

# Regimes of Microstructural Evolution as Observed from Rheology and Surface Morphology of Crosslinked Poly(vinyl alcohol) and Hyaluronic Acid Blends During Gelation

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**ABSTRACT:** Hyaluronic acid (HA)-based materials are being investigated because of their role in biological fluids and tissues. Poly(vinyl alcohol) (PVA) when blended with HA at different compositions leads to superior mechanical properties compared to pure HA. The PVAHA blend hydrogels are potential candidates for pharmaceutical, biomedical, and cosmetic applications. It is essential to understand the structure, gelation time, and morphological properties of these hydrogels. In this work, a blend system of PVA crosslinked with glutaraldehyde in the presence of HA is studied. Semidilute solutions of PVA and HA are blended, followed by gelation due to crosslinking. The crosslinked gels as well as the gel cast membranes were examined. The effect of HA on the gelation process is investigated using rheological characterization. It is shown that kinetics of gelation is influenced by HA content, though storage modulus of the gels is influenced marginally. The structural features of PVAHA gels were also probed with scanning electron microscopy and dynamic light scattering. It is argued that there is a complex interplay between intra- and intermolecular crosslinking of PVA and PVA–HA interactions during the gel formation. Based on the insights obtained from various probing techniques for PVAHA gels with different HA content, three broad structural features were identified. It is shown that the hydrogel is semi-interpenetrating network at lower HA content (<10% HA), cocontinuous morphology at moderate HA content and with domains at high HA content (>20% HA). © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 41081.

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## INTRODUCTION

Hydrogels are made from physical or chemical crosslinking in the presence of water or in aqueous biological fluids. The physical network implies entanglements, crystallites, or weak associations such as hydrogen bonding or van der Waal's attractive forces. The chemical networking is due to the covalent bonding among various functional groups. Chemically crosslinked hydrogels are swollen polymer networks. Hydrogels, depending on the structure and network formation, exhibit viscoelastic fluid like or viscoelastic solid like characteristics. Hence, the viscoelastic properties-like modulus and damping factor of the gels provide an insight into the key structural features.

Hydrogels of highly swellable polymeric materials have been studied extensively for the drug delivery systems.<sup>1–3</sup> The gelation time and the kinetics of gelation are important aspects to be considered for, as the solutions are injected to the target and are allowed to form gel.<sup>4</sup> Poly(vinyl alcohol) (PVA), a synthetic polymer, hyaluronic acid (HA), a natural polymer, and their blends are among the most common polymeric materials in drug delivery systems.

Kinetics of gelation and network formation for various polymeric systems have been reported earlier.<sup>5</sup> Considering the biocompatibility, various researchers reported the preparation and properties of blends, interpenetrating networks (IPNs), semi-interpenetrating networks (SIPNs), and swelling of PVA and HA systems.<sup>6–12</sup> The gels can also be cast into membranes, which can be stored and hydrated to different degrees before application.<sup>13–15</sup>

Glutaraldehyde (GA) is extensively used in various drug delivery systems to crosslink biological polymers such as proteins and polysaccharides. Use of GA crosslinked PVA in oral drugs for sustained release has also reported recently.<sup>16,17</sup> HA or “sodium salt of HA,” is a natural polymer of linear disaccharide type. It consists of D-glucuronic acid and N-acetyl-D-glucosamine. The HA in the form of a free macromolecular chain is capable of delivering the drug as the drug molecules are covalently bonded to the HA, a system called “polymeric prodrug.”<sup>18</sup> GA can crosslink both HA and PVA, but at two different conditions. It forms acetal bridges among PVA molecules under acidic conditions, while it reacts with HA in basic conditions.

**Table I.** Composition and Nomenclature of Hydrogels

| Sample        | 5% PVA solution (vol %) | 1% HA solution (vol %) | Wt % of PVA (water-free basis) | Wt % of HA (water-free basis) |                     |
|---------------|-------------------------|------------------------|--------------------------------|-------------------------------|---------------------|
| Pure PVA      | 100                     | 0                      | 100                            | 0                             | Low HA content      |
| PVAHA 71      | 87.5                    | 12.5                   | 97.33                          | 2.67                          |                     |
| PVAHA 62      | 75                      | 25                     | 93.99                          | 6.01                          |                     |
| PVAHA 53      | 62.5                    | 37.5                   | 89.67                          | 10.33                         |                     |
| PVAHA 4.5 3.5 | 56.25                   | 43.75                  | 87.01                          | 12.99                         | Moderate HA content |
| PVAHA 44      | 50                      | 50                     | 83.9                           | 16.1                          |                     |
| PVAHA 3.54.5  | 43.75                   | 56.25                  | 80.21                          | 19.79                         |                     |
| PVAHA 35      | 37.5                    | 62.5                   | 75.76                          | 24.24                         | High HA content     |
| PVAHA 26      | 25                      | 75                     | 63.46                          | 36.54                         |                     |

The determination of gel time is very important for all the geling systems, and rheology is used very often in its determination.<sup>19</sup> The gel time can be determined by measuring storage modulus ( $G'$ ), loss modulus ( $G''$ ) and damping factor ( $\tan \delta$ ) in oscillatory shear, or measuring viscosity in steady shear. Several criteria have been used for determining the gel time using these measurements.<sup>20,21</sup>

Criterion 1: Gel time is determined from the crossing point between the baseline and the tangent drawn from the turning point of  $G'$  curve.

Criterion 2: Gel time is the time required for viscosity to reach a very large value.

Criterion 3: Gel time is measured as the time where  $\tan \delta$  equals 1, or  $G'$  and  $G''$  curves crossover.

Criterion 4: Gel time is determined as the time when  $\tan \delta$  is independent of frequency.

Examination of the rheological characteristics during gelation, and for the gel, can also lead to insights about the microstructure. The main focus of the present study is to investigate the blends comprising HA and PVA and their gels during the crosslinking reaction with GA.

It is shown that the rheological variation of properties leads to insights about different types of microstructures. A SIPN, a cocontinuous morphology and a domain formation in these blends could be discerned from the rheological property variation. Three different types of surface morphology were also observed using environmental scanning electron microscopy (ESEM). Similarly, correlation function obtained from dynamic light scattering (DLS) demonstrated three different types of structural features. The effect of the addition of HA on gelation, gel, and membrane properties are also studied.

## EXPERIMENTAL

### Materials

PVA with an average molecular weight of 146–186 kDa (99% hydrolyzed) was purchased from Sigma Aldrich. HA with an average molecular weight of 1270 kDa (44.2% glucuronic acid) was purchased from Linyi Taihao International Trading Com-

pany, Shandong, China. GA solution 25% with a molecular weight of 100.12 was supplied by FINAR Chemicals, Ahmedabad. All other chemicals that were used are laboratory reagent grade and used as purchased. For the preparation of solutions, Millipore® water was used.

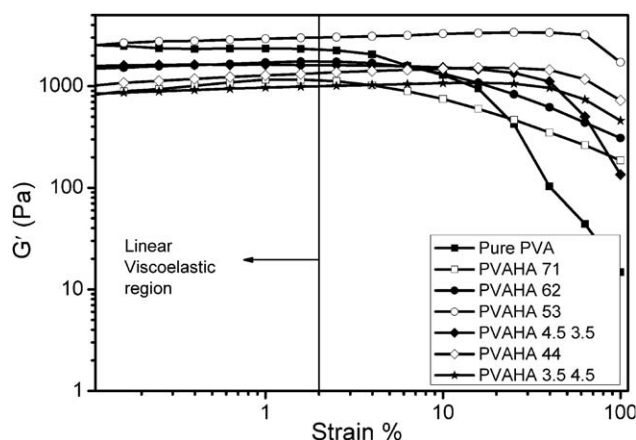
### Preparation of Gel/Membrane Systems

The gel systems were prepared according to the methods mentioned elsewhere.<sup>22,23</sup> A crosslinking solution consisting of 3 mL of 50 vol % methanol (quencher), 2 mL of 10 vol % acetic acid (buffer), 1 mL of 25 vol % GA, and 1 mL of 10 vol % sulfuric acid was prepared. PVA solution (5 wt %) was prepared by dissolving 5 g of PVA in 95 g of water at 70°C for 8 h of stirring. HA solution (1 wt %) is prepared by dissolving 1 g of sodium hyaluronate in 99 g of water at room temperature for 5 h of stirring.

PVAHA solutions were prepared by mixing the 5% PVA solution with 1% HA solution according to the volume ratios given in the Table I. For example, PVAHA 62 was prepared by mixing 6 mL of 5% PVA solution and 2 mL of 1% HA solution. The weight percent of PVA and HA on water-free basis are also shown in the table. Mixing was carried out for 2 h at room temperature before the reaction.

While crosslinking these solutions, PVA to the crosslinker solution ratio of 10:2.8 by volume was maintained.<sup>23</sup> The crosslinking was carried out in a 10 mL glass vial with continuous stirring for 30 s to get homogeneous mixture, after the addition of crosslinker. The mixed solution was subjected to rheological analysis. These polyelectrolyte solutions show time dependent behavior, and therefore, they were allowed to be equilibrated for 6 h. During this period molecular interactions, such as hydrogen bonding, would lead to an equilibrated structure. It was confirmed that the properties like viscosity and modulus of these solutions do not change for 48 h after the equilibration time. Therefore, all the rheological studies and gel preparations were done within 48 h of equilibration time. If the solutions are stored longer, biodegradation of HA can lead to changes in the properties.<sup>24</sup>

The solutions that were prepared in a glass vial are poured into a Petri dish to cast membranes. After keeping the membranes



**Figure 1.** Strain sweep of PVAHA gels (sample nomenclature is shown in Table I).

for 3 h under ambient conditions, the rheological properties were measured. The weight loss due to water evaporation in these PVAHA membranes, under ambient conditions, was monitored for 3 days. For all the membranes, no further loss was observed after this duration. The membranes after 3 days are referred to as the dried membranes, and were used for characterizing the degree of swelling (percent increase in weight with respect to the weight of the dried membrane). The PVAHA membranes were stable after placing them in water. The fully swollen membranes were kept in water for 4 days, and no loss in their weight was observed.

### Rheological Experiments

The viscoelastic properties and the gel point of the blends were estimated using Anton Paar MCR 301 rheometer (with a cone and plate geometry of 25 mm diameter and 1° cone angle). Shear experiments were performed on the PVAHA solutions by varying the shear from 1 to 100  $s^{-1}$  to measure viscosity. Oscillatory strain sweep, from 1 to 100%, was performed on the PVAHA solutions to evaluate the linear viscoelastic regime at a frequency of 100  $rad\ s^{-1}$ . An oscillatory frequency sweep on PVAHA solutions was performed in the linear viscoelastic regime by varying the frequency from 1 to 100  $rad\ s^{-1}$ . While evaluating gel properties, the PVAHA solutions, immediately after the addition of crosslinker, were transferred to the rheometer and allowed to gel. An oscillatory strain sweep was performed to all such PVAHA gels at 10  $rad\ s^{-1}$ , by varying the strain % from 1 to 100, as shown in Figure 1. An oscillatory frequency sweep on PVAHA gels was also performed in the linear viscoelastic regime by varying the frequency from 1 to 500  $rad\ s^{-1}$ . To evaluate transient property evolution during the crosslinking reaction, the variation in  $G'$  and  $G''$  in the linear viscoelastic regime was followed for 20 min. This experiment was conducted at frequencies of 0.5, 1, 3, and 8  $rad\ s^{-1}$ .

For the measurement of viscoelastic properties of the membranes, parallel plate geometry (25 mm diameter) was used. Oscillatory strain sweep and frequency sweep on the membranes were performed with the similar conditions as that of gels. All the rheological experiments were conducted at physiological temperature of 37°C, unless mentioned.

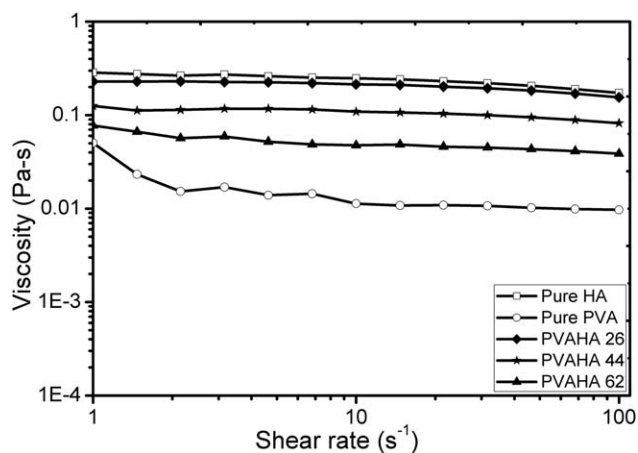
### Microscopy and Light Scattering

PVAHA gels after different drying times were observed in an FEI Quanta 400 FEG (ESEM mode, gaseous secondary electron detector [GSED] at 0.96 Torr vacuum). After the addition of crosslinker to the solutions of PVAHA, they are poured in to Petri dishes. A small portion of the gel was cut and placed on the stub and is subjected to microscopic analysis at different intervals of drying time. Entire surface of the sample was scanned to observe the surface features at different magnifications.

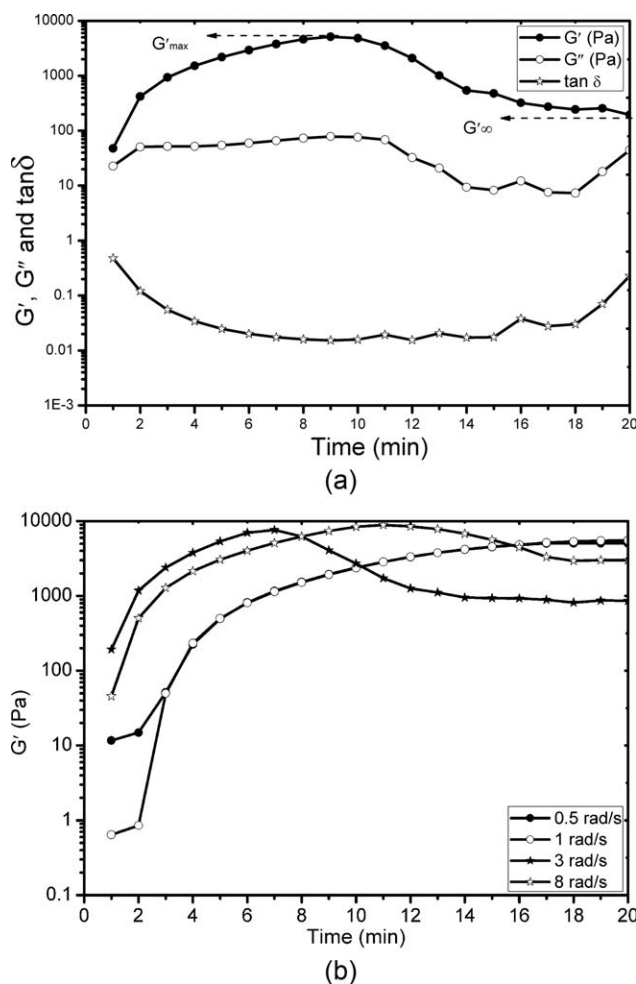
DLS from PVAHA samples (samples used in rheology) was analyzed using Horiba nano partica SZ 100. The first-order correlation function was measured for the solutions, and at different times during gelation. Laser light with a wavelength of 532 nm was passed through a disposable cuvette open at four sides and the intensity of scattered light was measured at 90°. During the experiments, the cell temperature was maintained at 37°C.

## RESULTS AND DISCUSSION

Initially, rheological properties of PVAHA solutions were characterized. Figure 2 shows the viscosity of PVAHA solutions at different HA content. With higher HA content, the viscosity of PVAHA solutions is higher. All the solutions were mildly shear thinning, indicating the presence of physical interactions. While mixing PVA and HA solutions, the presence of hydroxyl groups in PVA and carboxylic and hydroxyl groups in HA are responsible for hydrogen bond formation. These solutions were used to prepare gels by chemical crosslinking. It should be noted that, though the gel formation is due to these covalent crosslinks, the microstructure of resulting hydrogels can be influenced by the physical interactions.<sup>6</sup> Hong et al.<sup>25</sup> have reported the overlap concentration of PVA/water to be 0.99 g/100 mL. The concentration of PVA solutions used in the present work (PVA with the same molar mass and the identical degree of hydrolysis as in Refs. 6, 25) is 5.3 g/100 mL, ~5 times higher than the overlap concentration. Therefore, semidilute polymer solutions have been used in this work for making crosslinked gels. The effect of physical gelation due to chain entanglements is, therefore, limited.  $G'$  for PVA solution (0.35 Pa) and PVA crosslinked gel



**Figure 2.** Viscosity versus shear rate for PVAHA solutions (sample nomenclature is shown in Table I).



**Figure 3.** (a) Storage modulus, loss modulus, and  $\tan \delta$  for PVAHA 71 during gelation, at 8 rad  $s^{-1}$ , (b) Evolution of  $G'$  at different frequencies for PVAHA 62 (sample nomenclature is shown in Table I).

(14,100 Pa) are different by orders of magnitude, and this difference is due to chemical crosslinking. Such orders of magnitude difference between  $G'$  of the solution and  $G'$  of the gel were also observed for all PVAHA compositions. The focus in the present work is on chemically crosslinked PVAHA gels while PVAHA gels with physical crosslinks have been studied earlier.<sup>13,26</sup>

The mechanical properties of hydrogels, and the gelation process, are affected by the initial polymer concentration, temperature and polymer to crosslinker ratio. In the present study, PVA to crosslinker ratio is maintained constant as the system forms a strong gel at this ratio. The effect of composition and temperature with varying network density (as the crosslinking of PVA is done in the presence of HA of this blend system) is studied. The hydrogels thus prepared, based on the methods outlined in the previous section, were optically clear and transparent.

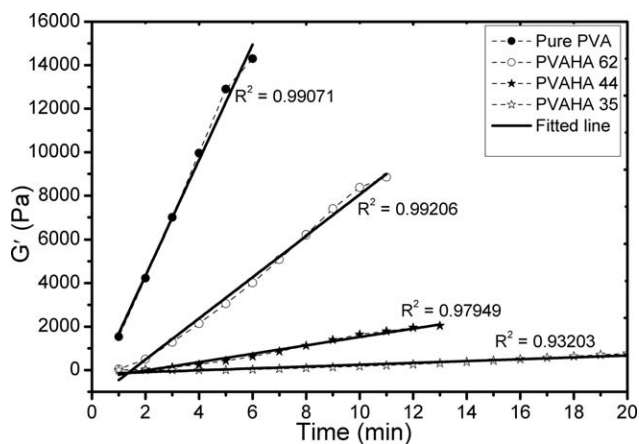
### Gelation Rheology

Rheological measurements were carried out to evaluate the gel time with varying amounts of HA while gelation occurred due to crosslinking reaction in PVA.<sup>23</sup> For an example, evolution of  $G'$ ,  $G''$ , and  $\tan \delta$  for PVAHA 71 (at 8 rad  $s^{-1}$ ) is shown in Fig-

ure 3(a). It can be observed that  $G'$  becomes 1–2 orders of magnitude larger than  $G''$  as the crosslinking reactions proceed (in this system, the crossover was not observed). Since the material becomes more elastic with crosslinking reactions,  $\tan \delta$  decreases with time. After a certain period of increase, a decrease in  $G'$  was also observed in some cases. As crosslinking proceeds, the decrease in  $G'$  could be due to the elution of water from the crosslinked network,<sup>27</sup> and was observed only for lower HA content. It could also be due to physical interactions among PVA and HA as the crosslinking reaction proceeds. However, this needs to be explored further. The variation of  $G'$  during gelation of PVAHA 62 at different frequencies is shown in Figure 3(b). Since  $G'$  is a function of frequency during gelation, the overall evolution also seems to be different at different frequencies.  $G'$  at higher frequency is higher at the initial stage, and remains higher throughout the gelation process.

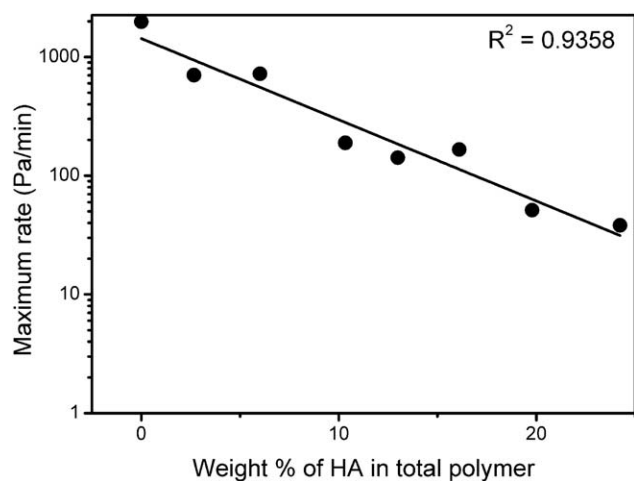
Generally, three regimes were observed during modulus increase to the maximum value; initial induction regime, a regime of constant rate of modulus increase (maximum rate), regime of slower increase. The maximum rates measured in the second regime, as function of HA content, are shown in Figure 4. It can be seen that the rate is very high (2652 Pa/min) for pure PVA, and it decreases with the addition of HA. For PVAHA 71 and PVAHA 62, the presence of HA molecules delay the reaction process to greater extent, when compared to higher contents of HA. The decrease in the maximum rate is exponential with the HA content, as shown in Figure 5, representing a straight line in semilogarithmic coordinates. It was observed that the addition of HA generally delayed gelation, with PVAHA 26 not forming a gel at all. The effect of temperature on gelation time was modest as observed from gelation experiments conducted at different temperatures from 25 to 42°C.

To examine the evolution during gelation, it is of interest to observe variation from initial  $G'$  to the maximum  $G'$ . However, from an application point of view,  $G'$  of the gel after elution (when  $G'$  becomes constant with respect to time) is important. In further discussion, maximum in  $G'$  ( $G'_{max}$ ) is used for normalizing  $G'$  as a function of time, while plateau in  $G'$  ( $G'_{\infty}$ ) is used for examining properties of the gel.



**Figure 4.**  $G'$  (8 rad  $s^{-1}$ ) versus time during the regime of constant rate of modulus increase (the figure shows straight line fits to estimate the maximum rate. Sample nomenclature is shown in Table I).





**Figure 5.** Maximum rate of modulus increase during gelation for various compositions of PVAHA.

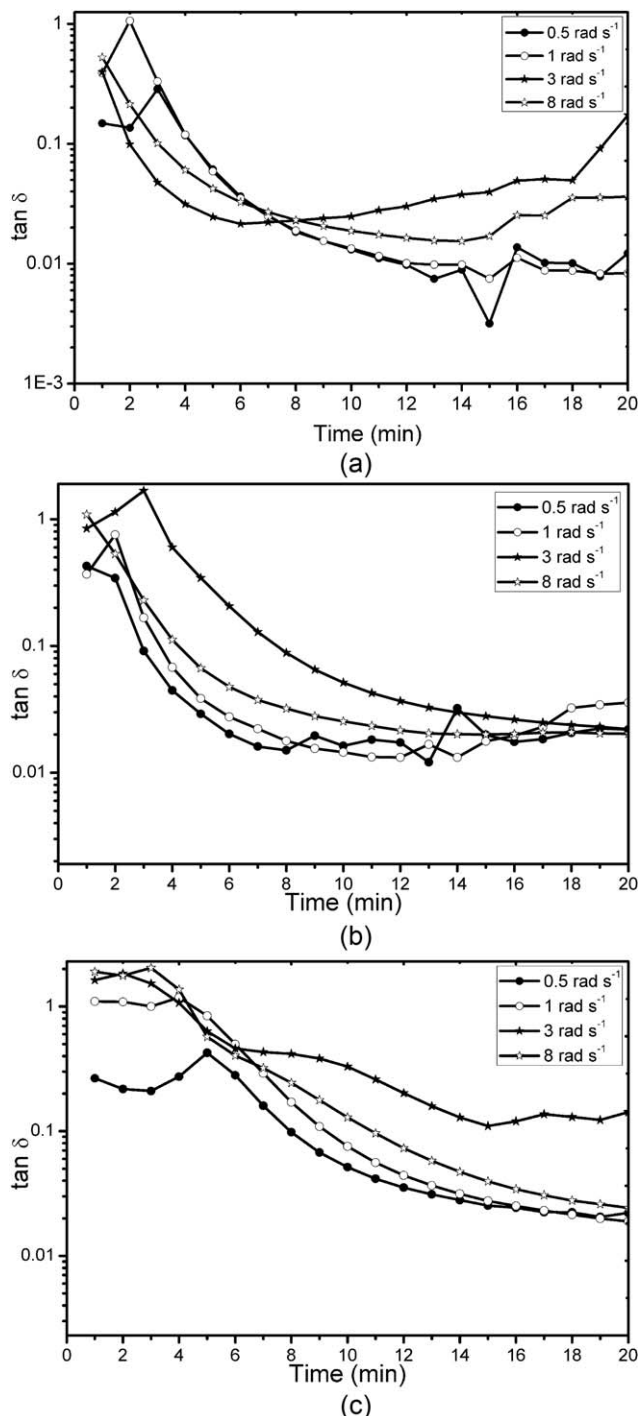
One way is to follow  $G'$  and evaluate the gel time by drawing the tangent at the turning point (onset of gelation) as discussed in Criterion 1.<sup>28</sup> Since  $G'$  is a function of frequency, the gel time for each frequency is different, when this criterion is used. With many soft hydrogels such as investigated in this work, the measurement of viscosity (Criterion 2) is difficult due to slip and material failure. For all the systems analyzed, the crossover of  $G'$  and  $G''$  (Criterion 3) was not observed. Therefore, in this work, Criterion 4 ( $\tan \delta$  being independent of frequency at the gel time) was used, whenever possible. An example for the evolution of  $\tan \delta$  with time for PVAHA 62 is shown in Figure 6(a). At the gel time,  $\tan \delta$  is observed to be independent of frequency, and this is indicated in the figure.

At moderate and higher HA contents, the independence of  $\tan \delta$  at a given time (or Winter–Chambon) was not observed, as shown in Figure 6(b,c) for PVAHA 44 and PVAHA 35. Figure 7 shows the variation of  $G'$  for PVAHA 44 at different frequencies. A remarkable feature is observed, in that  $G'$  is “independent of frequency throughout the gelation process.”  $G'$  evolution at different HA content is shown in Figure 8(a–c). To highlight the evolution with time,  $G'$  has been normalized with respect to  $G'_{\max}$ . Similarly, time has been normalized by the time required to reach  $G'_{\max}$ . Though the evolution of  $G'$  is a function of frequency for PVAHA 62 [Figure 3(b)], the normalized  $G'$  evolution [Figure 8(a)] is the same for all frequencies. For PVAHA 44,  $G'$  evolution was observed to be independent of frequency (Figure 7), and therefore, the normalized modulus is also independent of frequency during the gel formation [Figure 8(b)]. As shown in Figure 8(c), the normalized modulus is function of frequency for PVAHA 35. Therefore, the evolution of  $G'$  is different for low, moderate, and high content of HA during the gel formation.

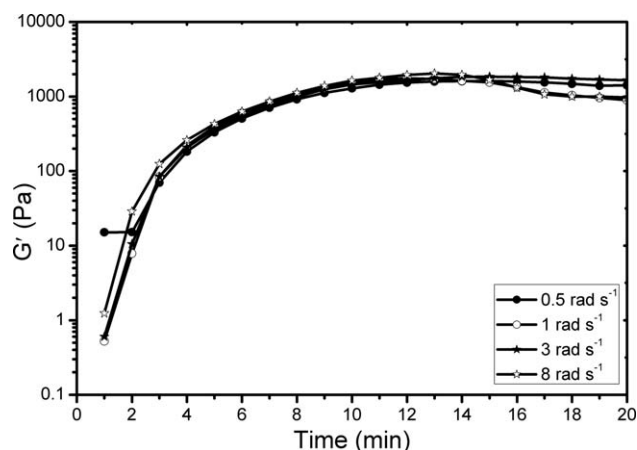
### Characterization of Gels and Membranes

Elastic or storage moduli for different compositions were analyzed by forming the gels in the rheometer (gel formation was allowed for 15 min, since the gel times were observed to be less than this for all compositions). As shown in Figure 9,  $G'$  is independent of frequency for all the gels (rheological signature

of the gel formation). This is expected for crosslinked and swollen hydrogels. The  $G'$  of pure PVA is high at 12,900 Pa. With the addition of HA, the  $G'$  is lower by an order of magnitude, and varies from 894 to 1940 Pa. For PVAHA 26, the  $G'$  was found to be very low, since the gel did not form till 20 min. As



**Figure 6.** (a)  $\tan \delta$  versus time for PVAHA 62. The gel time is indicated by vertical lines, (b)  $\tan \delta$  versus time for PVAHA 44, showing that Winter–Chambon criterion is not followed, (c)  $\tan \delta$  versus time for PVAHA 35, showing that Winter–Chambon criterion is not followed (sample nomenclature is shown in Table I).



**Figure 7.** Evolution of storage modulus for PVAHA 44 at different frequencies (sample nomenclature is shown in Table I).

many of the drug delivery hydrogel systems have  $G'$  in the range from 500 to 2000 Pa, most of the PVAHA hydrogels investigated in this work can be used for drug delivery applications.<sup>29–33</sup>

Swelling characteristics of the membranes were evaluated, as these characteristics provide an insight into stability of the membrane structure and diffusion of water. The degrees of swelling for different compositions are listed in Table II. The degree of swelling is higher for PVAHA gels with higher HA content. This is expected due to higher hydrophilicity of HA as well as lower concentrations of PVA and crosslinker.

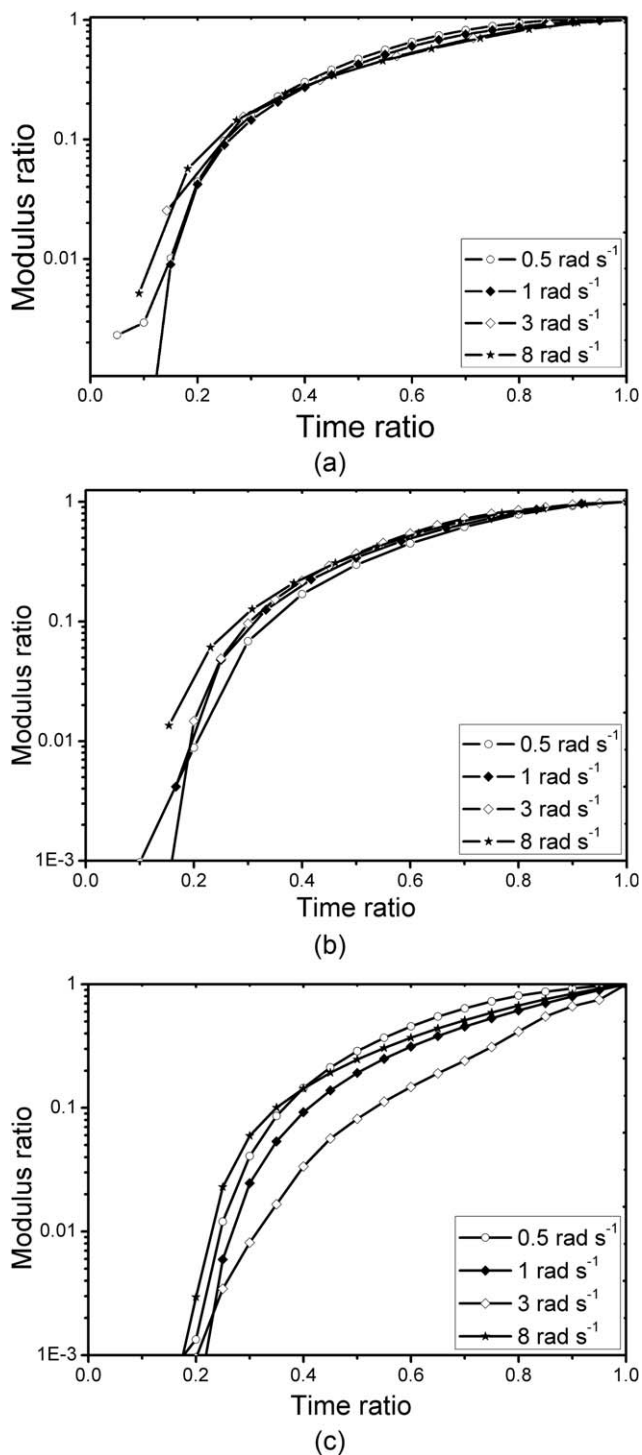
Though the PVAHA gels were transparent, the membranes become translucent as well as shrink during the casting process (as described in Experimental section). The shrinkage is due to the loss of water. The weight loss due to water evaporation was monitored from the initial gel state to the dried membrane state. Figure 10 shows the weight loss of water during the membrane casting process. It is evident from the figure that this gel cast membranes dry at different rates. It can also be seen that about 68–75 wt % of water loss occurred in first 3 h.

#### Surface Morphology and Structural Correlation

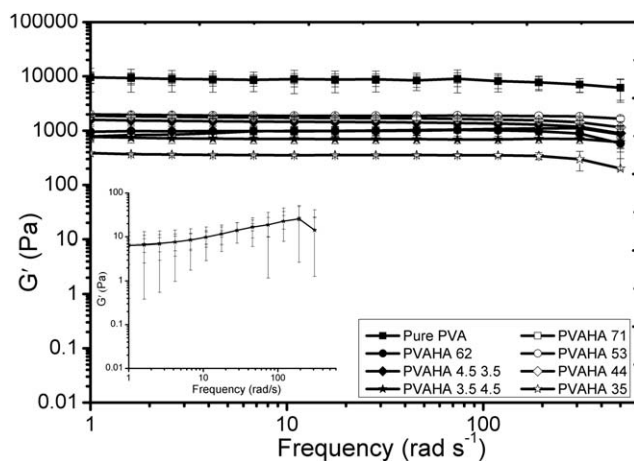
Figure 11 shows the ESEM images of pure PVA, PVAHA 62, PVAHA 44, and PVAHA 35 at various drying times. The ESEM analysis was done for different batches of samples, various locations, and at magnifications, only the representative results are shown in the figure. It can be observed that the surface morphology of these samples is different in comparison at any drying time. It can be also seen that the surface of pure PVA and PVAHA 62 is smooth without exhibiting any feature while the samples PVAHA 44 and PVAHA 35 has distinct features compared to the PVAHA 62.

Figure 12 shows the first-order correlation function obtained by DLS experiments on PVAHA samples with different HA contents. In each of the graphs, PVAHA solution and PVAHA samples during gelation are shown. It is evident from these figures that PVAHA solutions, irrespective of HA content, show correlation with two characteristic decay times. In other words, the correlation function decays as combination of two functions. As shown in Figure 12(a), PVAHA 62 after 3 and 19 min of gela-

tion continue to show the correlation with two decay times. Conversely, for PVAHA 44 and PVAHA 35 samples after 3 min of gelation, appear to have less prominent two decay times. With 19 min of gelation, both these samples do not show any signature of two decay times. Therefore, the structural features



**Figure 8.** (a) Storage modulus versus time (normalized) for PVAHA 62, (b) storage modulus versus time (normalized) for PVAHA 44, (c) storage modulus versus time (normalized) for PVAHA 35 (sample nomenclature is shown in Table I).



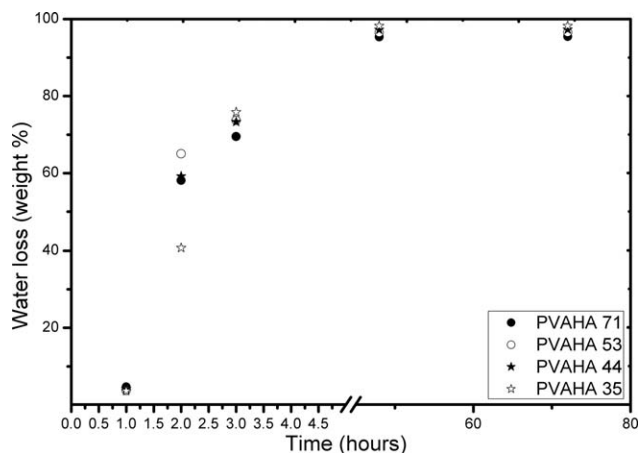
**Figure 9.** Frequency sweep of the gel for PVA and HA compositions (the inset shows the storage modulus versus time of PVAHA 26. Sample nomenclature is shown in Table I).

observed with DLS confirm that PVAHA 62 is very different from PVAHA 44 and PVAHA 35.

#### Microstructure at Different HA Content

Intramolecular and intermolecular interactions are important in determining the structure of network, especially in semidilute polymer solutions. It is helpful to examine the polymer concentrations used in this work, and compare them with literature results on intra- and intermolecular crosslinking. It has been shown that the lower the concentration of the polymer, the higher is the intramolecular crosslinking or cyclization.<sup>34,35</sup> Specifically, PVA crosslinking with GA was investigated at various concentrations of PVA (2.2–17.8 g/100 mL) and GA ( $\sim 0.006$ – $0.06$  [CHO]/[OH]).<sup>32</sup> It was shown that for lower concentration of PVA (2.2 g/100 mL), 100% gel was not obtained even when highest amount of crosslinker was used. This is due to the intramolecular crosslinking reactions. At higher concentrations of PVA, as long as a critical concentration of GA was used, 100% gel formation was observed.<sup>32</sup> Kim et al.<sup>6</sup> have shown the importance of HA aggregation in determining the rheology and morphology of PVAHA-borax systems. In the present work also, intra- and intermolecular crosslinking and aggregation would be expected to play a role in determining microstructures of PVAHA systems with different HA content. In the following discussion, we present our hypothesis regarding PVAHA microstructures at three different regimes of HA content.

At lower HA content (10 wt % of HA in total polymer on water-free basis, as shown in Table I), rheological characterization during gelation showed that the Winter–Chambon criterion was observed. It can be argued that the aggregation of HA molecules is suppressed by the effective chemical crosslinking of PVA molecules. Therefore, at lower HA content, a SIPN of HA in crosslinked PVA is formed. ESEM analysis also led to the



**Figure 10.** Drying rate curves for various PVAHA samples (Sample nomenclature is shown in Table I).

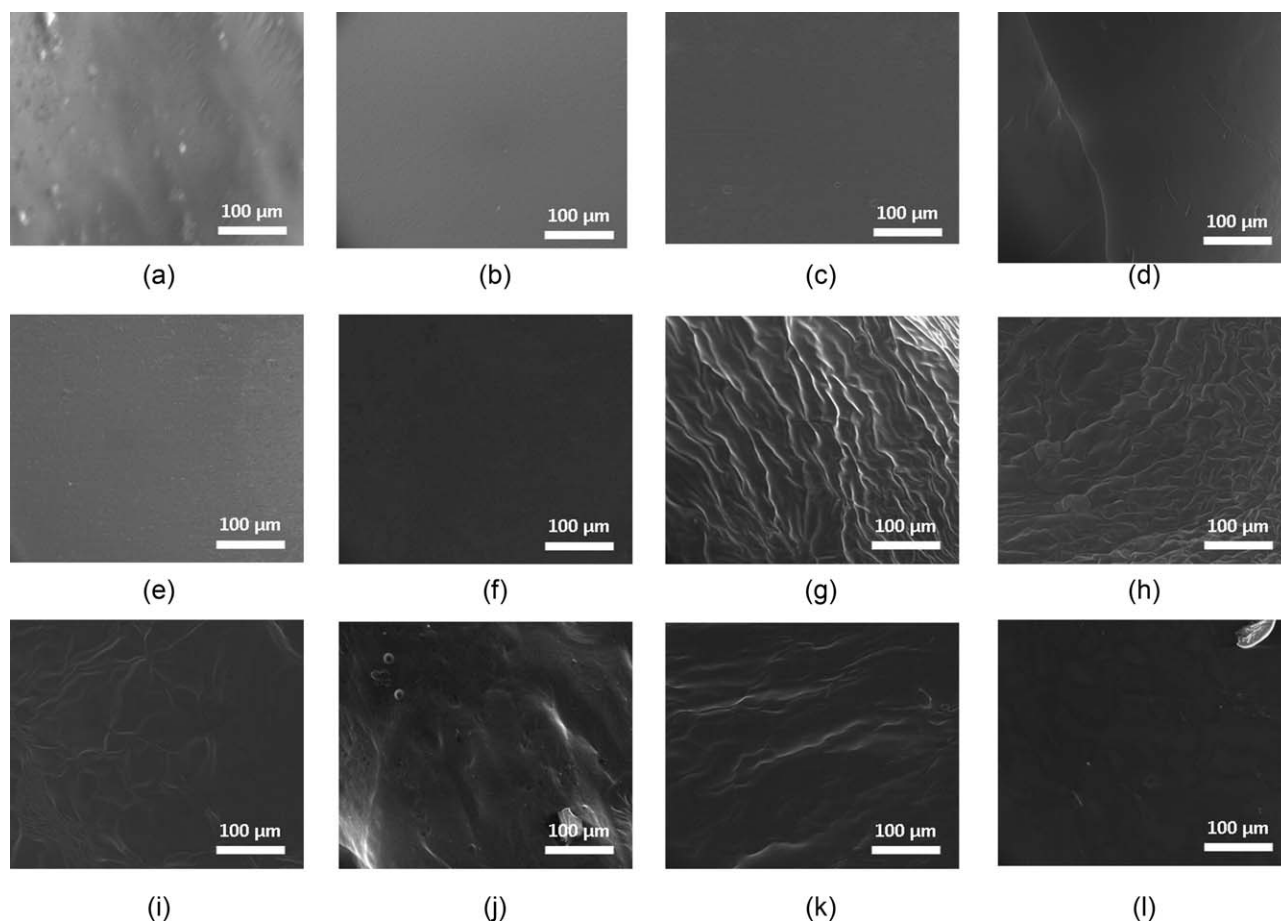
observation that PVAHA gels with lower HA content exhibit no distinct surface features. Similarly, the intensity correlations function, before and after the gelation process was observed to be similar for PVAHA with lower HA content. Therefore, both ESEM and light scattering also support formation of a homogeneous SIPN of PVAHA at lower HA content. Therefore, the molecular organization at lower HA content is likely to be as depicted in schematic of Figure 13(a).

As the HA content increases in the blend, intramolecular cyclization would become dominant, possibly allowing the HA molecules to form aggregates. The effective concentration of PVA (on HA-free basis) used in this work was 1.75–5.26 g/100mL. It should be emphasized that the overall polymer concentration (i.e., both PVA and HA) used in this work was 2.07–5.26 g/100mL. Another point to note is that the crosslinker ratio (moles of CHO in GA/moles of OH in PVA) was constant for all compositions, 0.35. Therefore, in the present work, the effect of intramolecular cyclization is likely to be stronger with higher contents of HA, purely on the basis of the lower effective concentration of PVA.

At moderate HA content (10–20% HA), striking surface features possibly indicating cocontinuous morphology. These surface features were observed at different drying times and could be related to cocontinuous microstructure of the PVAHA system. An interesting feature observed during gelation rheology, was that the evolution of  $G'$  was independent of frequency (Figure 7). The difference of correlation functions for the solution and gel at moderate HA content also points to significant molecular aggregations taking place during gelation. Based on these results, therefore, a cocontinuous morphology at moderate HA content is hypothesized and is represented schematically in Figure 13(b). This cocontinuous microstructure morphology arising from semidilute polymer solutions is very unique, and needs to be explored further.

**Table II.** Degree of Swelling for PVAHA Membranes

| Sample             | Pure PVA | PVAHA 71 | PVAHA 62 | PVAHA 53 | PVAHA 44 | PVAHA 35 | PVAHA 26 |
|--------------------|----------|----------|----------|----------|----------|----------|----------|
| Degree of swelling | 7.24     | 11.5     | 10.25    | 15.37    | 78.06    | 109.28   | 152.62   |



**Figure 11.** (a), (b), and (c) The ESEM images of pure PVA at  $\times 400$  magnification during 1, 2, and 3 h of drying, respectively. (d), (e), and (f) The ESEM images of PVAHA 62 at  $\times 400$  magnification during 1, 2, and 3 h of drying, respectively. (g), (h), and (i) are the ESEM images of PVAHA 44 at  $\times 400$  magnification during 1, 2, and 3 h of drying, respectively. (j), (k), and (l) are the ESEM images of PVAHA 35 at  $\times 400$  magnification during 1, 2, and 3 h of drying, respectively (sample nomenclature is shown in Table I).

At higher HA content ( $>20\%$  HA), rheological characterization showed that neither the Winter–Chambon criterion was followed nor  $G'$  was independent of frequency. Also, normalized modulus was also shown to be not independent of normalized time. These results, therefore, indicate that morphology at higher HA content is very different from SIPN or cocontinuous morphology observed at low and moderate HA contents, respectively. The surface morphology of PVAHA at higher HA content indicated nonuniform features. Unlike the surface morphology of PVAHA 44, the surface morphology of PVAHA 35 did not show cocontinuous features. The surface heterogeneities are, therefore, most likely due to improper formation of gels or due to significant aggregation of HA domains. The HA domain formation would be higher at the higher content of HA, due to intracycle crosslinking of PVA, as mentioned earlier. In DLS also, the correlation function of solution and gel was observed to be different, indicating domain formations. The heterogeneous structural features of the PVAHA gel at higher HA content are shown schematically in Figure 13(c).

Starting with semidilute solutions of PVAHA, we have demonstrated that it is possible to obtain three different types of qualitative behavior in rheology, surface morphology, and DLS. All

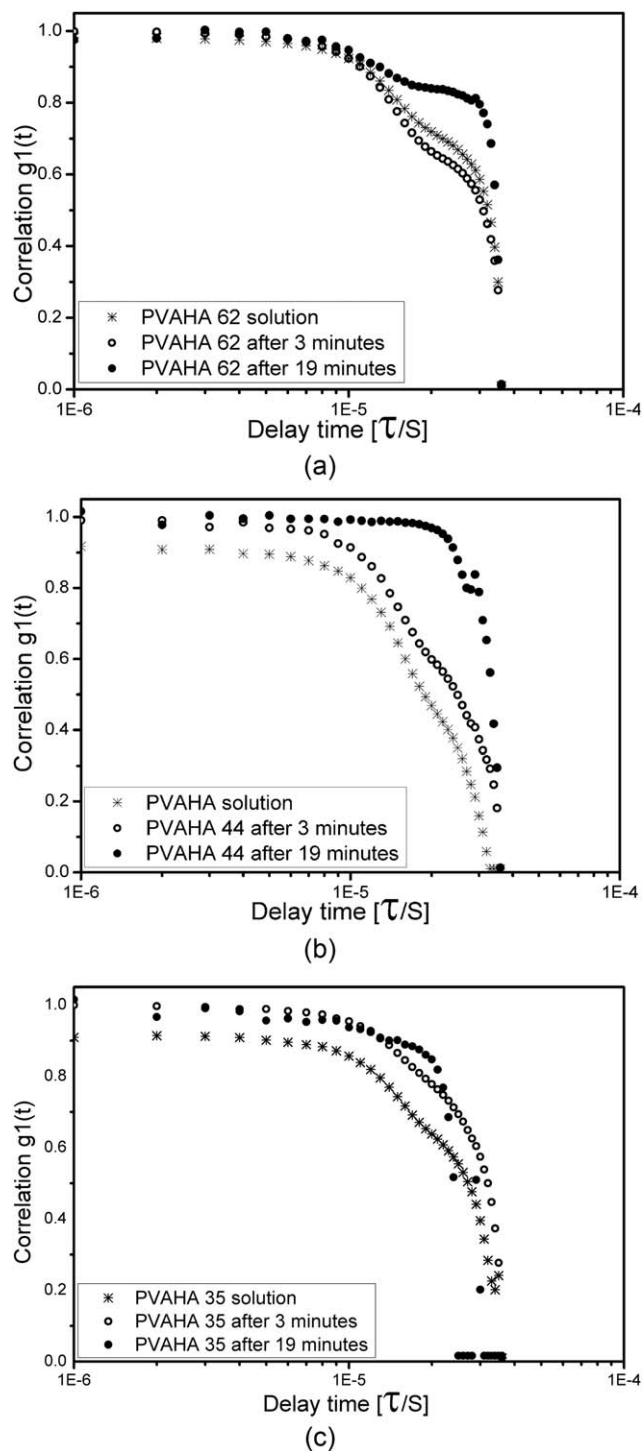
these results can be used to surmise three different molecular organizations as depicted in Figure 13. Among these, formation of cocontinuous morphology at moderate HA content is very interesting. The mechanism that leads to such morphology needs to be investigated by directly examining the molecular organization.

## CONCLUSIONS

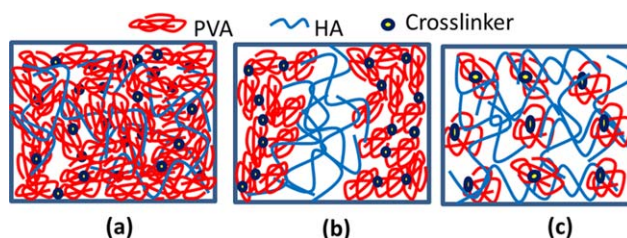
Gels of PVA and HA were investigated at various HA content, with the chemical crosslinking of PVA. Rheological properties, surface morphology, and correlation function were analyzed to show that different structural features are observed at different HA content when PVAHA gels are formed. For blend compositions with  $<10$  wt % HA (on water-free basis), SIPN of PVAHA were observed. During gelation of SIPNs, the Winter–Chambon criterion was observed. At HA contents from 10 to 20%, cocontinuous morphology was observed, with rheological property evolution being independent of frequency. At concentrations of HA above 20%, intracyclic crosslinking leads to formation of phase separated domains. The surface morphology observed with ESEM and structural correlation observed using DLS also supported three different types of features in PVAHA systems.



The results are analyzed in terms of relative role of intra- and intermolecular crosslinking for semidilute polymer solutions. Postgelation analysis on gels and membranes demonstrated the robustness of these hydrogels, indicating similar modulus for various compositions.



**Figure 12.** Intensity correlation functions for (a) PVAHA 62 at solution, after 3 and 19 min of gelation, (b) PVAHA 44 at solution, after 3 and 19 min of gelation, (c) PVAHA 35 at solution, after 3 and 19 min of gelation (sample nomenclature is shown in Table I).



**Figure 13.** Schematic representation of PVA and HA at various concentrations of HA (a) at low HA content, significant intermolecular PVA crosslinking leading to SIPN of PVA and HA, (b) at moderate HA content, cocontinuous distribution of PVA and HA, and (c) at high HA content, significant intramolecular crosslinking leading to domain formation. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

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