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Description	

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

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Running head title: Transparent PVA hydrogels by hot-pressing

Abstract

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 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.

Keywords

Biomaterials/crystallization/hydrogel/poly(vinyl alcohol)

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 cross-linked poly(2-hydroxyethyl methacrylate) hydrogels in 1960 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] etc.

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

Chemical gels are covalently cross-linked 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 cross-linking with chemical cross-linkers, 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 cross-linking agents, which are toxic and need to be extracted or neutralized before using the gels for their intended applications. Moreover, chemical cross-linking 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 cross-linked, have several advantages over chemically cross-linked gels, especially for biomaterial applications. Since Bray and Merrill first reported the use of PVA-Hs in artificial cartilages in 1973,[17] 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]

In 1975, 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 °C and is 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, and excellent transparency.[31,32] In this method, PVA was first dissolved in a water/ DMSO solvent at a low temperature (−20 °C). This promoted the crystallization and cross-linking 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 hydrogels 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)

15 g PVA, with a viscosity-average degree of polymerization of 1700 and a degree of saponification of 98.5 mol% (Japan VAM & POVAL Co., Ltd., Osaka, Japan), was dissolved in 135 g H_2O 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 (Fig. S1). Thus-prepared PVA hydrogels are hereafter denoted as PVA-H (FT).

・Low-temperature crystallization method: PVA-H(LTC)

15 g PVA was dissolved in a solvent mixture of DMSO (108 g) and H_2O (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 (Fig. S2). Thus-prepared PVA hydrogels are hereafter denoted as PVA-H (LTC).

・Hot pressing method: PVA-H(HP-W) / PVA-H(HP-D/W)

20 g PVA powder was swollen in 20 g of solvent (either water or DMSO/H_2O 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) 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 one week. Subsequently, thus-obtained hydrogels were dried in air for 2 days, followed by vacuum drying for 2 days (Fig. 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/H_2O 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 (Fig. S4). PVA hydrogels 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 of PVA molecules into water from PVA-H (HP-W).

Test piece ($20 \times 20\ \mathrm{mm}^2$) 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 elution ratios (ER) of the PVA gels were calculated from the equation (1),

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

・Small-angle X-ray scattering (SAXS)

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

SAXS measurement was performed with a NANO-Viewer (RIGAKU) at a voltage of 45 kV, current of 60 mA, with irradiation time of 12 h to produce Cu Kα radiation (λ = 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.

・Wide-angle X-ray scattering (WAXS)

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

WAXS measurement was performed with a FR-E (RIGAKU) at a voltage of 45 kV, current of 45 mA, with irradiation time of 10 min to produce Cu Kα radiation (λ = 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 water content (WC) of the PVA-H was calculated using equation (3).

$$\mathrm{WC}(\%) = \frac{w_{wet} - w_{dry}}{W_{wet}} \times 100 \qquad (3)$$

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

・Differential Scanning Calorimetry (DSC)

Crystallinities of PVA-H (FT) and PVA-H (HP-W) were measured by differential scanning calorimetry (DSC, DSC8500, Perkin Elmer) under N_2 gas.

About 2 mg of dried grain sample was cut, and was sealed in an aluminum DSC pan. This pan was heated from 10 °C to 280 °C at a rate of 10 °C/min. The crystallinity (CR) was calculated using equation (4) 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)[35].

$$\mathrm{CR}(\%) = \frac{\Delta H}{H} \times 100 \ldots\ldots\ldots\ldots \ (4)$$

Results and discussion

・Preparation of PVA hydrogels

To precisely evaluate PVA hydrogels 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).

Fig. 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 (Fig. 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 non-uniform polycrystalline PVA structures that give visible light scatterings.[36] On the other hand, PVA-H prepared by low-temperature crystallization showed good transparency in the formed hydrogel (Fig. 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 (Fig. 1c). However, in this method, the high initial concentration of PVA might accelerate crystallization without spinodal decomposition. Due 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 (Fig. 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 elution ratio 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. Since PVA gelation progressed during this period due to increased entanglement of the chains in the hydrogel, which were not physically cross-linked 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 7 repetitive freeze-thaw cycles showed ~20% dissolution after 30 days.[39] Thus, comparing these studies, PVA-H (HP-W) showed a relatively higher gelation speed with better gelation efficiency.

・Crystallinity and water content

Crystallinity is a key physical quantity to indicate the degree of cross linking in PVA-H. Therefore, the crystallinity of PVA-H prepared by each method was measured by DSC. The DSC results (Fig. 3) clearly showed that the crystallinity 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 crystallinity of the gels: crystallinity 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 crystallinity of the hot pressed PVA-H[40]. This result is closely synonymous with the water contents data shown in Fig. 4. Water contents of PVA-H (FT), PVA-H (LTC), and PVA-H (HP-W) showed no significant difference; the lowest water content was observed for PVA-H (HP-W). The water molecules probably interacted with the amorphous region of PVA rather than the crystalline part; therefore, the higher crystallinity of PVA-H (HP-W), as seen previously, could be the reason for the low water interaction and consequent lower water content in PVA-H (HP-W). Moreover, as small crystals play an important role in the cross-linking of PVA molecules, the presence of crystalline microstructure in the hydrogels is crucial. Since DSC showed the total crystallinity in the hydrogel (not just in the microcrystals), we employed 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 (Fig. 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).

Fig. 5a shows the SAXS profile of PVA-H (HP-D/W) at 1 to 6 days after hot-pressing. A broad scattering at 2θ = 0.35° (25.2 nm) was observed at day 1 after preparing PVA-H (HP-D/W). With increasing time, the broad peak shifted to 2θ = 0.49° (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 (Fig. 5b). The small-angle scattering also shifted towards 2θ = 0.88° (10 nm) for the next 7 days (Fig. 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 (Fig. 1d) of PVA-H (HP-W) due to the inhibition of Rayleigh scattering. Here, the scattering lower than 2θ = 0.2° 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. Fig. 6a shows that the peak corresponding to ($10\bar{1}$) (2θ = 19.3° (0.46 nm)) and the shoulder corresponding to (101), characterized from PVA crystallites, were observed in both hydrogels.[41,42] These peaks were clearly observed, suggesting that both hydrogels have high crystallinities. The peak at 2θ = 5.58° 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) (Fig. 6b, 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[43]. Hence, the mobility of PVA molecule in water under the hot pressing conditions induces high homogeneity and homogeneous crystallization.

Crystallinity of PVA-H (HP-W), which leads to fusion enthalpy, was ~20% (crystallinity in dried gel: ~40% and water contents of hydrogel: ~50%) and water content was ~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 (Fig. 6a). Here, we converted the weight fraction of crystallinity obtained from DSC to volume fraction of crystallinity using following formula.

$X_w = X_v\rho_c/[X_v\rho_c + (1 - X_v)\rho_a]$

where, X_w and X_v are weight fraction and volume fraction of crystallinity, respectively, ρ_c (1.345) and ρ_a (1.269) are crystal and amorphous density[44], respectively.

And we assumed that solvent exists only in the amorphous region and volume fraction of crystallinity 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 ~12.8 nm for PVA-H (HP-D/W) and 10^3-nm^3 space to be 7.14 nm for PVA-H (HP-W) (Fig. S5).

Based on these results, schematics of the microstructure in both the hydrogels are shown in Fig. 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 cross-linking structures have been reported to have high mechanical properties.[45–47] 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 pf PVA hydrogels with high water content.[48] 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, water content, and crystallinity. 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 cross-linked by small crystallites decreased drastically.

We also found higher crystallinity in PVA-H (HP-W) than in PVA-H (FT). Although their water contents showed no significant difference, the lowest water content 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 towards 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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Table 1 Preparation processes of various PVA-Hydrogels: Low-temperature crystallization method, freezing-and-thawing method, hot-pressing method (only water), and hot-pressing method (DMSO and water).

Process	Low temperature crystallization method (LTC)	Freezing and thawing method (FT)	Hot pressing method (only water) (HP-W)	Hot pressing method (DMSO and water) (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)

Title and legends to figures

Figure 1. Photographs of 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 DMSO. (d) % light transmittance at 550nm of each PVA-H.

Figure 2. Time-dependent elution of PVA molecules from PVA-H (HP-W) into water.

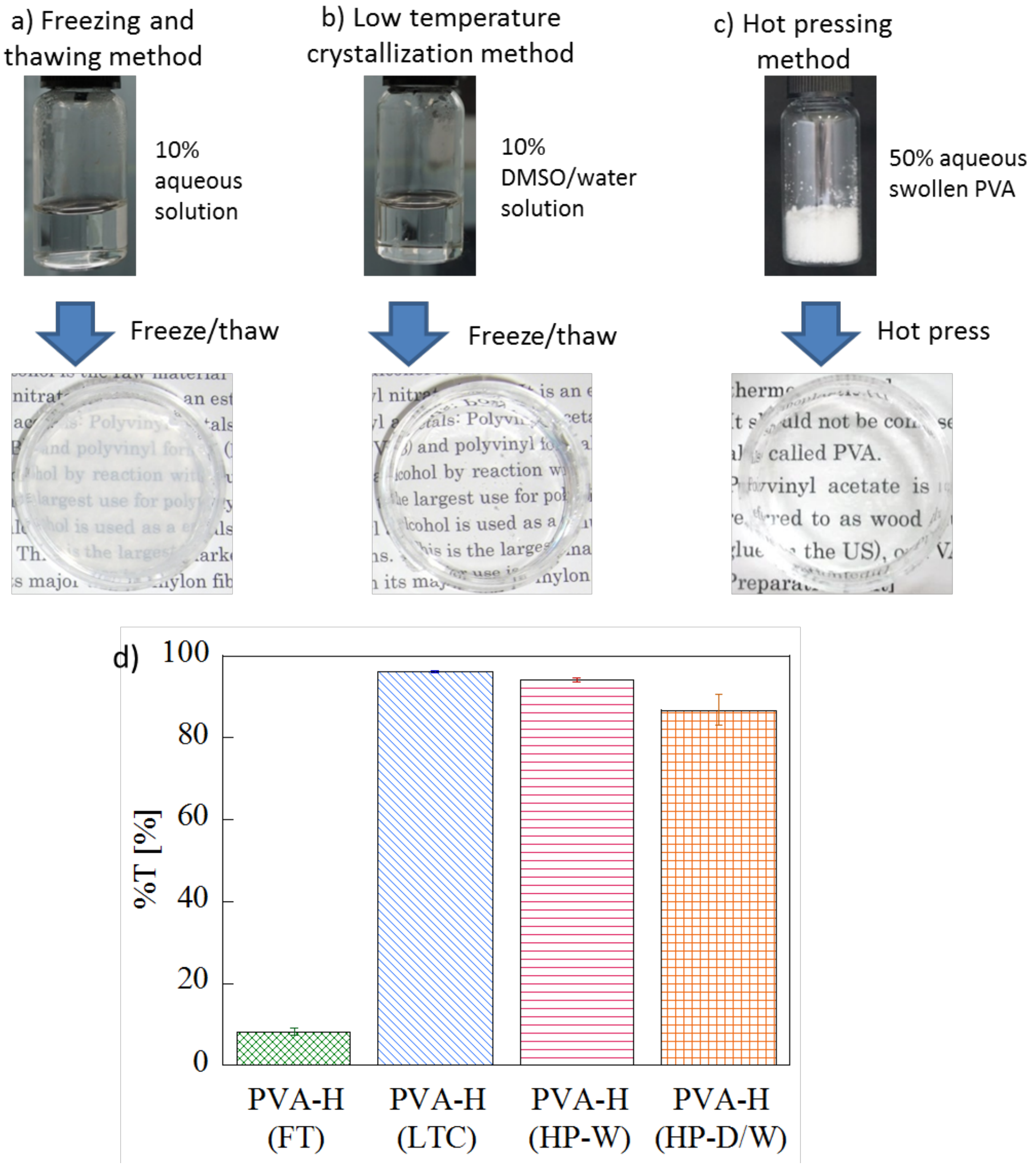
Figure 3. Crystallinities of PVA-H (HP-W) and PVA-H (FT) measured by DSC. (mean ± SD, n=3,**= $p<0.05$ analyzed by Student's T-test)

Figure 4. Water contents of PVA-H prepared by various methods. (mean ± SD, n=3, N.S.: not significant, analyzed by analysis of variance (ANOVA))

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.

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.

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 includes amorphous and water.



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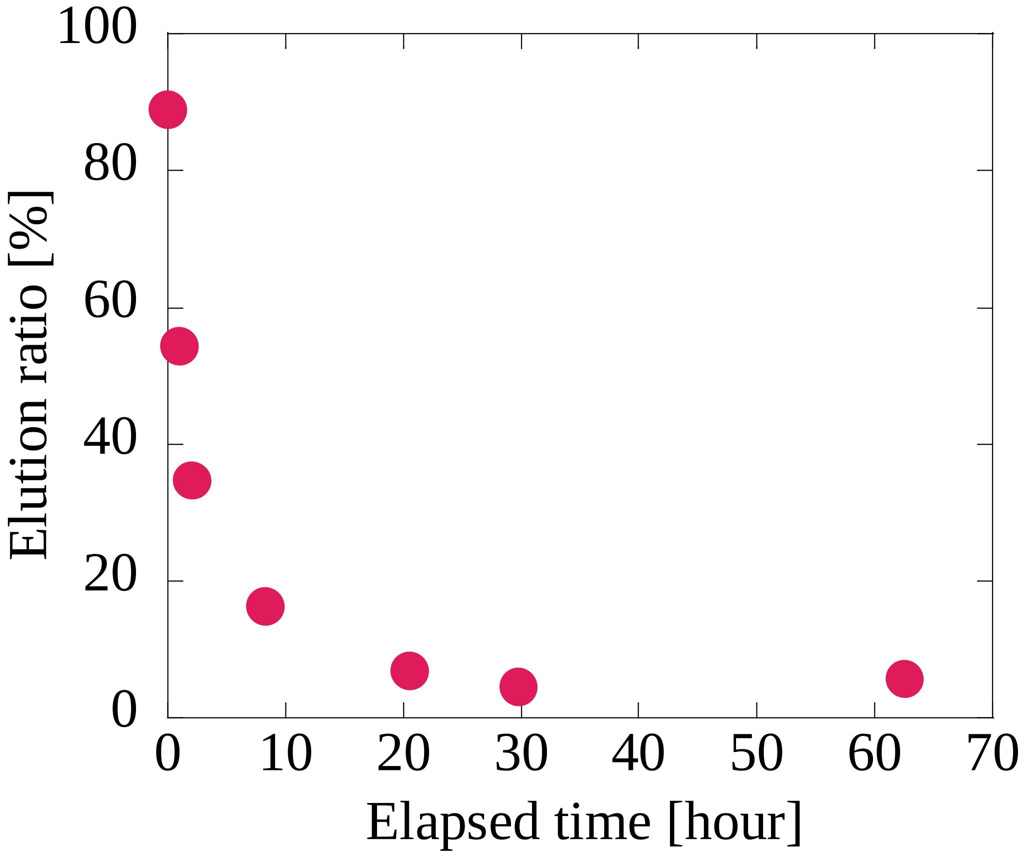

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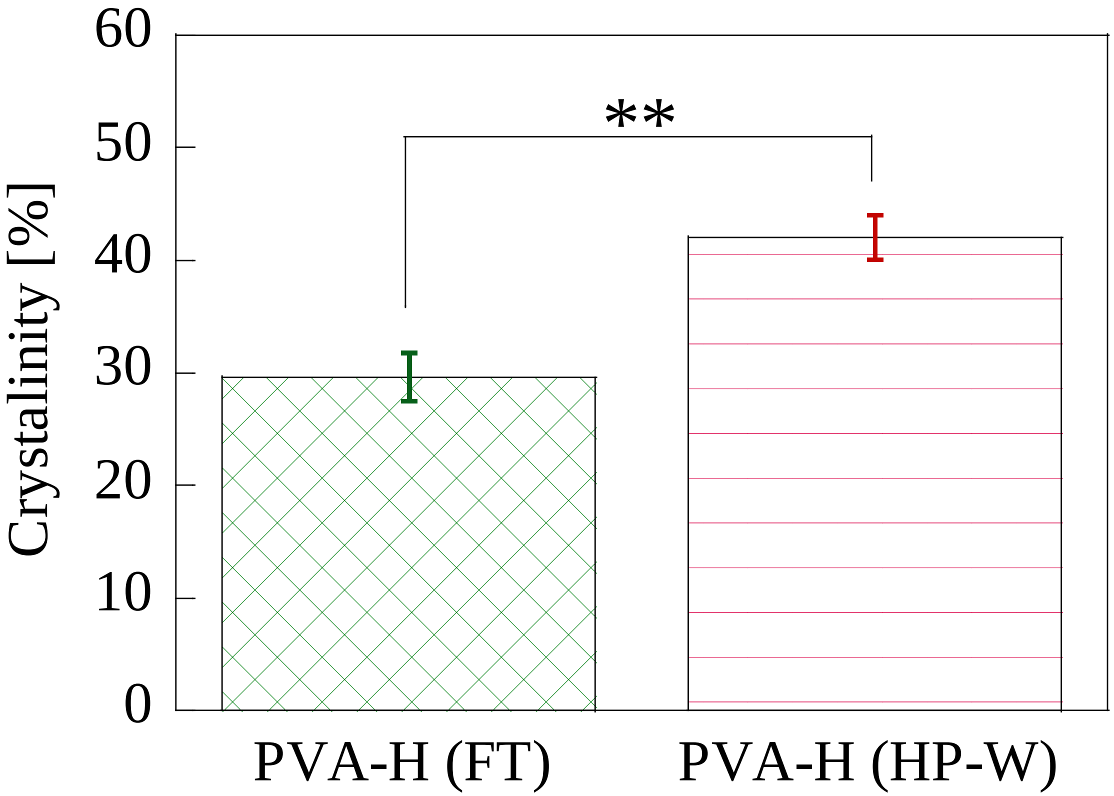

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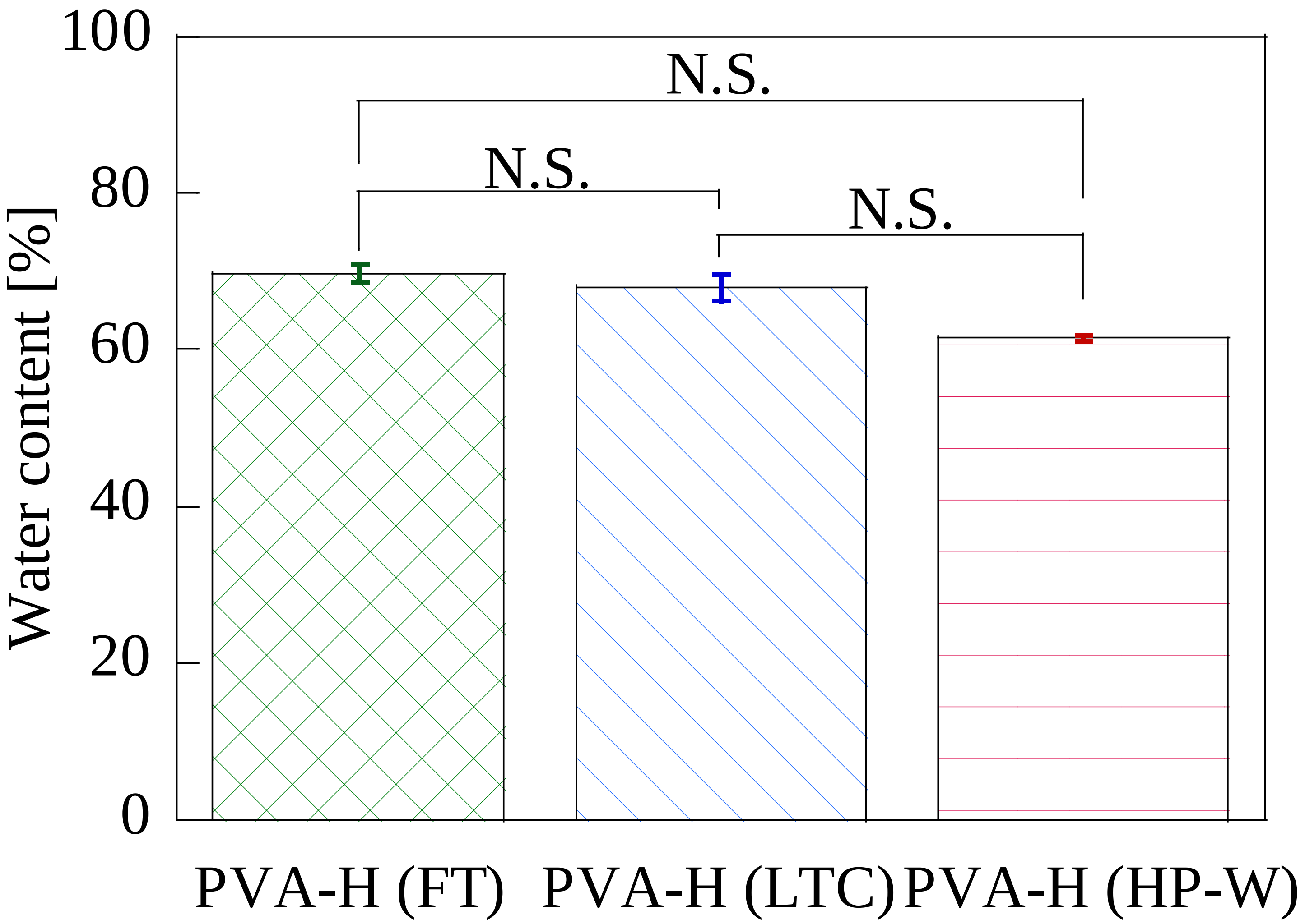

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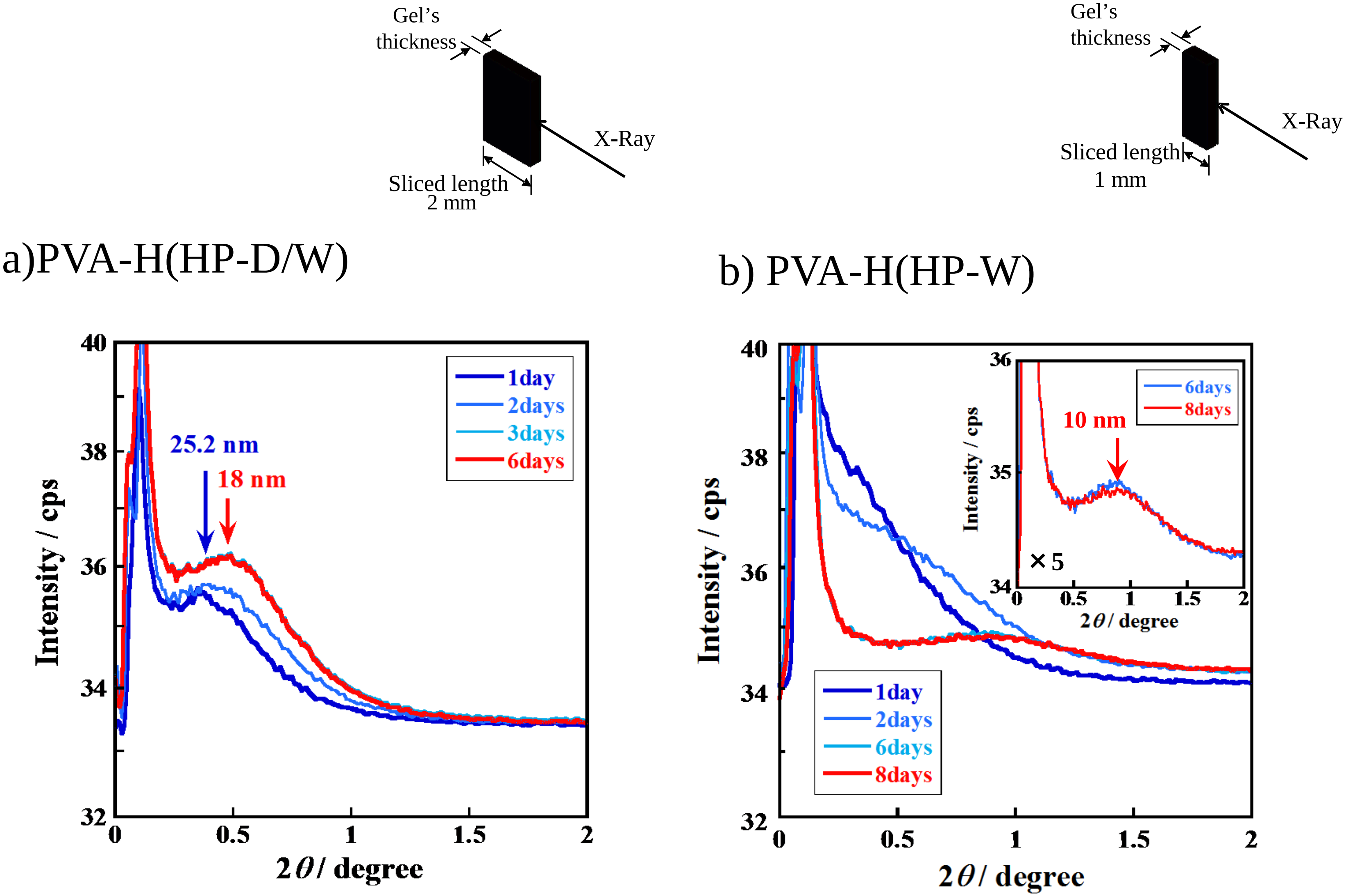

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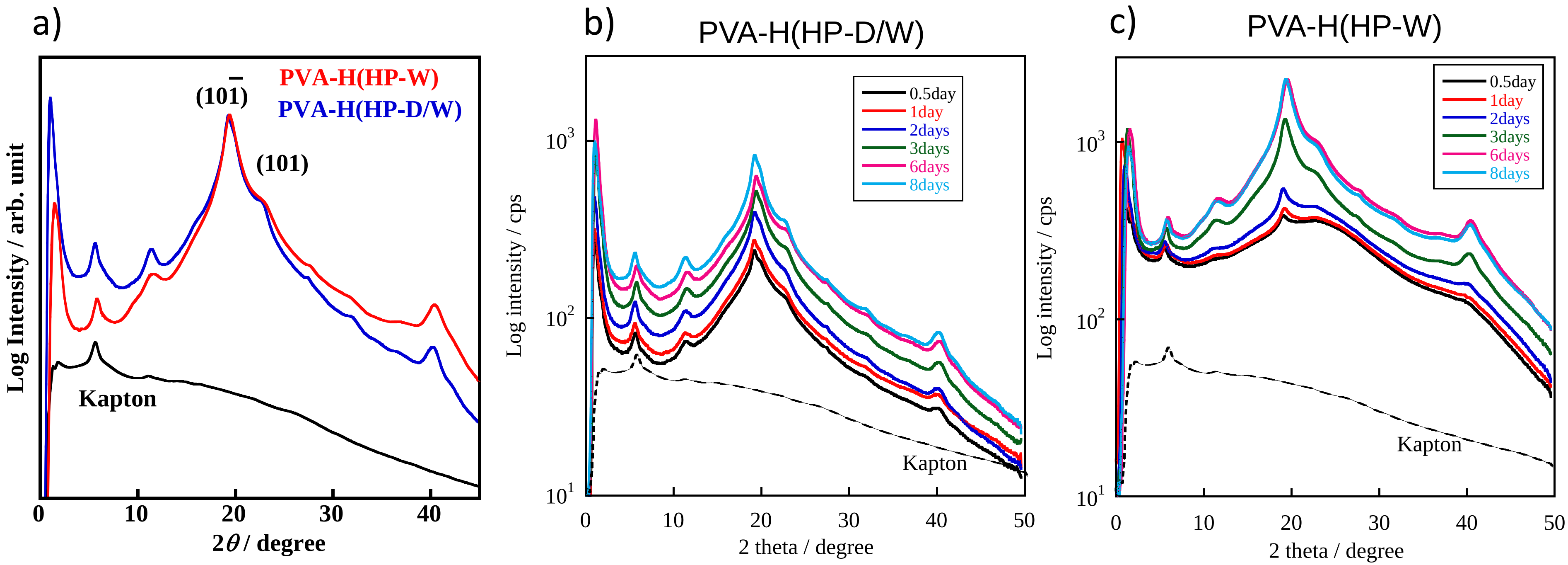
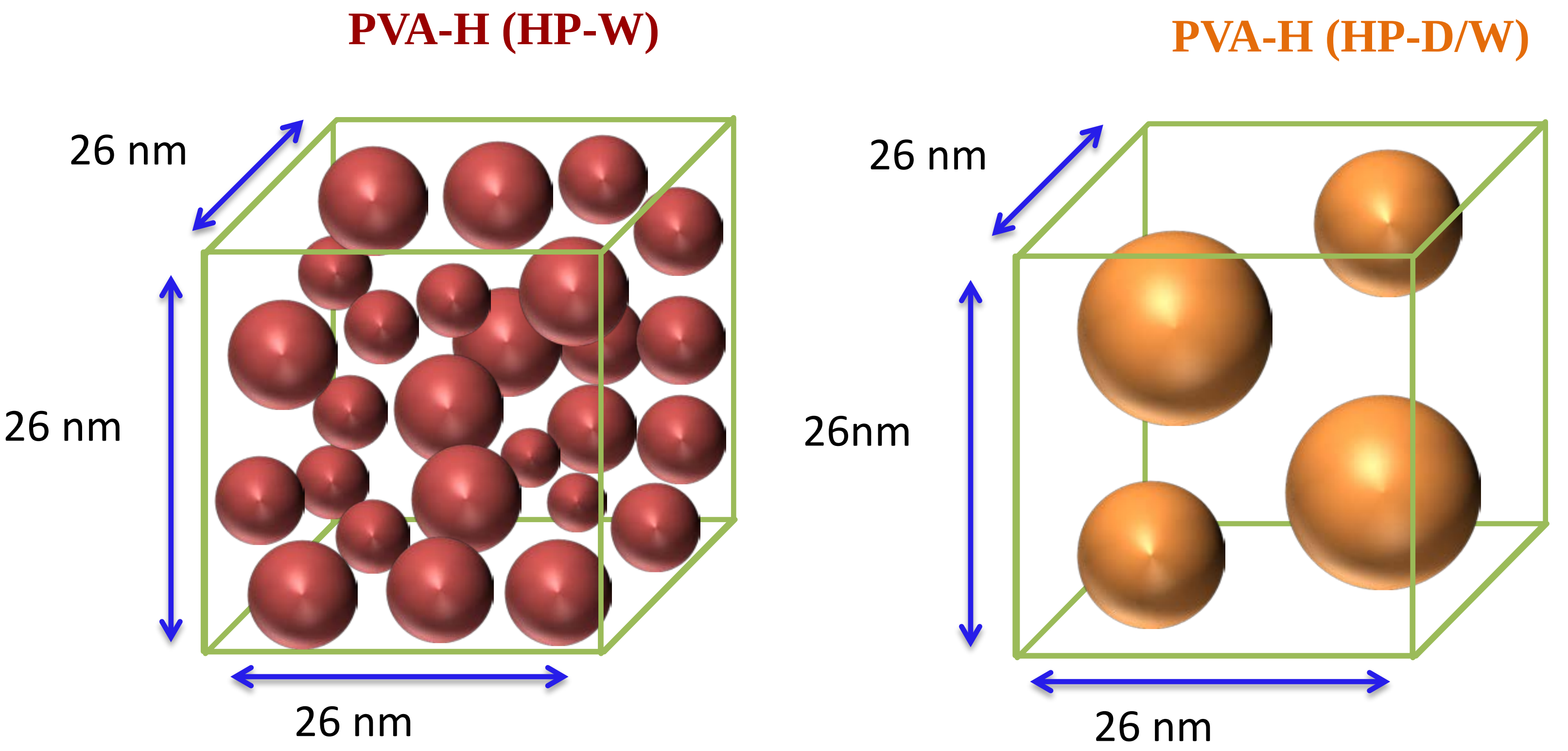


Figure 6. WAXS profiles of (a) PVA-H (HP-D/W) and PVA-H (HP-W) at 8 days after hot-pressing and time-dependent WAXS profiles of (b) PVA-H (HP-D/W) and (c) PVA-H (HP-W) after hot-pressing.


PVA-H (HP-W)

PVA-H (HP-D/W)

Average crystal diameter 7.14 nm

Average crystal diameter 12.8 nm

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 includes amorphous and water.

Supplementary Information for

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

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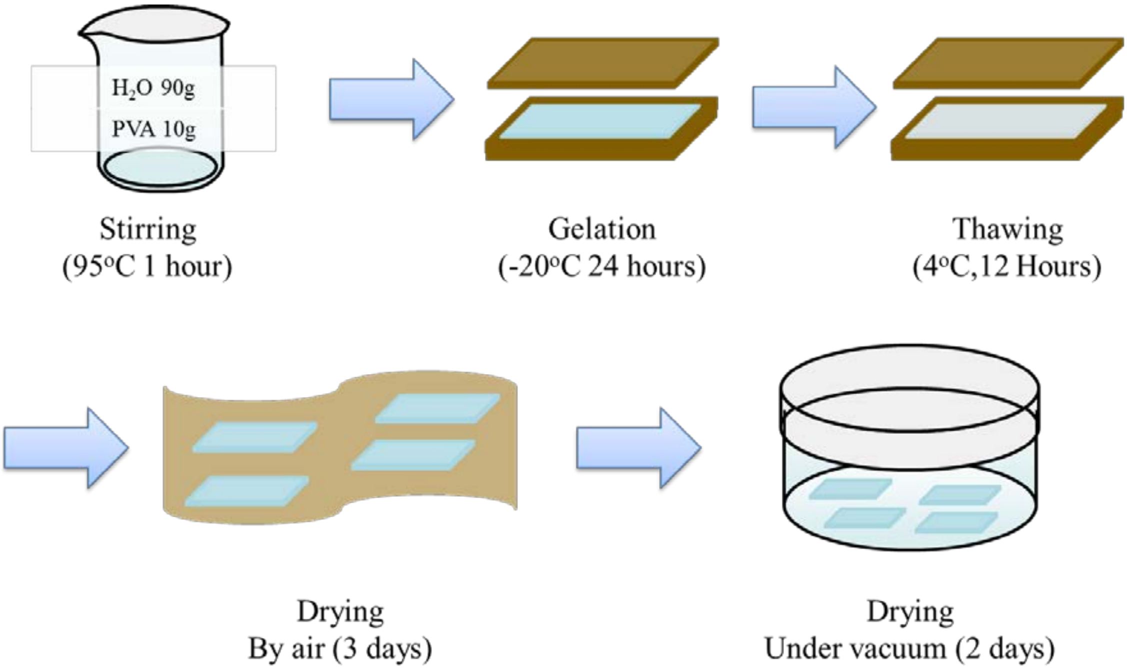

Figure S1. Preparation of PVA-H (FT)

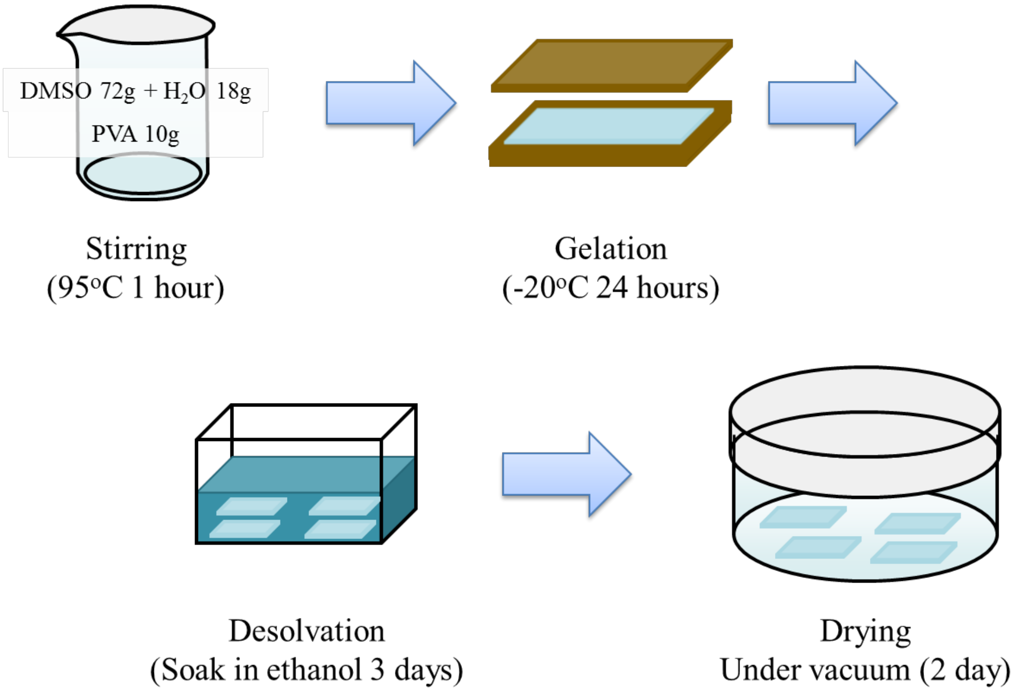

Figure S2. Preparation of PVA-H (LCT)

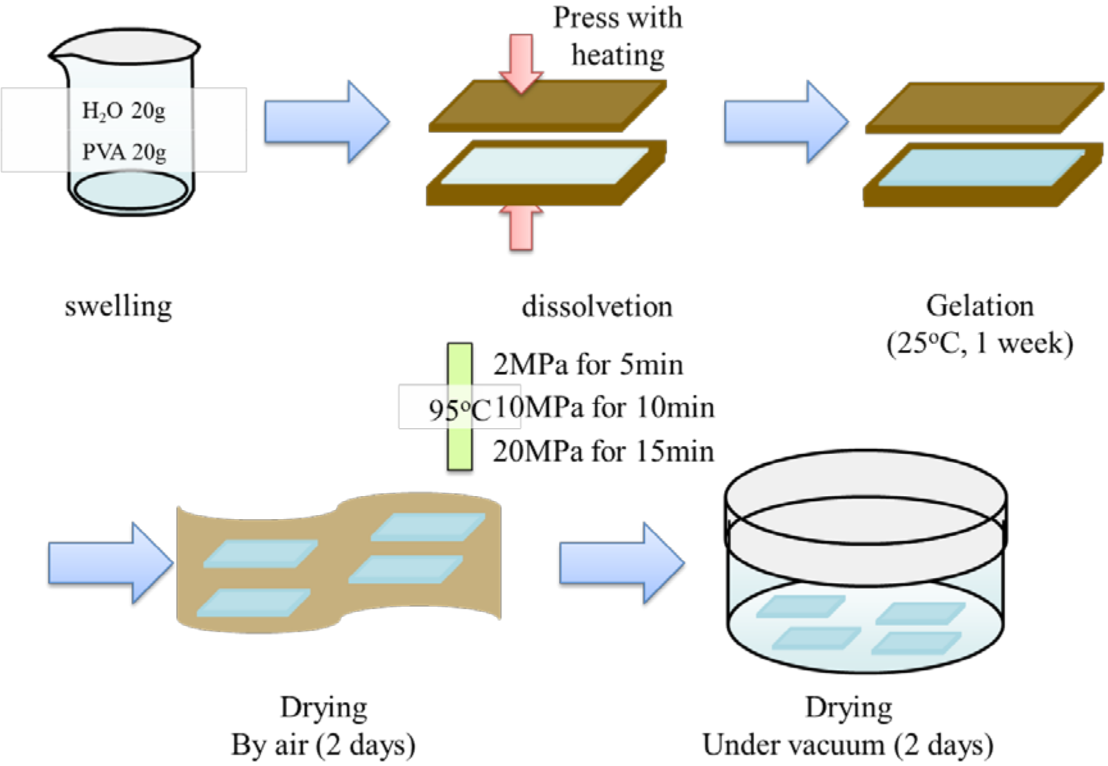

Figure S3. Preparation of PVA-H (HP-W)

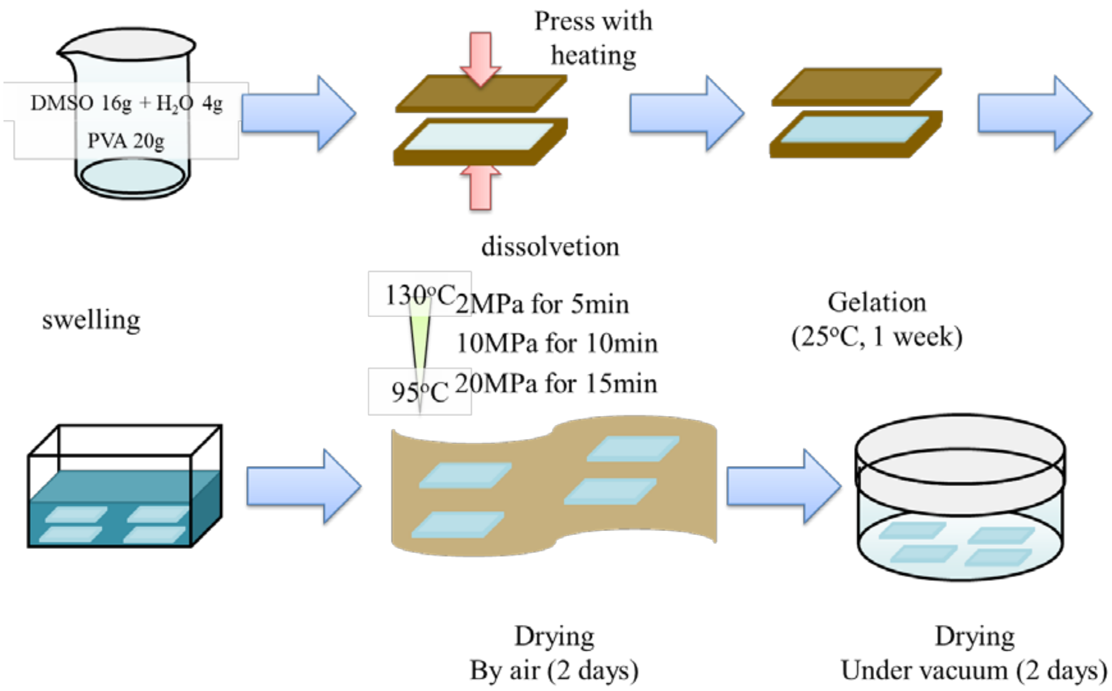

Figure S4. Preparation of PVA-H (HP-D/W)

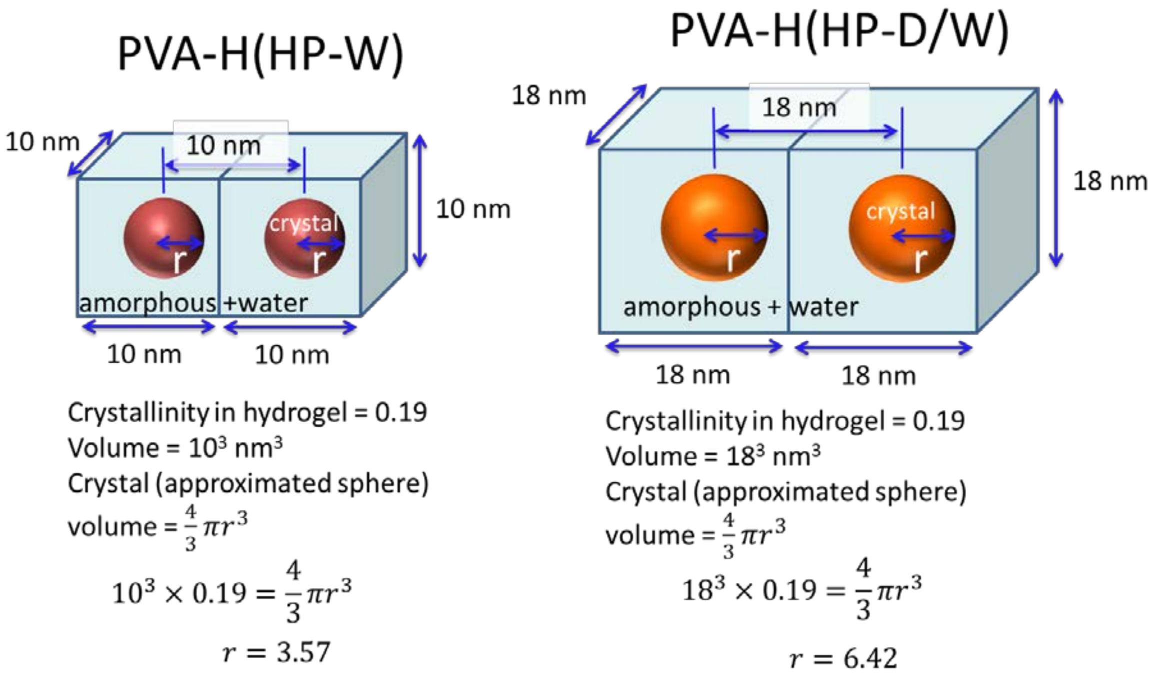

Figure S5. Schematic of crystal domain size calculation in the PVA-H(HP-D/W) and PVA-H(HP-W).