-prepared PVA hydrogels are hereafter denoted as PVA-H (FT).

Low-temperature crystallization method: PVA-H (LTC). A unit of 15 g PVA was dissolved in a solvent mixture of DMSO (108 g) and H₂O (27 g) (80/20 w/w) at 95 °C. The solution (PVA concentration was 10% w/w) was poured between two brass plates with a 3 mm-thick spacer and was cooled to –20 °C for 24 h. The PVA gel sheets obtained by low-temperature crystallization were immersed in excess ethanol at 25 °C for 3 days to remove the solvents from the gels. Ethanol was removed from the hydrogel by vacuum

drying for 2 days (Supplementary Figure S2). Thus-prepared PVA hydrogels are hereafter denoted as PVA-H (LTC).

Hot-pressing method: PVA-H (HP-W)/PVA-H (HP-D/W). A unit of 20 g PVA powder was swollen in 20 g of solvent (either water or DMSO/H₂O mixture (80/20 w/w)) at room temperature. To obtain the hydrogel, a brass frame mold (183 mm × 134 mm) with 2 mm thickness was used.

In the case of hydrogel prepared with water as a solvent, the temperature of hot-pressing machine (AH-2003, AS ONE, Osaka, Japan) was set at around 95 °C. The swollen PVA (40 g) was placed on the plate of the hot-pressing machine and was pressed at 2 MPa for 5 min, followed by 10 MPa for 10 min and finally at 20 MPa for 15 min. After removing from the hot-pressing machine, the PVA solution was kept in the mold at room temperature for gelation, without drying for 1 week. Subsequently, thus-obtained hydrogels were dried in air for 2 days, followed by vacuum drying for 2 days (Supplementary Figure S3).

When the mixed solvent was used, the temperature of the hot-pressing machine was set at ~130 °C. The swollen PVA was set on the pressing plate of the machine and pressing was started, allowing the temperature to gradually decrease to 95 °C during pressing. During this gradual decrease, a similar pressing pattern was applied as for water-swollen PVA. After this, the pressed PVA was kept in the mold for gelation at room temperature for 1 week. Subsequently, DMSO/H₂O mixture was removed by immersing the gels in excess ethanol at 25 °C for 3 days, followed by ethanol removal from the samples by vacuum drying for 2 days (Supplementary Figure S4). PVA-Hs prepared by hot-pressing with water solvent and mixed solvent are hereafter denoted as PVA-H (HP-W) and PVA-H (HP-D/W), respectively. Hydrogel preparation processes are summarized in Table 1.

PVA elution into water. To determine the gelation speed, we measured time-dependent elution ratio (ER) of PVA molecules into water from PVA-H (HP-W).

Test pieces (20 × 20 mm²) were cut from the PVA gels at definite intervals during gelation of PVA (at room temperature) after completion of the pressing and were soaked in distilled water. After 2 days of soaking, the test pieces were removed, dried completely and the weights of PVA-H (W_{gel}) and of eluted PVA gels (W_{elu}) were measured.

The ERs of the PVA gels were calculated from the equation (1)

$$\text{ER}(\%) = \frac{W_{\text{elu}}}{W_{\text{elu}} + W_{\text{gel}}} \times 100 \quad (1)$$

Small-angle X-ray scattering. Micro-crystalline structure in PVA-H (HP-W) and PVA-H (HP-D/W) was determined by time-dependent small-angle X-ray scattering (SAXS) analysis for 1–8 days after hot-pressing.

SAXS measurement was performed with a NANO-Viewer (Rigaku, Tokyo, Japan) at a voltage of 45 kV and current of 60 mA, with irradiation time of 12 h to produce Cu K α radiation ($\lambda = 0.154$ nm). The camera length was 960 mm. Test samples cut with 1 mm thickness (PVA-H (HP-W)) or 2 mm thickness (PVA-H (HP-D/W)) were irradiated from the cross-sectional direction.

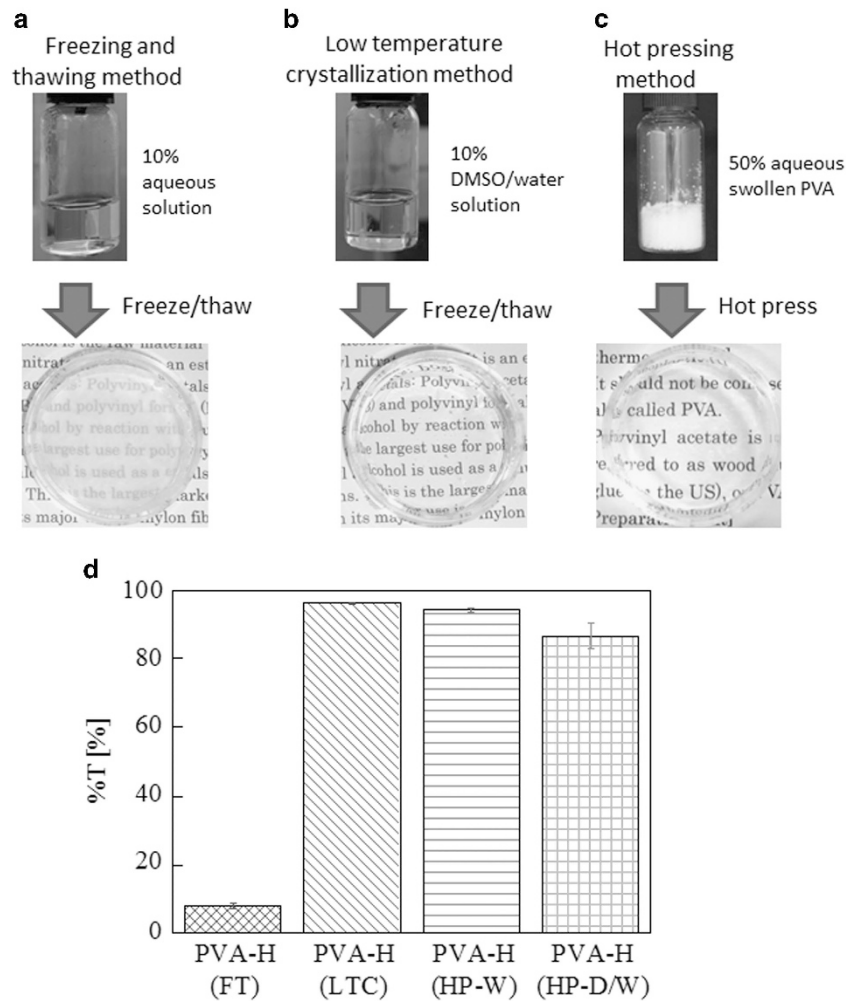


Figure 1 Photographs of poly(vinyl alcohol) hydrogels (PVA-Hs) prepared by various methods: (a) Translucent PVA-H (FT); (b) PVA-H (LTC) transparent hydrogel; (c) PVA-H (HP-W) transparent hydrogel prepared without using dimethylsulfoxide. (d) Percent light transmittance at 550 nm of each PVA-H. HP-W, hot-pressing method (water); FT, freezing and thawing method; LTC, low-temperature crystallization method. A full color version of this figure is available at *Polymer Journal* online.

Wide-angle X-ray scattering. Amount of crystallization in PVA-H (HP-W) and PVA-H (HP-D/W) were determined by time-dependent wide-angle X-ray scattering (WAXS) analysis for 1–8 days after hot-pressing.

WAXS measurement was performed with a FR-E (Rigaku) at a voltage of 45 kV and current of 45 mA, with irradiation time of 10 min to produce Cu K α radiation ($\lambda = 0.154$ nm). The camera length was 180 mm. Test samples cut with 1 mm thickness (PVA-H (HP-W)) or 2 mm thickness (PVA-H (HP-D/W)) were irradiated from the cross-sectional direction.

Water contents of PVA-H. Dried PVA-H prepared by each method was immersed in excess water at 25 °C for 2 days to obtain swollen PVA-H. The WC of the PVA-H was calculated using Equation (2).

$$\text{WC}(\%) = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100 \quad (2)$$

where W_{wet} is the weight of hydrated PVA-H and W_{dry} is the weight of dried PVA-H.

Differential scanning calorimetry. Crystallinities of PVA-H (FT) and PVA-H (HP-W) were measured by differential scanning calorimetry (DSC; DSC8500, Perkin Elmer, Norwalk, CT, USA) under N₂ gas.

About 2 mg of dried grain sample was cut and was sealed in an aluminum DSC pan. This pan was heated from 10 to 280 °C at a rate of 10 °C min⁻¹. The crystallinity (CR) was calculated using Equation (3) as the ratio between the

heat required to melt the polymer (ΔH) with the heat required to melt a 100% crystalline PVA ($H = 138.6$ J g⁻¹).³⁵

$$\text{CR}(\%) = \frac{\Delta H}{H} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Preparation of PVA-H

To precisely evaluate PVA-Hs by our method, we compared the characteristics of the hydrogels prepared by hot-pressing method (our method) with the gels prepared by conventional methods (freeze-thawing method and low-temperature method).

Figure 1 shows physical appearances of the precursors for preparing PVA-H by all three methods (top) and those of the resulting gel (bottom). The gels prepared by the freeze-and-thaw method were obviously translucent (Figure 1a). The freezing process necessarily induces condensation of the PVA solution due to formation of ice crystals, which causes phase separation of the solution into frozen phase (ice) and concentrated polymer solution phase during gelation.³¹ The resulting PVA-H (FT) comprises nonuniform polycrystalline PVA structures that give visible light scatterings.³⁶

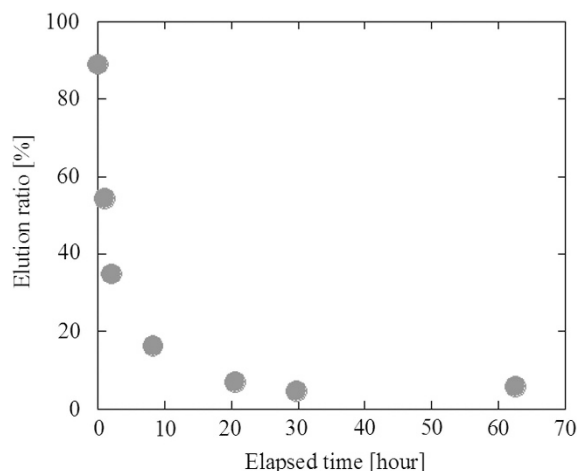


Figure 2 Time-dependent elution of PVA molecules from PVA-H (HP-W) into water. HP-W, hot-pressing method (water); PVA-H, poly(vinyl alcohol) hydrogel. A full color version of this figure is available at *Polymer Journal* online.

On the other hand, PVA-H prepared by low-temperature crystallization showed good transparency in the formed hydrogel (Figure 1b). The mixed solvent (DMSO/water) exhibits a freezing point lower than -20°C and prevents phase separation, unlike the freeze-and-thaw method. In this method, the crystallization of water to ice was avoided, enhancing the formation of small crystals of PVA at low temperatures, without the phase separation due to spinodal decomposition.^{31,37}

The hot-pressing method successfully produced PVA-H with high transparency without using DMSO (Figure 1c). However, in this method the high initial concentration of PVA might accelerate crystallization without spinodal decomposition. Owing to the high initial concentration of PVA, high molecular entanglement can be achieved in the resulting transparent hydrogel without resorting to low temperatures. In this paper, we compare the process of gelation of transparent PVA-H (Figure 1d) by hot-pressing method with the conventional freeze-thaw and low-temperature crystallization methods.

PVA elution into water

To further compare the gels prepared by hot-pressing method with those prepared by the other two methods, the gelation time and efficiency of the novel method needs to be tested. The gelation time of PVA-H (HP-W) was determined by studying the elution rate of PVA molecules from PVA-H (HP-W) after removal from the hot-pressing machine. Figure 2 shows that the ER decreased drastically with increase in the resting time after hot-pressing. PVA-H (HP-W) after 30 min from hot-pressing showed more than 80% elution of PVA chains from the gels, in contrast to almost no elution from PVA-H (HP-W) after 2 days from pressing. During the resting period after pressing, the gels probably mature significantly. As PVA gelation progressed during this period due to increased entanglement of the chains in the hydrogel, which were not physically crosslinked in the gel by small crystallites, elution of PVA molecules has decreased to a great extent. As previously reported, PVA cast gel also showed elution as low as 8% after the gel reached equilibrium state.³⁸ On the other hand, PVA-H prepared by seven repetitive freeze-thaw cycles showed $\sim 20\%$ dissolution after 30 days.³⁰ Thus, comparing these studies, PVA-H (HP-W) showed a relatively higher gelation speed with better gelation efficiency.

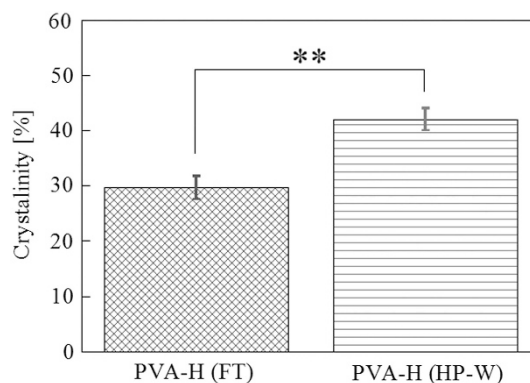


Figure 3 Crystallinities of PVA-H (HP-W) and PVA-H (FT) measured by differential scanning calorimetry (mean \pm s.d., $n=3$, $**P<0.05$ analyzed by Student's *t*-test). FT, freezing and thawing method; HP-W, hot-pressing method (water); PVA-H, poly(vinyl alcohol) hydrogel. A full color version of this figure is available at *Polymer Journal* online.

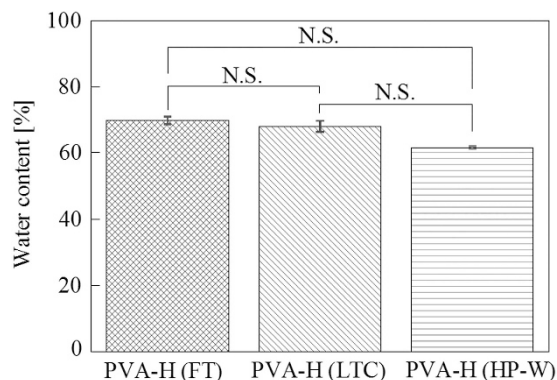


Figure 4 Water contents of PVA-H prepared by various methods. (mean \pm s.d., $n=3$, NS, not significant, analyzed by analysis of variance). PVA-H, poly(vinyl alcohol) hydrogel. A full color version of this figure is available at *Polymer Journal* online.

CR and WC

CR is a key physical quantity to indicate the degree of crosslinking in PVA-H. Therefore, the CR of PVA-H prepared by each method was measured by DSC. The DSC results (Figure 3) clearly showed that the CR of PVA-H (HP-W) was significantly higher than that of its equivalent gels prepared by the freeze-thaw method (PVA-H (FT)). For PVA-H (LTC), the remaining DMSO affected DSC measurement; therefore, no clear conclusion can be inferred from the obtained data. As a matter of fact, dried sample is a mandatory requirement for DSC measurement; thus, the hydrogel must be dried before measurement. The drying process is known to influence the CR of the gels: CR could increase during drying due to an increase in molecular density. Therefore, higher initial PVA concentration in the hot-pressing method than that in the freezing-and-thawing method might lead to enhanced CR of the hot-pressed PVA-H.³⁹ This result is closely synonymous with the WC data shown in Figure 4. Water contents of PVA-H (FT), PVA-H (LTC) and PVA-H (HP-W) showed no significant difference; the lowest WC was observed for PVA-H (HP-W). The water molecules probably interacted with the amorphous region of PVA rather than with the crystalline part; therefore, the higher CR of PVA-H (HP-W), as seen previously, could be the reason for the low water interaction and consequent lower

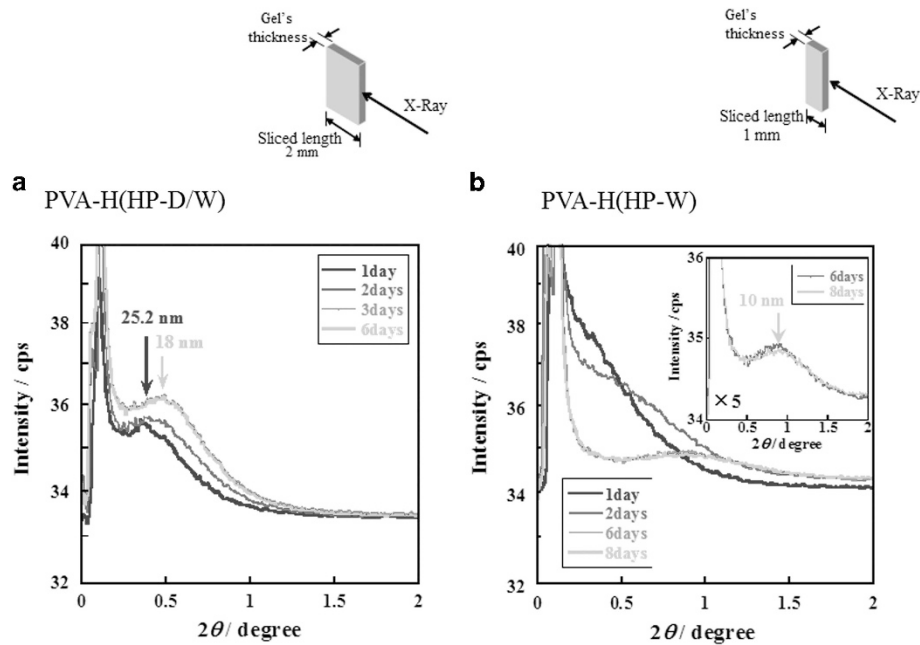


Figure 5 SAXS profiles of (a) PVA-H (HP-D/W) and (b) PVA-H (HP-W) from 1 to 6 days after hot-pressing. HP-DW, hot-pressing method (dimethylsulfoxide and water); HP-W, hot-pressing method (water) solvent; PVA-H, poly(vinyl alcohol) hydrogel; SAXS, small-angle X-ray scattering. A full color version of this figure is available at *Polymer Journal* online.

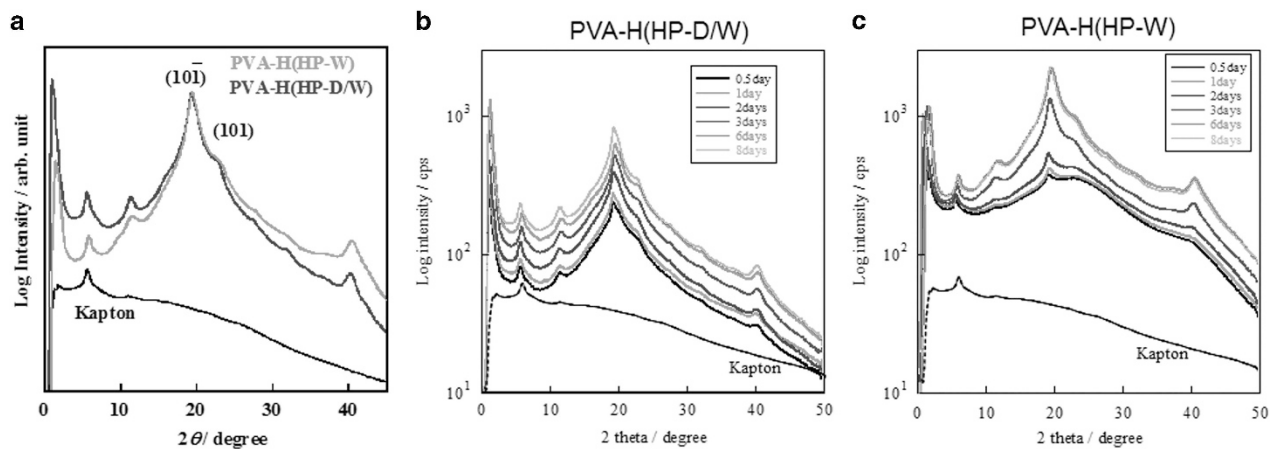


Figure 6 WAXS profiles of (a) PVA-H (HP-D/W) and PVA-H (HP-W) at 8 days after hot-pressing and time-dependent WAXS profiles of (b) PVA-H (HP-D/W) and (c) PVA-H (HP-W) after hot-pressing. HP-DW, hot-pressing method (dimethylsulfoxide and water); HP-W, hot-pressing method (water) solvent; PVA-H, poly(vinyl alcohol) hydrogel; WAXS, wide-angle X-ray scattering. A full color version of this figure is available at *Polymer Journal* online.

WC in PVA-H (HP-W). Moreover, as small crystals have an important role in the crosslinking of PVA molecules, the presence of crystalline microstructure in the hydrogels is crucial. As DSC showed the total CR in the hydrogel (not just in the microcrystals), we used X-ray scattering methods to determine the hydrogel structure and the amount of small (micro) crystallites produced during gelation to elucidate the gelation mechanism for the hot-pressing method.

SAXS and WAXS

Transparency of PVA-H (HP-W) strongly correlates with the homogeneity of the gel structure. The crystal sizes and the distances between the crystalline domains in PVA-H are small enough so that visible light is not scattered. In our method, the hydrogel precursor of swollen PVA (50% w/w) was heated to dissolve in the

high-concentration solution during hot-pressing. Then, the macroscopic hydrogel was formed within 48 h at room temperature after removal from the hot-pressing machine (Figure 2). Therefore, dynamic changes in the spatial distributions and sizes of the microcrystals in PVA-H (HP-W) were evaluated using SAXS and WAXS after hot-pressing. For the low-temperature crystallization method, DMSO/water mixed solvent was necessary. Here we measured SAXS and WAXS in PVA-H (HP-W) and PVA-H (HP-D/W) during the gelation process after the hot-pressing step. The initial gels were prepared from lower-concentration solutions in the conventional methods than in our hot-pressing method. Therefore, comparison of the gelation conditions is difficult. We compared the solvent effects on the gel structure and crystalline structure between PVA-H (HP-W) and PVA-H (HP-D/W).

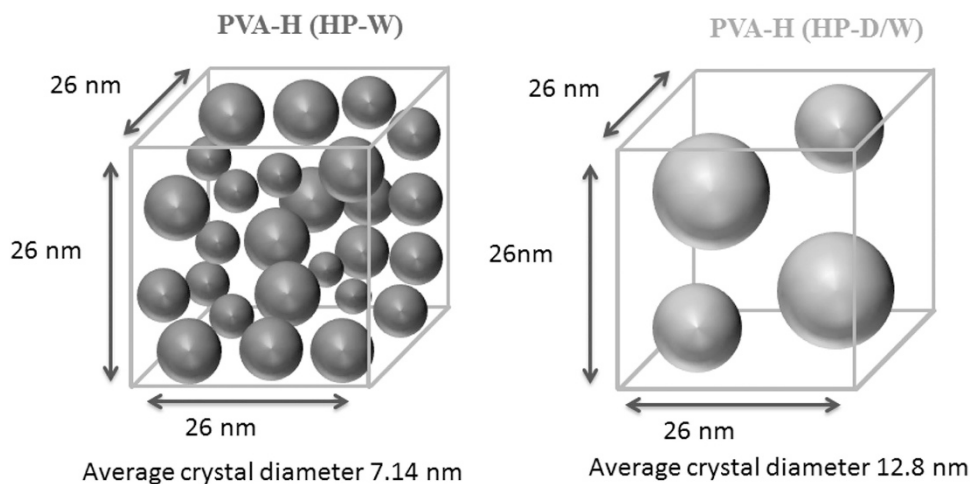


Figure 7 Schematic of microstructures in the PVA-H (HP-D/W) and PVA-H (HP-W) derived from SAXS and WAXS results. Microcrystallites are represented by circles and other parts include amorphous and water. HP-DW, hot-pressing method (dimethylsulfoxide and water); HP-W, hot-pressing method (water) solvent; PVA-H, poly(vinyl alcohol) hydrogel; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering. A full color version of this figure is available at *Polymer Journal* online.

Figure 5a shows the SAXS profile of PVA-H (HP-D/W) at 1–6 days after hot-pressing. A broad scattering at $2\theta=0.35^\circ$ (25.2 nm) was observed at day 1 after preparing PVA-H (HP-D/W). With increasing time, the broad peak shifted to $2\theta=0.49^\circ$ (18 nm). This scattering reflects the average distance between the microcrystalline structures formed in the hydrogel; after 3 days, PVA-H (HP-D/W) has microcrystalline structures dispersed with an average distance of 18 nm. The wide-angle shift indicates a decrease in the average distance between the microcrystals with increasing time, due to an increase in the number of microcrystallites produced by crystallization. On the other hand, PVA-H (HP-W) exhibits smaller angle and broader scatterings than those observed for PVA-H (HP-D/W) in the early stages (Figure 5b). The small-angle scattering also shifted toward $2\theta=0.88^\circ$ (10 nm) for the next 7 days (Figure 5b), suggesting that the microcrystalline distance in PVA-H (HP-W) is 10 nm, smaller than that in PVA-H (HP-D/W). This weak scattering intensity also agreed with the better transparency (Figure 1d) of PVA-H (HP-W) due to the inhibition of Rayleigh scattering. Here the scattering lower than $2\theta=0.2^\circ$ represents the leakage light from the beam stopper of X-ray direct beam.

The crystalline structures of PVA-H (HP-D/W) and PVA-H (HP-W) were compared by WAXS. Figure 6a shows that the peak corresponding to $(10\bar{1})$ ($2\theta=19.3^\circ$ (0.46 nm)) and the shoulder corresponding to (101) , characterized from PVA crystallites, were observed in both hydrogels.^{40,41} These peaks were clearly observed, suggesting that both hydrogels have high crystallinities. The peak at $2\theta=5.58^\circ$ was assigned to the scatter from the kapton film used for prevention of drying. When we compared scattering intensities, a clear peak from the $(10\bar{1})$ plane was observed immediately after pressing in PVA-H (HP-D/W). The PVA crystal growth in PVA-H (HP-D/W) was fast and instantaneous, without any induction period of crystallization. In contrast, PVA-H (HP-W) required 3 days to show the same profile as PVA-H (HP-D/W) (Figure 6b and c). PVA-H (HP-W) exhibited relatively slow crystallization rate and a longer induction period. The induction period of crystallization should be present under highly random conditions in bulk or solutions by preventing nucleation in systems. Therefore, the crystal growth of PVA-H (HP-W) involves the longer induction period because water is relatively better solvent compared with DMSO/water mixed solvent.⁴²

Hence, the mobility of PVA molecule in water under the hot-pressing conditions induces high homogeneity and homogeneous crystallization.

CR of PVA-H (HP-W), which leads to fusion enthalpy, was $\sim 20\%$ (CR in dried gel: $\sim 40\%$; and WCs of hydrogel: $\sim 50\%$) and WC was $\sim 50\%$. From the WAXS data, we obtained almost similar profiles for PVA-H (HP-W) and PVA-H (HP-D/W) after 8 days of hot-pressing, suggesting that the crystalline structures in PVA-H (HP-W) and PVA-H (HP-D/W) are essentially the same (Figure 6a). Here we converted the weight fraction of CR obtained from DSC to volume fraction of CR using following equation (4).

$$X_w = X_v r_c / [X_v r_c + (1 - X_v) r_a] \quad (4)$$

where X_w and X_v are weight fraction and volume fraction of CR, respectively, ρ_c (1.345) and ρ_a (1.269) are crystal and amorphous density,⁴³ respectively.

And we assumed that solvent exists only in the amorphous region and volume fraction of CR in PVA-H is 19% with the distance between microstructures being 10 nm. From the distance between microstructures observed by SAXS, we can roughly estimate the average size of crystal domains in the 18^3 nm^3 space to be $\sim 12.8 \text{ nm}$ for PVA-H (HP-D/W) and 10^3 nm^3 space to be 7.14 nm for PVA-H (HP-W) (Supplementary Figure S5).

On the basis of these results, schematics of the microstructure in both the hydrogels are shown in Figure 7. Small crystallites are represented by spheres, whereas the outer space includes amorphous PVA and solvent. After the hot-pressing step, both the hydrogels reached equilibrium almost in the same time. The crystallinities of both PVA gels were almost similar, as confirmed by the WAXS results. Therefore, the total volumes of the crystalline regions (spheres) are depicted to be the same in both the PVA-H (HP-W) and PVA-H (HP-D/W) models. In contrast, the size distributions of the microcrystallites in PVA-H (HP-W) are smaller due to the relatively short distances between the microcrystallites, as confirmed by the SAXS data. According to these models, PVA-H (HP-W) forms more uniform structure than in PVA-H (HP-D/W). Recently, hydrogels possessing uniform crosslinking structures have been reported to have high mechanical properties.^{44–46} The present results show that our hot-press method provides highly uniform and

transparent PVA hydrogel without using toxic DMSO. Therefore, in the next stage, we anticipate that the proposed method should facilitate and accelerate the preparation of PVA hydrogels with higher mechanical properties, and expedite the biomedical application of PVA hydrogels with high WC.⁴⁷ Further investigations exploring the mechanical properties are now in progress and will be reported in due course.

CONCLUSION

Through our experiments, the gels prepared by the novel hot-pressing method were evaluated in terms of transparency, elution of PVA chains, WC and CR. From the above studies, it can be concluded that we successfully prepared transparent PVA-H without using toxic DMSO using the hot-pressing method. After 2 days from pressing, almost no elution was observed from PVA-H (HP-W), which signifies quick and efficient gelation, because PVA gelation progressed rapidly, and the amount of PVA molecules that were not physically crosslinked by small crystallites decreased drastically.

We also found higher CR in PVA-H (HP-W) than in PVA-H (FT). Although their WCs showed no significant difference, the lowest WC was observed in PVA-H (HP-W), leading to better gelation. SAXS and WAXS profiles of PVA-H (HP-W) and PVA-H (HP-D/W) during the gelation process after hot-pressing confirmed that the total amount of crystallites was the same for PVA-H (HP-W) and PVA-H (HP-D/W). In contrast, size distribution of microcrystallites in PVA-H (HP-W) was smaller than that in PVA-H (HP-D/W), which clearly signifies that better gelation and greater strength of PVA hydrogels can be achieved by our method without using toxic DMSO.

This could be a crucial step toward overcoming the current challenges for the use of PVA hydrogels prepared without using toxic DMSO for biomedical applications, especially in the field of artificial cartilages.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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