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Morphology–rheology relationship in hyaluronate/poly(vinyl alcohol) /borax polymer blends

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Abstract

In this work, we have prepared bioartificial polymer blends using hyaluronate (HA) as a biological component and poly(vinyl alcohol)–borax association (PVAs) as a synthetic component, and investigated the rheological properties as well as morphology of the blends. When plotted against the blend composition, the rheological properties showed both positive and negative deviation from the linear additive mixing rule depending on thermal history. The blend showed enhanced viscosity at the composition of 20 wt% of HA and 80 wt% of PVAs, when PVA was dissolved at high temperature. The viscosity enhancement was caused by the network formation of HA aggregates in the micrometer scale. In addition, the network structure of HA aggregates was found to be fractal with the fractal dimension of 1.7. As PVA system also forms a network structure in the nanometer scale between hydroxyl groups of PVA and borate anions, the blend system is unique in that it has network structures in both micrometer and nanometer scales in one material. On the contrary, HA formed aggregates but not any network structure in the blend of the same composition but of the negative deviation. In conclusion, we showed that HA/PVAs blend system may have diverse morphology as well as very broad spectrum of rheological properties, and could suggest that the rheology and morphology of HA/PVAs blends can be designed not only by controlling composition but also by controlling thermal and deformation history of the components.

Keywords: Bioartificial polymer; Hyaluronate; Poly(vinyl alcohol)

1. Introduction

Hyaluronic acid (HA) is a naturally occurring biocompatible and biodegradable linear polysaccharide composed of repeating disaccharide units of glucuronic acid and *N*-acetyl glucosamine linked by β 1-3 and β 1-4 glycosidic bonds (Guss et al. [13]; Winter et al. [40]; Winter and Arnott [41]). Its structure is shown schematically in Fig. 1. HA can be obtained not only from animal sources, mainly extracted from umbilical cord, rooster comb, synovial fluid, or vitreous humor of the eyes with molecular weight in the range of $3–6\times10^6$, but also from biotechnological production of streptococcus (Hynes et al. [17]; Deangelis [15]).

Hyaluronic acid is used in many biomedical applications. One of the widespread clinical uses of HA is in the field of ophthalmic surgery. In cataract surgery, for example, the role of HA solution is to facilitate the procedure and to protect the corneal endothelium (Scott [32]). This procedure relies on the rheological properties of HA solution that allows it to be injected through small cannulae into the anterior chamber. Due to its high molecular weight, HA can maintain the space of the anterior chamber and absorb mechanical stress as an elastic solid that prevents cellular and surface injury (Scott [32]; Denlinger [14]). Another main application of HA is in the treatment of inflammatory and degenerative joint diseases (Balazs and Denlinger [3]; Balazs et al. [2]; Dahl et al. [8]).

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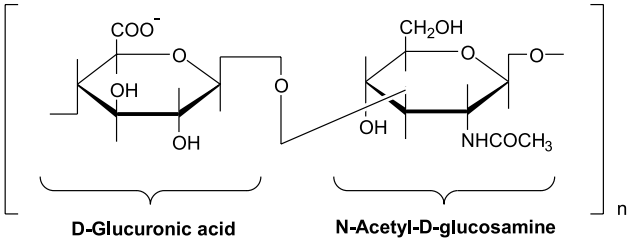

Fig. 1. The structure of hyaluronate.

When injected into a human body, HA degrades quickly and this limits possible biomedical applications. With the aim of producing a new biomaterial for biomedical applications, HA modifications have been introduced including alginate-HA association (Oerther et al. [21–23]; Pelletier et al. [26,27]), HA derivatives by chemical modification (Ambrosio et al. [1]), HA-synthetic polymer blends (Giusti et al. [11,12]; Cascone et al. [5]; Cascone [6]), and so on. For the last several decades, there has been a significant progress in the area of polymer blends. This was driven by the realization that new molecules are not always required, and that simple blending can sometimes be implemented more rapidly and economically than the development of a new chemistry. Blends of synthetic and natural polymers represent a new class of materials that has received little attention in the past, although their potential applications are now becoming evident. Polymeric materials are widely used in biomedical applications, but synthetic and natural polymers have been used separately. Bioartificial polymeric materials represent a new class of materials based on blends of synthetic and natural polymers, designed with the purpose of producing new polymeric materials that possess good mechanical properties as well as biocompatibility (Giusti et al. [11,12]; Cascone et al. [5]; Cascone [6]).

In this study, bioartificial polymer blends will be prepared using poly(vinyl alcohol)–borax association (PVAs; schematically shown in Fig. 2) as a synthetic component, and hyaluronate (HA) as a biological component. As PVA is water soluble, has many active sites for hydrogen bonding, and may form a network structure when

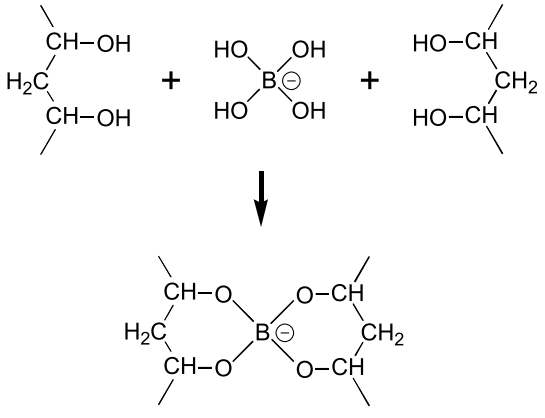

Fig. 2. The structure of poly(vinyl alcohol)–borax association.

sodium borate is added, its blends with HA are expected to have many attractive properties, and their rheological properties as well as morphology will be investigated.

2. Experiments

2.1. Materials

This study was carried out with hyaluronate (HA), poly(vinyl alcohol) and sodium borate (borax). HA (M_w: 3300 kDa) was used as received from LG Life Sciences. PVA (M_w: 12–186 kDa, 98–99% hydrolyzed) and borax were purchased from Sigma. The blend preparation method is as follows. First, 1 wt% HA aqueous solution was prepared by shaking at 300 rpm for 1 day at room temperature. Second, 4 wt% PVA aqueous solution was prepared by shaking at 200 rpm for 1 h, at 80 and 106 °C, respectively. We name the PVA solution which was dissolved at 80 °C as PVA[N], and the PVA solution which was dissolved at 106 °C as PVA[P]. Two weight percent borax stock solution was made at 60 °C. We mixed HA solution with PVA solution at 70 °C for 30 min, added borax stock solution into the HA/PVA solution, and then mixed blends at 70 °C for 30 min. The composition ratio of HA/PVA–borax (PVAs) was 20/80, 40/60, 60/40, 80/20, respectively. We call HA/PVAs blend that consists of PVA which was dissolved at 80 °C as HA/PVAs[N] and the blend in which PVA was prepared at 106 °C as HA/PVAs[P]. The blends were transparent irrespective of the preparation method as well as composition. Hyaluronate has carboxyl (COOH) groups which stain to deep blue with Alcian blue method (Clark [7]). For morphology investigation of the blends, we stained the blends with 1% Alcian blue 8GX in a 3% acetic acid solution. Alcian blue 8GX was purchased from Sigma.

2.2. Measurement

Rheological measurements were performed on a rheometer (RMS 800; Rheometrics Scientific, USA) with a cone and plate fixture. The cone diameter was 50 mm with an angle of 0.04 rad. A thin layer of silicon oil was deposited on the free surface of the samples in order to prevent solvent evaporation. All the measurements were carried out at 25 °C. The morphology was investigated with an optical microscope (BX51; Olympus). The staining of the sample was performed before investigation, but HA was stained after solvent casting when the film was under investigation. Measurements were carried out many times and the results were very reproducible.

3. Results and discussion

3.1. Rheological properties

HA solution can be used in biomedical applications

especially in the field of ophthalmic surgery and joint disease because of its high viscosity. It maintains the space of the anterior chamber and protects from the mechanical stress in cataract surgery, whose function depends on the rheological properties of HA solution. The dynamic properties of 1% HA solution are shown in Fig. 3(a). The strain was kept less than 10% to ensure linear viscoelasticity. As the molecular weight of HA is very high, the complex viscosity does not show a Newtonian plateau at low frequencies. The slope of storage modulus G' at low frequencies is much smaller than two in the log–log plot, which implies that there exist a significant amount of entanglements or microstructures. The loss modulus G'' shows a broad peak, almost a plateau over two decades of frequency, which implies a broad spectrum of relaxation times. It shows viscous behavior ($G'' > G'$) at low frequencies while elastic behavior ($G' > G''$) is observed at high frequencies. The transition, as indicated by the crossover of the G' and G'' curves, occurs at a crossover frequency (f^*), which is about 0.4 rad/s in this case. At frequencies lower than the crossover frequency (f^*), the polymer chains can release stress by disentanglement or molecular rearrangement during the period of oscillation, while the chains cannot disentangle during the short period of oscillation at higher frequencies. These results are in agreement with the observation reported previously (Ambrosio [1]). Another main application of HA is in the treatment of inflammatory and degenerative joint diseases (Balazs et al. [2]; Balazs and Denlinger [3]; Dahl et al. [8]). It can lubricate the joint and prevent mechanical damage in degenerative joint diseases. The physiological function of synovial fluids is to lubricate the joint at low frequencies (e.g. resting or walking) and to prevent mechanical damage at high frequencies (e.g. running). As it shows a liquid-like behavior at low frequencies and a solid-like behavior at high frequencies, the viscoelastic properties of HA solution satisfy above requirements (Weiss [39]). Upon the application of shear, a viscosity overshoot is observed at low shear rates, as shown in Fig. 3(b). However, over a critical shear rate of 7/s, a minimum viscosity or a viscosity undershoots is also observed in the transient flow curve. The appearance of the undershoot is favored at high shear rate. The viscosity undershoot has been observed in some highly concentrated emulsions as well as in some polymer solutions, however its mechanism is poorly understood (Partal et al. [25]; Stasiak and Cohen [35]; Strand et al. [37]). It is related to the microstructural change arising from the elastic contribution of the polymer chain or of the microstructural domains. The steady state viscosity decreases with increasing shear rate, and the solution shows a shear thinning behavior.

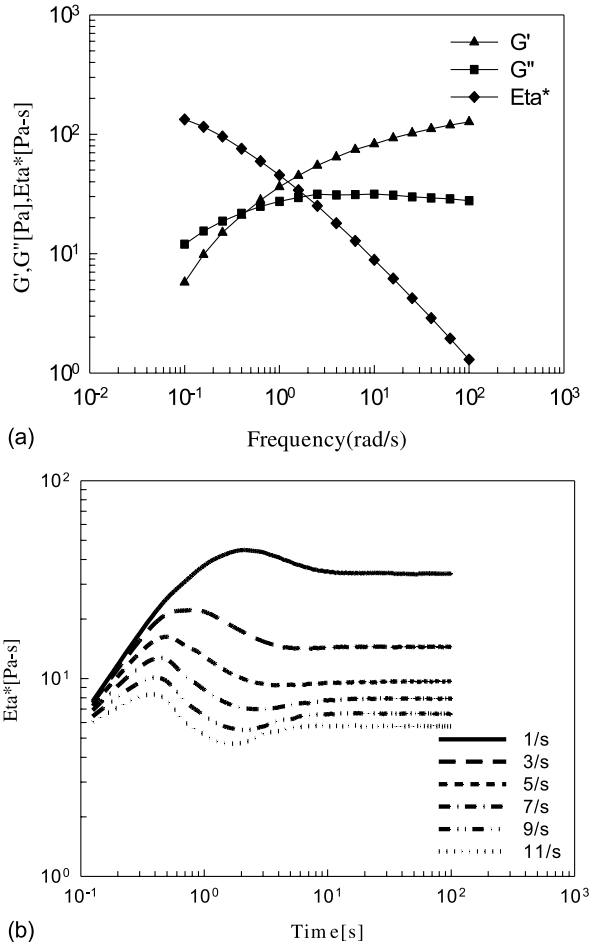

Fig. 3. Complex viscosity (Eta*), storage modulus (G') and loss modulus (G'') as a function of frequency (a), and the viscosity as a function of time at different shear rates (b) for 1% hyaluronate at 25 °C.

Poly(vinyl alcohol) solution forms a network structure with the addition of borax which condenses with organic hydroxyl groups in aqueous solution (Finch [10]; Sinton [34]; Shibayama et al. [33]). A schematic diagram of the complex formation is shown in Fig. 2. The complex viscosity shows a Newtonian plateau at low frequency and becomes thinning as frequency increases as shown in Fig. 4(a). The crossover frequency is about 2 rad/s, which is larger than that of HA solution. It reflects the difference in molecular weight, and consequently the relaxation time of PVAs solution is less than that of HA solution, as the relaxation time is roughly estimated as the inverse of the crossover frequency. The relaxation spectrum is narrow as evidenced by the relatively sharp peak of the loss modulus curve. The overall shape of the linear viscoelastic properties is common to that of ordinary polymer solutions, however, the behavior at large strain, or the nonlinear behavior is quite unique as shown in Fig. 4(b). At low shear rates, the viscosity increases and reaches a steady state value, while it increases rather sharply at high shear rates and does not reach a steady state. It shows a shear thickening behavior (Osaki et al. [24]). It seems that the crosslinks are formed upon the contact between the hydroxyl groups of PVA and borax during the early stage of solution preparation. However, the crosslinks rarely affect the rheological properties at small strain, while they influence significantly at large strain. In this sense, the crosslinks of PVA solutions

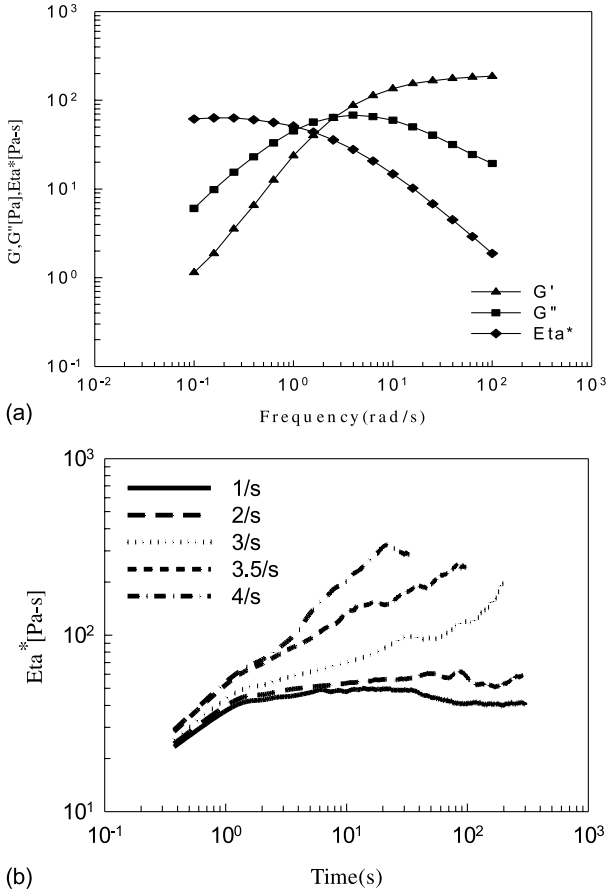

Fig. 4. Complex viscosity (Eta*), storage modulus (G') and loss modulus (G'') as a function of frequency (a), and the viscosity as a function of time at different shear rates (b) for 2% poly(vinyl alcohol)–1% borax association at 25 °C.

may be regarded as dynamic in nature, as is reported elsewhere (Osaki et al. [24]).

The tendency of poly(vinyl alcohol) to form microcrystalline structures depends on thermal history. For a highly hydrolyzed PVA (98–99%), a preparation temperature of above 92 °C is required for complete dissolution in water (Prokopova et al. [28]; Stern et al. [36]; Prokopova et al. [29]). Under this condition, the inter and intra hydrogen bonding of PVA chain is disrupted by thermal energy, thus solubility increases (Briscoe et al. [4]). When PVA is dissolved at 80 °C, undissolved microcrystallites are present, while the crystallites disappear when PVA is dissolved at 106 °C (Nijenhuis [20]). The microstructure of HA/PVAs blends that consist of PVAs, which have been prepared at different temperatures, is different. The rheological properties as well as the microstructure of the blends therefore depend on the PVA dissolution temperature.

Depending on thermal history, more specifically depending on the dissolution temperature of PVA during the sample preparation, the rheological properties of HA/PVAs blend differ significantly. The complex viscosities of the blends at 1 rad/s are compared as a function of HA concentration in Fig. 5(a). We clearly observe two types of curves, which deviate from the linear additive mixing rule. It should be noted that the only difference is the dissolution temperature of PVA. PVA was dissolved at 80 °C for HA/PVAs[N], while it was dissolved at 106 °C for HA/PVAs[P]. The complex viscosity of PVA–borax did not depend on the preparation temperature. However, when mixed with HA solution, HA/PVAs[P] blend shows a strong

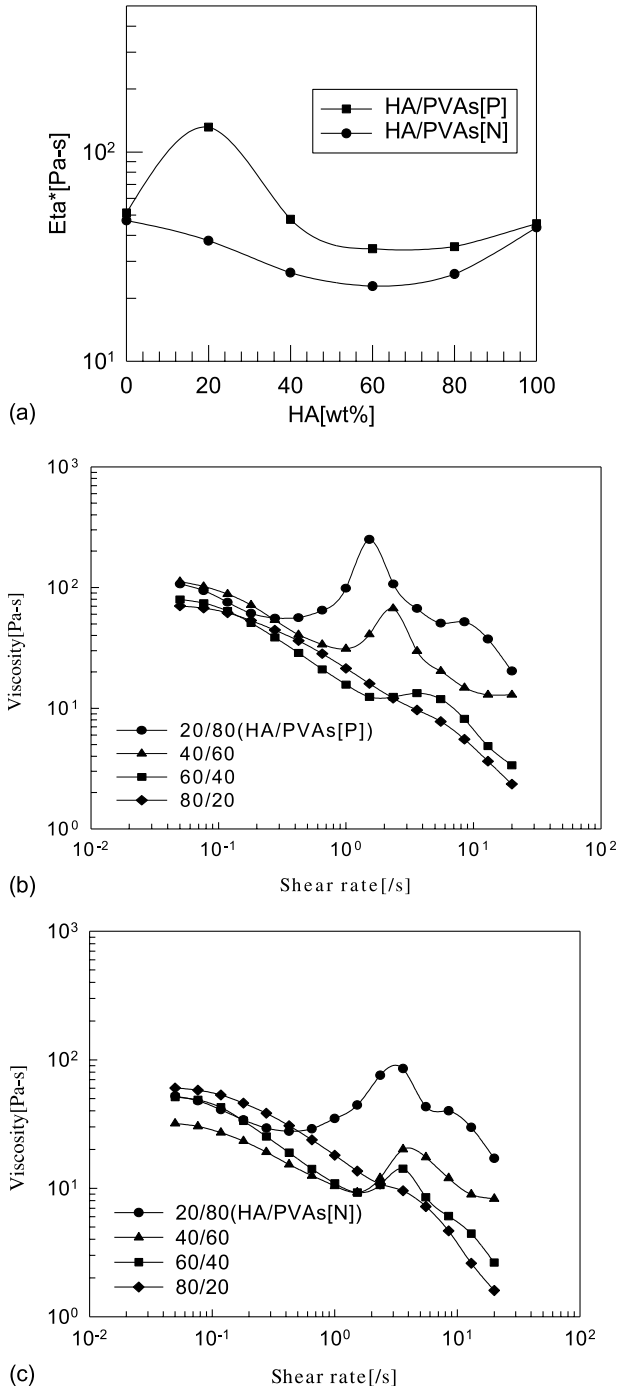

Fig. 5. Complex visocosity as a function of HA composition at 1 rad/s and 25 °C (a), and the corresponding viscosity curves of HA/PVAs[P] and HA/PVAs[N] blends.

positive deviation at 20 wt% of HA, while HA/PVAs[N] blend shows a negative deviation for all the blend composition. The positive deviation from the linear additive mixing rule means that there exists a strong interaction between the blend components, that is between HA and PVA–borax (Lapcik et al. [18]; Turner et al. [38]; Heatley and Scott [16]; Scott et al. [30]; Hyun et al. [31]). It is expected that the increase in viscosity for HA/PVAs[P] blend is due to the formation of hydrogen bonding during the mixing stage, and hence the interaction between HA and PVA chains. The viscosity curves are very complicated as shown in Fig. 5(b) and (c). When HA composition is high, the viscosity of the blend shows typical shear thinning behavior. However, as the composition of PVAs increases, there appears shear thickening followed by shear thinning. As the constituents show both shear thinning and shear thickening, respectively, the viscosity of the blend shows a complex behavior. It should also be noted that the curve is not a simple addition of contributions from each component but is more complicated. Furthermore, the shape of the curves looks similar for both HA/PVAs[P] and HA/PVAs[N]; the magnitude is higher and the shear thickening is more pronounced for HA/PVAs[P]. Even though their linear viscoelastic properties deviate from linear mixing rule in different ways, the interaction between each constituent is not physically different, but only the intensity of the interaction and the way they interact are different. This will be more evident when we compare the morphology altogether. The complex behavior of the nonlinear properties also implies the existence of strong interactions between the blend components. The details of the interactions are not yet completely understood, but may be reflected on the microstructure of the blend.

3.2. Morphology of HA/PVAs blends

As the rheological properties of 20/80(HA/PVAs) blends strongly depend on PVA preparation temperature, it is expected that the microstructure of the blends HA/PVAs[N] and HA/PVAs[P] be different. In addition, the positive deviation from the linear mixing rule means that there exists a strong interaction between the components. The morphology of two blends has been investigated in both solution and film states as shown in Figs. 6 and 7. The dispersed phase is HA which has been stained using Alcian blue method, and the matrix is the PVA–borax complex. HA forms micron-size aggregates, and there seems to exist an attractive interaction between the aggregates as can be seen in Fig. 6. It is well known that HA easily forms aggregates due to hydrogen bonding, and the aggregates tend to floc together as well (Lapcik et al. [18]; Turner et al. [38]; Heatley and Scott [16]; Scott et al. [30]). The formation of aggregates and the floc structure arises from hydrogen bonding and thermodynamic compatibility. The chaining of aggregates is distinctly observed in the blend HA/PVAs[P] as shown in Fig. 6(b), which means that the interaction between the aggregates is more attractive and stronger in the blend HA/PVAs[P] than in the case of HA/PVAs[N]. Chaining of HA aggregates is believed to be caused by the interaction between HA and PVA, because the only difference between the two blends lies in PVA, that is in the different microstructure of PVA depending on the dissolution temperature (Stern et al. [36]; Prokopova et al. [29]). It seems that the hydroxyl group of PVA forms hydrogen bonding with that of HA aggregates, and then added borax condenses and crosslinks with PVA chains (Finch [10]; Sinton [34]; Shibayama et al. [33]); that is, HA aggregates form a network structure or a chaining structure by hydrogen bonding with PVA chains through PVA–borax crosslinking as a bridge. During the preparation of PVA[N], PVA does not dissolve completely and some amount of crystallites are still present (Nijenhuis [20]), which reduces the number of active sites of PVA to interact with the hydroxyl group of HA. However, PVA[P] does not have crystalline structure (Nijenhuis [20]), and has enough sites to interact with HA, leading to a chaining structure of HA. So, the morphology of blend HA/PVAs[P] shows a chaining structure of HA aggregates, while that of HA/PVAs[N] is less pronounced, even though the aggregates still tend to form flocs due to hydrogen bonding between HA aggregates as well as thermodynamic origin (Lapcik et al. [18]; Turner et al. [38]; Heatley and Scott [16]; Scott et al. [30]). This is in accordance with the viscosity curves as discussed in the previous section. Though the interaction is weaker for HA/PVAs[N], the aggregates still tend to form chaining structure with less pronounced attractive forces. The morphological difference becomes more significant when compared in the film state after evaporating the solvent as shown in Fig. 7. HA aggregates are dispersed in the HA/PVAs[N] blends as can be seen in Fig. 7(a), while they are chaining and form a network structure in the HA/PVAs[P] blends in Fig. 7(b). The network structure looks similar to the particle gel system like a protein-stabilized emulsion gel (Dickinson [9]). Therefore, it can be concluded that the positive deviation of rheological properties or the enhancement of complex viscosity arises from the formation of network structures among HA aggregates, which is facilitated by hydrogen bonding of HA aggregates with PVA through PVA–borax crosslinking as a bridge as explained earlier. The network formation is also observed when we change the composition and molecular parameters such as MW of HA, MW and the degree of hydrolysis of PVA, and the borax concentration. Though the detailed morphology shows a broad spectrum depending on situation, the network structure looks quite general (not shown here) and the details will be reported elsewhere.

As HA aggregates are dispersed in the blend whether they are separated or networked, it would be necessary to investigate morphology in a more quantitative way. The fractal analysis of the blend morphology will be discussed in the next section.

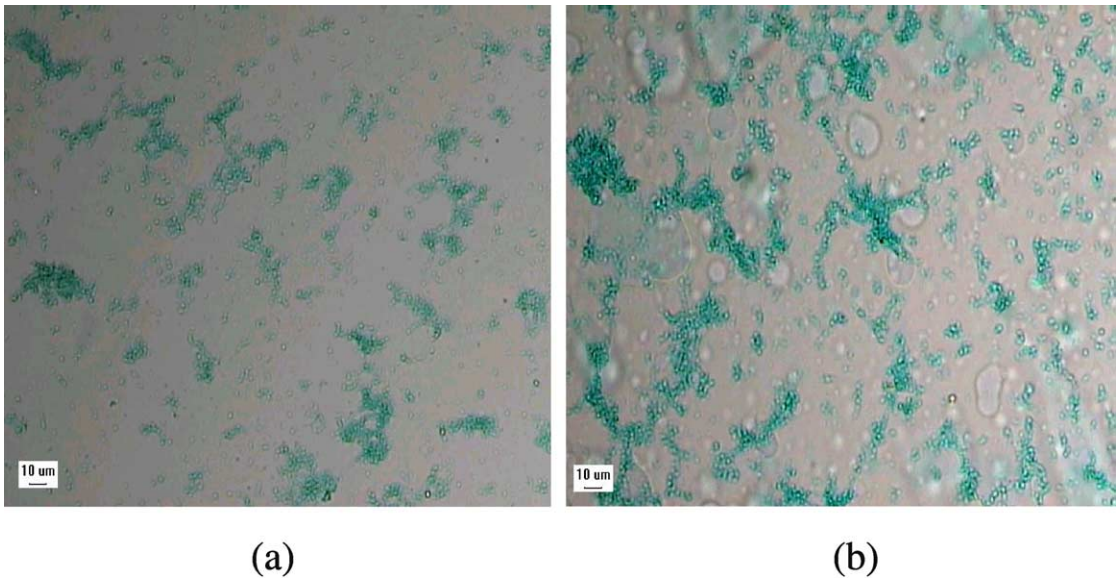

Fig. 6. Morphology of 20/80 HA/PVAs[N] (a) and 20/80 HA/PVAs[P] (b) blends in the solution state, magnified by 200 times.

3.3. Fractal analysis of HA/PVAs blends

We characterized the morphological textures of Figs. 6 and 7 in terms of the fractal dimension. According to the fractal theory, the relation between the length of each piece r and the number of self-similar pieces required to cover the texture $N(r)$ is expressed by the following equation (Mandelbrot [19]),

$$N(r) = r^{-D}$$

where D stands for the fractal dimension. Fig. 8(a) and (b) show the relation between r and $N(r)$ for HA/PVAs solutions and films, respectively. The morphological patterns presented in Figs. 6 and 7 satisfy the above relation indicating the patterns formed by HA are fractal. By means of the least mean squares method, the fractal dimension D of the solution was determined as 1.4 and 1.6 for HA/PVAs[N] and HA/PVAs[P], respectively. Correlation coefficients obtained by the least mean squares fitting was more than 0.9975 as listed in Table 1. The fractal dimension of HA/PVAs[N] was much lower than the value 5/3 of three-dimensional self-avoiding random walks (SARW), which means that the pattern made by HA aggregates is not well developed enough to fill into PVAs matrix. It is probable that PVA–borax aggregation prevents HA aggregates from forming network structures. The fractal dimension of HA/PVAs[P] was close to the value of SARW, suggesting HA molecules are well-dispersed and formed network structures

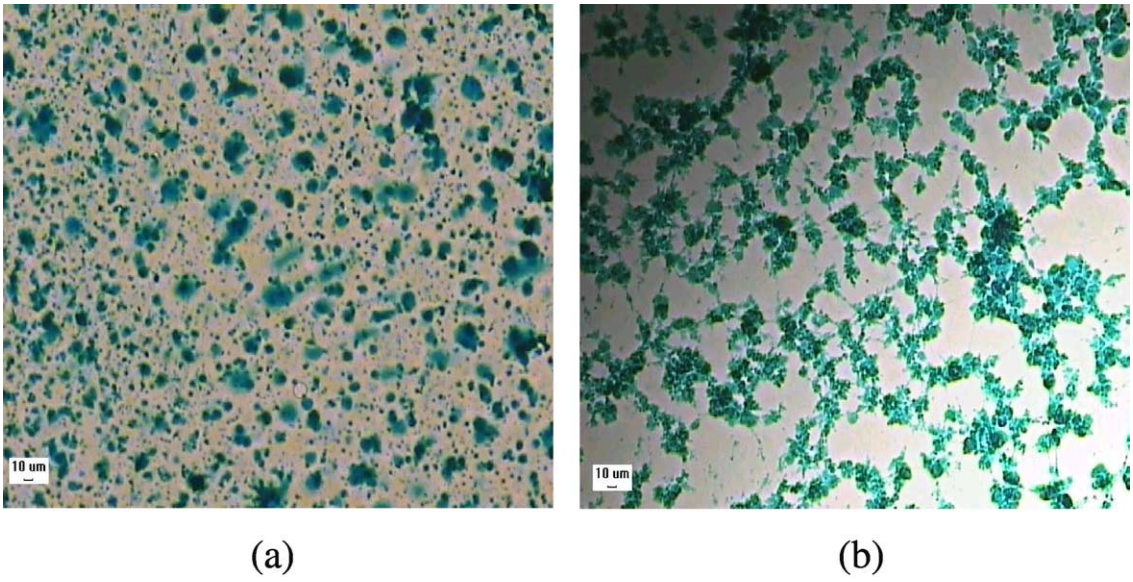

Fig. 7. Morphology of 20/80 HA/PVAs[N] (a) and 20/80 HA/PVAs[P] (b) blends in the film state, magnified by 200 times.

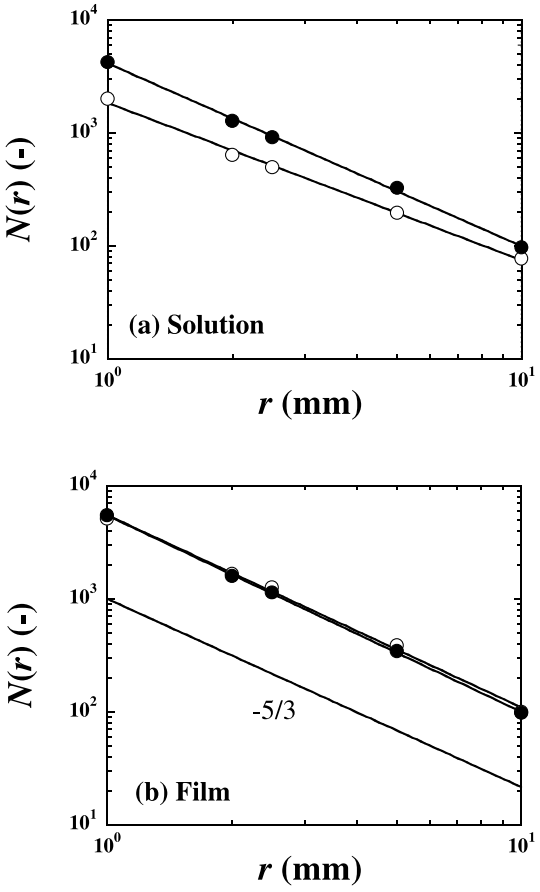

Fig. 8. Relation between the length of each piece and the number of self-similar pieces for 20/80 HA/PVAs[N] (○) and 20/80 HA/PVAs[P] (●) blends in the solution state (a) and film state (b).

compared to HA/PVAs[N]. In Fig. 8(b), the slope of HA/PVAs[P] film is slightly higher than that of HA/PVAs[N], which indicates that HA aggregates in HA/PVAs[P] are well-dispersed and better developed than HA/PVAs[N]. However, both fractal dimensions of HA/PVAs[P] and HA/PVAs[N] are almost identical, very close to the value of SARW; the slope of 5/3 is also shown in the figure. Significant change in the fractal dimension of HA/PVAs[N] is observed before and after evaporating solvents. It is considered that the morphology of HA/PVAs[N] film was generated in the process of evaporation. Therefore, the morphology is affected not only by molecular interactions but also by the heterogeneity of HA/PVAs[N] solution. For example, the difference in evaporation rate between HA and PVA domains may lead to different aggregation speed, as a result the morphology would be complex with higher fractal dimensions. On the contrary, no clear changes in the fractal dimension was observed in HA/PVAs[P] before and after evaporating solvents. This suggests that HA/PVAs[P] solution was dried by keeping the morphological structure as uniform as possible.

Table 1
Fractal dimensions of HA/PVAs solutions and films

	Type	Fractal dimension	Correlation coefficients
Solution	Negative	1.4	0.9975
	Positive	1.6	0.9996
Film	Negative	1.7	0.9994
	Positive	1.7	0.9999

Finally it needs to be noted that the microstructure of HA/PVAs[P] forms a network structure in two different length scales. HA aggregates form a network structure on a micrometer scale as can be seen in Fig. 7(b), while the matrix forms a network structure between PVA and borax in a nanometer scale as evidenced by the shear thickening behavior (Fig. 4(b)) that is caused by the network formation whose mechanism is schematically proposed in Fig. 2 (Nijenhuis [20]; Osaki et al. [24]). So the blend HA/PVAs[P] has network structures in both micrometer and nanometer scale in one material. Our result also suggests a way to control the rheology and microstructure of HA/PVAs blend systems. The key design parameter will be the processing condition (e.g. dissolution temperature) as well as the molecular parameters (e.g. molecular weight, degree of hydrolysis) of the constituent materials. It will be possible to obtain quite a broad spectrum of morphology and rheology by controlling the design parameters mentioned above, which will be effective for the better design of bioartificial polymeric systems. This paper focused on the effect of processing conditions, and the details of the effect of molecular parameters will be reported elsewhere.

4. Conclusions

The rheological properties of bioartificial polymer blends based on hyaluronate (HA) as a biological component and poly(vinyl alcohol)–borax association (PVAs) as a synthetic component have been investigated. The blend system shows different rheological properties depending on the PVA dissolution temperature during the sample preparation. The complex viscosity shows a positive deviation from the linear additive mixing rule at the composition of 20/80 (HA/PVAs) when PVA was dissolved at 106 °C, while it shows a negative deviation at all compositions when PVA was dissolved at 80 °C. For the blend system with a strong positive deviation, there exists a strong attractive interaction, and HA aggregates form a network structure. It is caused by the interaction between HA and PVA. The hydroxyl group of PVA seems to form hydrogen bonding with that of HA aggregates through PVA–borax cross-linking as a bridge, leading to a network structure. The network structure of HA aggregates was found to be fractal with fractal dimension of 1.7. For the blend system with a negative deviation, PVA chains do not dissolve completely and coexisting crystallites reduce the number of active sites of PVA to interact with the hydroxyl group of HA, leading to a morphology in which the HA aggregates are well-dispersed in the matrix. The network structure of HA

aggregates means that the material has network structures in two different length scales. The HA aggregates form a network structure on a micrometer scale, while the matrix forms a network structure between PVA and borax on a nanometer scale. Thus there exist network structures both in a micrometer and a nanometer scale in one material. This study also suggests a way to control the morphology and rheology of HA/PVAs blend systems. As there are a lot of parameters in the molecular design including molecular weight and the degree of hydrolysis, it will be possible to obtain quite a broad spectrum of morphology and rheology, which will be effective for the better design of bioartificial polymeric systems.

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