

DSC Analysis on Water State of Salvia Hydrogels

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Abstract: The role of the water structure present in hydrogels from nutlets of three species of salvias, *S. miltiorrhiza* (SM), *S. sclarea* (SS) and *S. viridis* (SV), was analyzed by differential scanning calorimetry (DSC). The sharp endothermic peaks that appeared at 5.9 °C (SM), 2.8 °C (SS) and 1.8 °C (SV) in each 1.0% hydrogel of 10.4-15.8% were not affected by addition of 0.1 M urea and alkali-metal salts. The order-disorder portions in the network were slightly affected by the distribution of freezable and non-freezable water in the hydrogel networks. The SV hydrogel was further used to investigate the effects of additives (0.1-8.0 M urea and 0.1-5.0 M NaCl) on its melting behavior. At 0.5-4.0 M urea and 1.0-3.0 M NaCl, two endothermic peaks appeared, corresponding to unbound (high temperature) and bound (low temperature) water in the gel networks, and eventually merged into one endothermic peak at 5.0-8.0 M urea and 4.0-4.5 M NaCl. After this merger, the endothermic peak shifted to 3.7, 4.0 and 5.6 °C at 5.0, 6.0 and 8.0 M urea, respectively. In the case of NaCl, a combination of peaks that occurred at 4.0-4.5 M were accompanied by a shift to lower temperature (-14.4 and 15.3 °C) and the endothermic peak finally disappeared at 5.0 M NaCl due to the strong binding of water in the gel networks.

Keywords: salvia, hydrogels, differential scanning calorimetry, freezable water, free freezable water.

Introduction

Salvia is one of genera in Lamiaceae family found wild in South America, Southern Europe, Northern Africa and Asia which utilized for medical, ornamental and culinary qualities. Morphological and chemical properties of hydrogels isolated from three species of Salvias, *S. miltiorrhiza* (SM), *S. sclarea* (SS) and *S. viridis* (SV), are reported by Yudianti, *et al.*^{1,2} Such hydrogels are belong to anionic polysaccharides which have approximately 25-30% of uronic acid in their acidic polysaccharides. According to the previous works,^{2,3} such hydrogels were identified as polysaccharide gels containing glucuronic acid linked to xylan as dominant polysaccharide. Such hydrogels are rich in divalent magnesium and calcium cations, at concentrations of 0.81 (SM), 0.13 (SS), 0.17% (SV) (w/w) and 1.12 (SM), 0.46 (SS), 0.44 (SV)% (w/w), respectively. These divalent metal ions have possibility of making dense cross-linkages between two carboxyl groups by ionic bonds to form three dimensional network structure as described in gellan gel using computer modeling.⁴ Similar cross-linking was also revealed by Loizou *et al.* and Torres *et al.* who studied cross-linking

formation by addition of iron, dichromate to protein gel and glutaraldehyde to chitosan solution, respectively.^{5,6}

Neutral and acidic polysaccharides present in the gel form closely associated network by hydrogen bonding, divalent cationic bridge and electrostatic interaction. Hydrate state of the gel is also formed as a result of the interactions between hydrophilic-hydrophobic groups and water. Most dominant feature of the hydrogels is importance of water included within network to form interactions with constituents of the hydrogels. Hence, state of water provides useful information on behavior of hydrogels to investigate water-polymer interaction. Previously, the investigations on state and role of water have been conducted on poly(vinyl alcohol),⁷ poly(2-hydroxyethyl methacrylate),⁸ chitosan⁹⁻¹¹ and curdlan.¹² Generally, property of water is investigated by thermal analysis to know melting behavior, phase transition of bulk polymer and sol-gel transition. Properties of hydrogels are known to be influenced not only by their equilibrium water contents (EWC) but also by the state of water in the gels. Water exists in more than two states, classified as unbound and bound water states in the polymer network. According to the thermodynamic properties, water molecules absorbed by hydrophilic region are known to be present in three conditions, i) non-freezable bound water, ii) freezable bound

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water, and iii) free water. Non-freezable bound water is defined as tightly hydrogen bonded water with undetectable phase transition. Free water, however, does not take contribution in the hydrogen bonds on the hydrogel structure while freezable bound water interacts weakly with the hydrogel molecules. The last two water groups are categorized as freezable water.

In the present study, we focused on melting behavior of Salvia hydrogels in native state and salt-rich environment by addition of urea, K_2SO_4 , KSCN or alkali-metal salts by using differential scanning calorimeter (DSC). DSC is the most common technique used to monitor the distribution of different type of water in swollen hydrogel. Melting behavior of the SV hydrogels was further analyzed in the presence of urea (0.1-8.0 M) and NaCl (0.1-5.0 M). Estimation of the amount of water state was also conducted in computerized way by DSC. The purpose of this study is to know interactions, including intra- and inter-molecular hydrogen bonds, that operate in the networks of Salvia hydrogels.

Experimental

Materials and Methods. Hydrogels were prepared from nutlets of three species of Salvias, *S. miltiorrhiza* (SM), *S. sclarea* (SS), *S. viridis* (SV) as described previously.¹⁻³ To control amount of water in gel structure, the hydrogel solution was concentrated above 1.5% w/w using vacuum evaporator.

Melting behaviors of the native hydrogels and hydrogels containing various kinds of additives (K_2SO_4 , KSCN, urea, alkali-metal salts) were observed by using differential scanning calorimeter (DSC) at concentration of 1.0% (w/w). The SV hydrogel was further used to analyze the effects of high concentrations of urea (0.1-8.0 M) and NaCl (0.1-5.0 M) on the network.

Thermal analysis was performed using DSC, Rigaku DSC 8240B. Each sample (8.3-8.7 mg) was filled in an aluminum pan, and its thermogram was obtained by scanning from -30 to 80 °C with heating rate of 2 °C/min, by using an empty aluminum pan as a reference. Freezable water content in total water was approximately calculated as the ratio of the endothermic peak areas for water-swollen hydrogel to the endothermic heat flow for pure water. The following equation was applied to quantify non-freezable bound water in the hydrogel system.

$$W_b(\%) = W_t - (Q_{endo}/Q_{pure} \times 100\%)$$

Where, W_b and W_t are amount of non-freezable bound water and equilibrium water content in swollen hydrogel, respectively; Q_{endo} is enthalpy of freezable water (freezable free water and freezable bound water) calculated by DSC computer and Q_{pure} is defined as enthalpy of pure water (340.1 J/g).

Results and Discussion

DSC heating traces of 1.0% (w/w) concentration of the three Salvia hydrogels (SM, SS and SV) are given in Figure 1. Each species showed similarity on melting behavior having only one transition peak at 5.9 °C (SM), 2.8 °C (SS) and 1.8 °C (SV). The existence of endothermic peak in the native hydrogels is considered as freezable free water.

Estimation of water state quantity in the Salvia hydrogels as freezable and non-freezable water is given in Table I. Absorbed water in the Salvia hydrogels gave different distribution in water state, approximately 10.4-15.8% as freezable water and 80.2-88.5% non-freezable water. High freezable to non freezable water ratio (F-NFW R) in the SM hydrogel meant high quantity of freezable water in comparison with others. Generally, amount of bound water depends on hydrophilic group contents in the hydrogel network.^{11,13}

According to the previous work, uronic acid contents of the Salvia hydrogels were estimated about 25-30%, almost similar with each species.³ Differences on quantity of freezable and non freezable water in the network are probably originated by order-disorder structure. This is closely related to increments of crystalline portion of each hydrogel, 8.1 (SM), 23.6 (SS) and 41.2% (SV), in each cellulose portion, 18.6 (SM), 25.3 (SS) and 35.4% (SV). Crystalline structure of

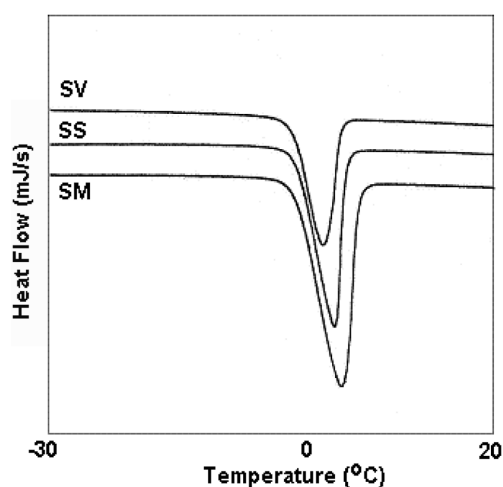


Figure 1. Endothermic curve of 1.0% (w/w) native Salvia hydrogels by differential scanning calorimetry (DSC).

Table I. Water State of 1.0% (w/w) Native Salvia Hydrogels Calculated by DSC

Native Hydrogel	FW	NFW	F-NFW R
SM	15.8	83.2	18.9
SS	12.2	86.8	14.0
SV	10.4	88.5	11.7

FW: Freezable water. NFW: Non freezable water. F-NFW R: Freezable-non freezable water ratio \times 100.

cellulose plays an important role to inhibit penetration of water into amorphous region. Amorphous structure of hydrogels may be built up from branching molecules of acidic polysaccharides. It contains carboxyl and hydroxyl groups responsible for water-polymer interaction, as elucidated by previous author.¹⁴⁻¹⁶ On the native hydrogel, 38-57% carboxylate was estimated to make crosslinkage through calcium ion,¹⁷ indicating that hydroxyl group of polymer dominantly determine water state in gel formation. It is reasonable that high crystalline contents in the SS and SV hydrogels caused lower quantity of freezable water than did the SM hydrogel. Absorbed water partially contributes in non-freezable water, suggesting interactions between water molecule and fraction of the polar carboxyl groups in the acidic polysaccharides. The interaction is strong enough to prevent movement of water to the other places.¹⁷ Otherwise, low crystalline content in the SM hydrogel leads penetration of water much easier than other hydrogels, so that the excess water is easily crystallized and eventually melted during heating. It contributes in ice crystal formation during cooling down. Analysis by wide angle X-ray diffraction indicates that free water in hydrogel forms hexagonal ice in network.¹⁸

To get more information about water state in hydrogel structure, effects of water content on freezable and non-freezable water in the SV hydrogel were analyzed at water contents of 0, 30, 50, 60, 70, 85, 90 and 99% (Figure 2). Upon initial absorption of water, water molecule disrupted the intermolecular hydrogen bonds and bounded with hydrophilic sites and uniformly distributed to the whole network with restricted mobility, (bound or non-freezable water). Increasing water content caused sufficient increase in non-freezable water content up to 30% and eventually reached almost saturated value. When water content reached above this critical value, a part of water began to crystallize at 50% water content, leading to appearance of freezable water. It indicates that hydrophilic groups interact with 30-40% water (water-polymer interaction), either directly or via other water molecule. Otherwise, increasing water content tends to increase freezable water content, confirming the existence of bulk-

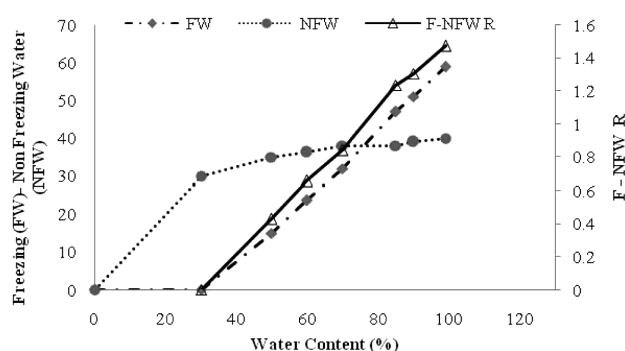


Figure 2. Effect of water content to freezable and non-freezable water of SV hydrogel.

like water or water cluster in water-saturated hydrogel.

Effects of various additives including urea and salts (urea, KSCN, K_2SO_4 , alkali-metal chloride) on melting peak of the hydrogels (1.0%) were also investigated at 0.1 M. Thermograms of pure aqueous urea, K_2SO_4 and KSCN solutions showed sharp peak at 2.2 °C (urea), -0.4 °C (KSCN) and 2.1 °C (K_2SO_4) corresponding to free freezable state of salt solutions (figures not shown). Addition of 0.1 M urea tends to decrease melting peak of the native hydrogels from 5.9 °C (SM), 2.8 °C (SS), 1.9 °C (SV) to 2.7 °C (SM), 2.6 °C (SS), 1.8 °C (SV), respectively, indicating that urea exists in free freezable state.

Similar profiles were also given in K_2SO_4 and KSCN, whereas K_2SO_4 and KSCN tended to occupy free freezable state. KSCN shifted melting point of the native hydrogel considerably to lower temperature of 2.2 °C (SM), 2.0 °C (SS) and 1.4 °C (SV) in comparison with K_2SO_4 (2.5 °C (SM), 2.1 °C (SS) and 19.9 °C (SV)). In all cases, the SV hydrogel gave the lowest melting peak. Salts gave restricted mobility to the SV hydrogel network, inducing longer time in evaporation during heating than in the cases of the other species. It may elucidate that freezable water in the SV hydrogel makes evaporation more difficult due to strong hydration of the polysaccharides contained in the gel (Tables II and III). Amount of freezable water in the SV hydrogel containing salts was somewhat lower than other species agreeing with limited water absorption due to higher cellulose content. In K_2SO_4 , a very small and broad shoulder appeared and overlapped with the main melting peak (figure not shown). K_2SO_4 may convert free to bound freezable state in the gel structure. Changing in distribution of water state in the hydrogels by additions of salts was caused by disruption of the interactions between salts and hydrogel network and

Table II. Water State of Salvia Hydrogels in 0.1 M Urea Solution Calculated by DSC

Urea Treated Hydrogel	FW	NFW	F-NFW R
SM	16.8	82.2	20.4
SS	16.6	82.4	20.1
SV	15.8	83.2	18.9

FW: Freezable water. NFW: Non freezable water. F-NFW R: Freezable-non freezable water ratio \times 100.

Table III. Water State of Salvia Hydrogels in 0.1 M KSCN and K_2SO_4 Calculated by DSC

Salvia Hydrogel	FW		NFW		F-NFW R	
	KSCN	K_2SO_4	KSCN	K_2SO_4	KSCN	K_2SO_4
SM	16.6	17.6	82.4	81.4	20.1	21.6
SS	16.1	17.3	82.9	81.7	19.4	21.2
SV	15.5	17.0	83.5	82	18.5	20.7

FW: Freezable water. NFW: Non freezable water. F-NFW R: Freezable-non freezable water ratio \times 100.

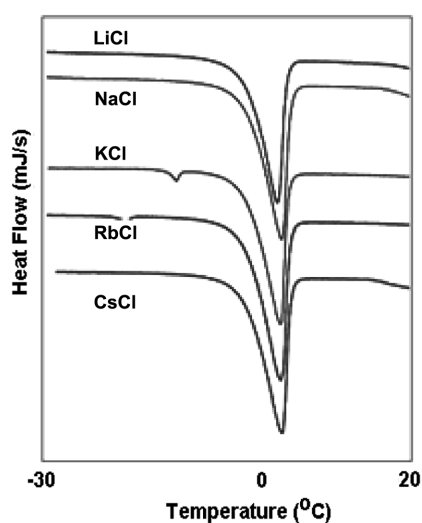


Figure 3. Endothermic curves of SV hydrogel in 0.1 M alkali-metal salt solution by DSC.

induced formation of ordered structure.

In order to know the effects of cation radius from Li^+ (smallest radius) to Cs^+ (largest radius) and water solubility of alkali metal chloride to the SV hydrogel, various alkali-metal salts (LiCl, NaCl, KCl, RbCl and CsCl) were added to the SV hydrogel at 1.0% (w/w). Melting behavior of the hydrogels showed similar profiles (Figure 3) in comparison with the native hydrogel and the other salts (K_2SO_4 , KSCN and urea). To analyze the changing of water state in the SV hydrogel induced by alkali-metal chlorides, estimation of water distribution was also conducted (Table IV). Non freezable water decreased by addition of LiCl to KCl, but subsequently increased by addition of RbCl and CsCl. Change in the amount of freezable and non freezable water probably correspond by difference of ion radius and solubility of alkali metal salts in water. Both NaCl and KCl have lower solubility in water than the other salts (LiCl, RbCl and CsCl). Less soluble salts were difficult to interact with water and easily form crystallized water cluster. Free freezable water is slightly higher for all species after addition of NaCl and KCl. By addition of RbCl and KCl, low endothermic peak appeared at -9°C , corresponding to freezable bound water, as crystallized water loosely bonded to the

hydrogel. The total content of freezable water was estimated by integration of the endothermic peak from -30 to 20°C and estimation of non-freezable water in the gel was conducted by subtracting freezable water from total water content in the swollen hydrogel.

Salts did not induce any change on water properties in the hydrogel network, only one transition being appeared at additive concentration of 0.1 M. If attentively observed, however, overall results showed considerable change in the state of water structure depending upon species. Hence, to get more information about the effects of additives on the hydrogels, additive concentration dependent effects on water in the hydrogels were analyzed by selecting urea and NaCl as representative additives.

Effect of Different Concentration of Salt Addition on Water State of SV Hydrogel. The SV hydrogel was selected to investigate the effects of concentration of additives on water state of Salvia hydrogels. Figures 4 and 5 showed endother-

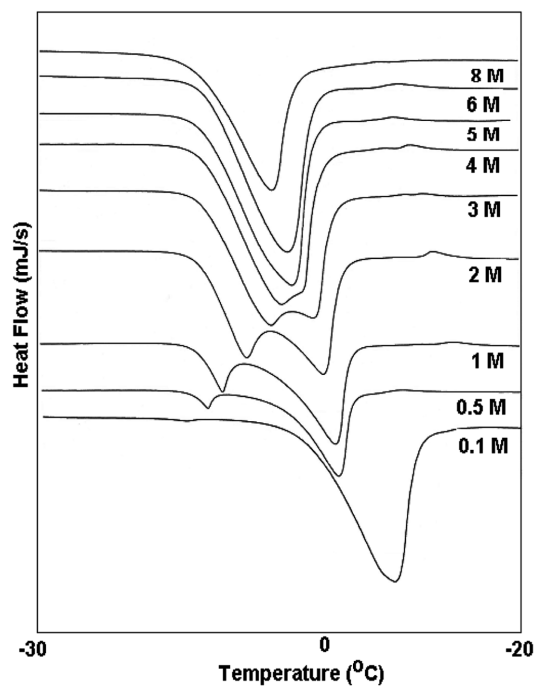


Figure 4. Endothermic curve of SV hydrogel under different urea concentration.

Table IV. State of Water in SV Hydrogel Containing 0.1 M Alkali-Metal Salts after Calculation by DSC

Alkali-Metal Salt	FW			NFW			F-NFW R		
	SM	SS	SV	SM	SS	SV	SM	SS	SV
LiCl	16.2	16.2	15.5	82.8	82.8	83.5	19.6	19.6	18.6
NaCl	17.1	16.3	16.5	81.9	82.7	82.5	20.9	19.7	20.0
KCl	17.8	17.9	16.9	81.2	81.1	82.1	21.9	20.9	20.6
RbCl	17.4	17.5	16.7	81.6	81.5	82.3	21.3	21.5	20.3

FW: Freezable water. NFW: Non freezable water. F-NFW R: Freezable-non freezable water ratio $\times 100$.

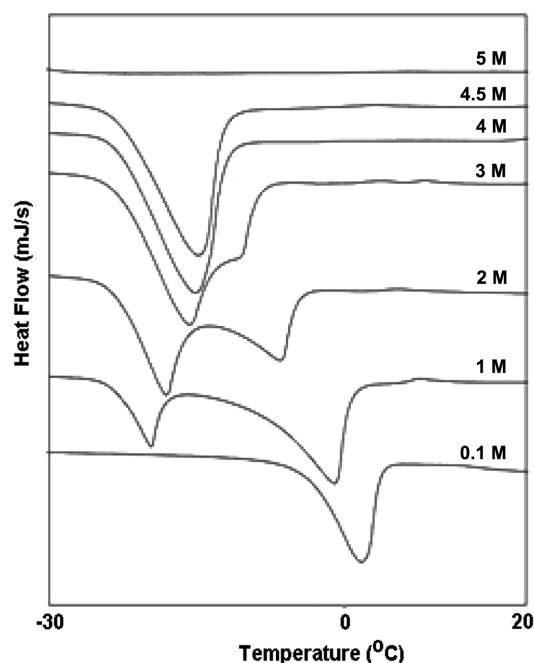


Figure 5. Endothermic curve of SV hydrogel under different NaCl concentration.

mic curves of the SV hydrogel containing urea (0.1–8.0 M) and NaCl (0.1–5.0 M). At the beginning of concentration (0.1 M) only one transition peak appeared. Above 0.1 M, state of water in the hydrogel split into well-resolved endothermic peaks at 0.5–3.0 M urea and 1.0–3.0 M NaCl.

The appeared peaks gradually shifted to low temperature side and merged into one transition peak above 3.0 M urea and NaCl. This suggests that partial water was displaced by loosely bound water at low temperature. Low melting temperature corresponds to melting of disorder frozen water, whereas high melting temperature to more stable water which may be present as hexagonal ice crystals.¹⁹ At 5.0 M NaCl concentration, the endothermic peak disappeared, indicating that absorbed water in the gel network could be assigned to take non freezable state and strongly bound to the network.

Profiles of salt solutions distributed in the hydrogel structure are shown in Figures 6 and 7. Peak area was calculated to know the effects of urea and NaCl concentrations in the 1.0% (w/w) hydrogel network. Generally, addition of salt in different concentrations changes the amount of freezable and non-freezable water in the network. Increasing concentration of urea and NaCl progressively enhanced the amount of non freezable water and reduced the amount of freezable water. The ratio of F to NFW (F-NFW) reduced with increase in concentration of NaCl and dropped to zero at 5.0 M NaCl. Increasing non freezable water was mostly followed by decreasing freezable water at constant amount of water relevant to formation of water structure through Na⁺ ion in

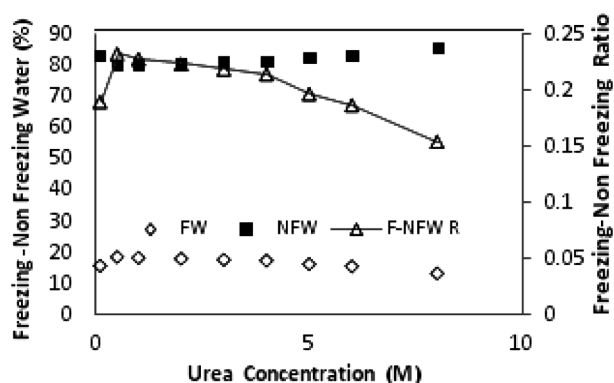


Figure 6. Concentration dependence of urea on freezable-non freezable water.

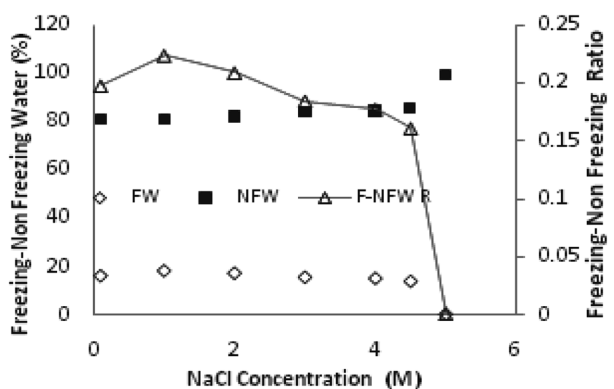


Figure 7. Concentration dependence of NaCl on freezable-non freezable water.

aqueous solution as elucidated by Annaka *et al.*²⁰ The results were interpreted in that both urea and NaCl affected water structure of Salvia hydrogels by changing water structure of the hydrogen bonded networks. Two mechanisms may occur in the gel networks: 1) Salt breaks water molecules bonded to the gel network; and 2) Salts change hydrogen bonded network of water.

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