Korea-Australia Rheology Journal
Vol. 17, No. 2, June 2005 pp. 79-85

Influence of preparation parameters on rheological behavior and microstructure of aqueous mixtures of hyaluronic acid/poly(vinyl alcohol)

Hyun-Ok Park[1], Joung Sook Hong[2], Kyung Hyun Ahn[1], Seung Jong Lee[1] and Seong Jae Lee*
Department of Polymer Engineering, The University of Suwon,
San 2-2, Wau-ri, Bongdam-eup, Hwaseong, Gyeonggi 445-743, Korea
[1]School of Chemical and Biological Engineering, Seoul National University,
San 56-1, Shillim-dong, Gwanak-gu, Seoul 151-744, Korea
[2]Applied Rheology Center, Korea University, 5-1, Anam-dong, Seongbuk-gu, Seoul 136-701, Korea

(Received June 1, 2005; final revision received June 21, 2005)

Abstract

Aqueous mixtures of hyaluronic acid and poly(vinyl alcohol) system and hydrogels thereof were introduced to obtain new bioartificial materials that have excellent mechanical properties, biocompatibility and enhanced rheological properties. The interactions between hyaluronic acid and poly(vinyl alcohol) and/or borax were investigated by rheological measurements. Preparation parameters of the aqueous mixtures were mixture composition, the degree of hydrolysis of poly(vinyl alcohol) and borax concentration. From the rheological behavior, it could be deduced that the key factor of the interaction between hyaluronic acid and poly(vinyl alcohol) was the hydrogen bonding between them and the effect was pronounced with borax. Enhanced viscosity was observed at the composition of 20 wt% of hyaluronic acid solution and 80 wt% of poly(vinyl alcohol) and borax solution. Rheological properties were influenced by the degree of hydrolysis of poly(vinyl alcohol) and borax concentration. As the degree of hydrolysis and borax concentration increased, rheological properties increased due to the increased hydrogen bonding and networking of hyaluronate aggregates. Physical hydrogels from hyaluronic acid and poly(vinyl alcohol) were prepared and the composition dependence of the gels was rheologically investigated as well.

Keywords : hyaluronic acid, poly(vinyl alcohol), bioartificial material, degree of hydrolysis, hydrogel

1. Introduction

Hyaluronic acid (HA) is a water soluble glycosaminoglycan composed of alternating 1,4-linked units of 1,3-linked glucuronic acid and N-acetyl glucosamine, as shown in Fig. 1 (Fouissac *et al.*, 1993). HA occurs naturally in many living substrates, such as synovial fluid, vitreous humor, connective tissues, rooster comb and umbilical cord. Indications of pharmacological activity have been demonstrated in the treatment of inflammatory and degenerative joint diseases (Hynes *et al.*, 2000; Dahl *et al.*, 1985). Its remarkable viscoelastic properties account for its importance in joint lubrication and its complete lack of immunogenicity makes it an ideal building block for biomaterials needed for tissue engineering and drug delivery systems (Denlinger, 1998). Typical molecular weight of HA in normal synovial fluids in human knees was reported as six million. The rheological properties of synovial fluids from normal and diseased joints have been extensively studied by clinical researches. The physiological function of synovial fluids is to lubricate the joint at low strain frequency (e.g., resting or walking) and to prevent mechanical damage at high strain frequency (e.g., running). Therefore, it behaves as a viscous liquid at low frequency and as an elastic shock absorber at high frequency (Weiss, 2000). It is also known that normal synovial fluids are shear thinning, showing a power law behavior at high shear rates and a constant zero-shear viscosity at low shear rates. However, the short residence time of HA due to biodegradability limits the possibility to

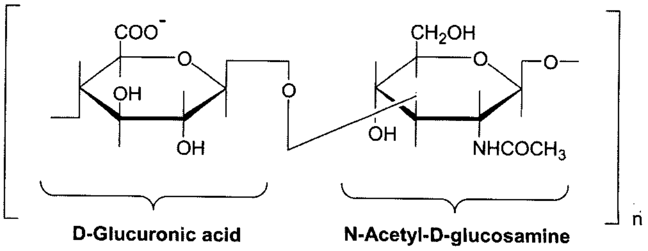


Fig. 1. Disaccharide unit of hyaluronic acid.

---

*Corresponding author: sjlee@suwon.ac.kr widen its range of biomedical application. To overcome this problem, many methods have been adopted by many researchers: associating HA with alginate (Oerther *et al.*, 2000), chemically modifying HA by crosslink or coupling reactions (Pelletier *et al.*, 2001), and blending HA with other polymers (Ambrosio *et al.*, 1999; Cascone, 1997).

Poy(vinyl alcohol) (PVA) is suitable for biocompatible materials because it has a large number of hydroxyl groups that can react with many kinds of functional groups. Hydrogen bonds related to the number of hydroxyl groups play an important role in the properties of PVA, such as high water solubility, a wide range of crystallinity and high crystal modulus (Shibayama *et al.*, 1988). In aqueous solutions with PVA concentration of more than 1%, entangled and aggregated supermolecular structures are formed. These structures contain amorphous and paracrystalline parts, the mutual ratio of which depends on experimental conditions during the preparation of solutions, and predominantly on the dissolution temperature (Kim *et al.*, 2005). Aqueous PVA solution shows various rheological behaviors with regard to temperature, molecular weight, degree of hydrolysis and so on (Yamaura *et al.*, 1981; Koopal, 1981; Prokopova *et al.*, 1985; Lozinsky *et al.*, 1986). Since PVA is prepared via the hydrolysis of poly(vinyl acetate), during which the acetate groups are progressively replaced by hydroxyls, the degree of hydrolysis of PVA is an important molecular parameter. It is thus natural that the degree of hydrolysis has a significant influence on the rheological properties of PVA as the difference in chemical composition gives rise to different physical properties. Moreover, PVA can react with borate ion to form a polymeric gel. The mechanism of PVA-borate crosslink is believed to be a 'di-diol' complexation, which is formed between two diol units of PVA chains and one borate ion (Pezron *et al.*, 1988; Pezron *et al.*, 1989) as schematically shown in Fig. 2. It is reported that the solution of PVA and borax exhibited a viscosity maximum followed by shear thinning as shear rate increased and a significant hysteresis occurred at higher viscosities and lower shear rates (Maerker and Sinton, 1986).

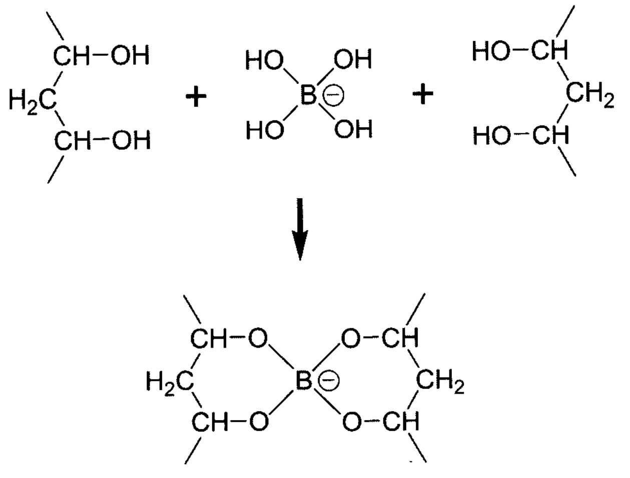


Fig. 2. Schematic representation of poly(vinyl alcohol)-borax complexation process.

In this study, bioartificial materials are prepared using HA as a biological component and PVA-borax association as a synthetic component and their rheological properties are investigated for potential biomedical applications. Rheological behavior of the aqueous mixtures of HA and PVA system is analyzed in connection with the microstructure of the materials. Finally, it is suggested that the rheological properties and morphology of the materials can be controlled by choosing suitable preparation parameters such as solution composition, the degree of hydrolysis of PVA, and borax concentration.

2. Experimental

2.1. Sample preparation

The main materials used in this study were HA, PVA and sodium borate (borax). HA (Mw: 3,300 KDa) was used as received from LG Life Sciences in Korea. PVA (Mw: 124-186 KDa, 98-99% hydrolyzed), PVA2 (Mw: 124-186 KDa, 87-89% hydrolyzed) and borax were purchased from Sigma-Aldrich. First, 1 wt% HA aqueous solution was prepared from the deionized water at room temperature by shaking at 300 rpm for 1 day. Second, 4 wt% PVA aqueous solution was dissolved at 200 rpm and 106°C for 1 hour. Borax stock solutions were prepared at 60°C for 30 min by varying the concentration of 0.2-2.0 wt%. The HA solution was mixed with the PVA solution at 70°C for 30 min, the borax solution was added into the HA/PVA solution, and then the resultant solution was mixed at 70°C for 30 min. The composition ratios of HA and PVA-borax system (HA/PVAb) were 20/80, 40/60, 60/40 and 80/20. For morphology investigation, the samples were stained with 1 wt% Alcian blue 8GX in 3 wt% acetic acid solution based on the Alcian blue staining method (Clark, 1981), where the dye, Alcian blue 8GX, was purchased from Sigma-Aldrich.

2.2. Measurements

Rheological measurements were performed by an RMS 800 (Rheometrics Scientific, USA) with a cone and plate fixture. The cone diameter and gap angle were 50 mm and 0.04 rad, respectively. For very low viscosity solutions, a stress-controlled rheometer (Bohlin C-VOR, UK) was also used, in which the fixture consists of a cone and plate geometry with 40 mm diameter and 4° angle. A thin layer of silicone oil was deposited upon the free surface of the samples to prevent solvent evaporation. All the measurements were carried out at 25°C. Rheological properties of hydrogels were measured by the stress-controlled rheometer with a parallel plate fixture having 40 mm diameter and 2 mm gap thickness. The morphology of the samples was investigated with an optical microscope (Olympus BX51, Japan). Prior to the investigation, the samples were stained with the Alcian blue solution because the dye solution can stain the carboxyl groups of HA in deep blue.

3. Results and discussion

3.1. Effect of solution composition

The rheological properties of 1 wt% HA solution are shown in Fig. 3(a). 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 frequencies, representing a broad spectrum of relaxation time. The steady state viscosity of 1 wt% HA solution decreases with increasing shear rate, and the solution shows shear thinning behavior.

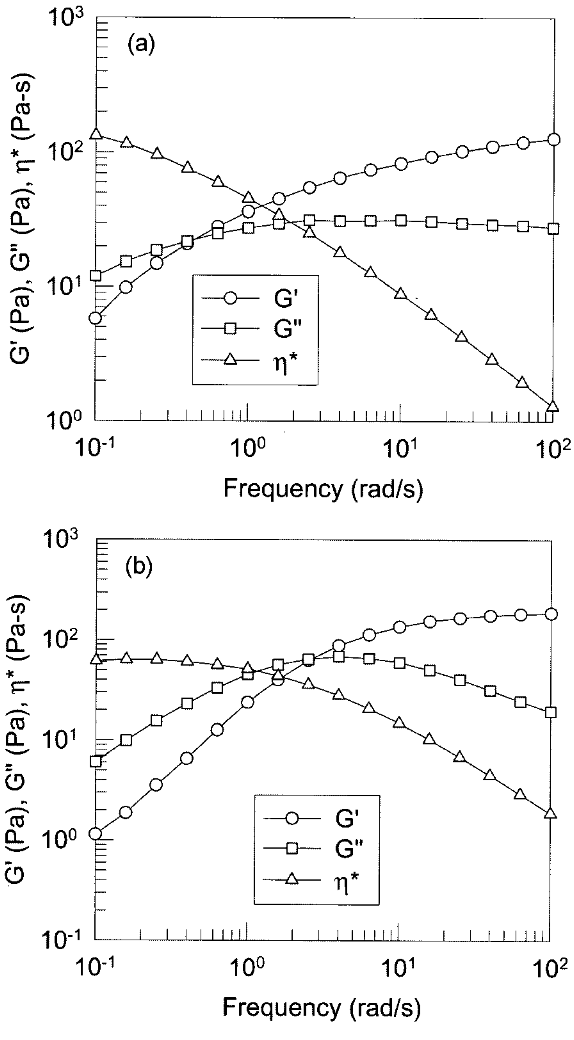


Fig. 3. Rheological properties of HA and PVAb solutions as a function of frequency at 10% strain: (a) 1% HA solution and (b) 2% PVA-1% borax solution.

PVA solution forms a network structure with the addition of borax which condenses with organic hydroxyl groups in aqueous solution (Finch, 1973; Sinton, 1987; Shibayama *et al.*, 1988) as schematically shown in Fig. 2. The complex viscosity of 2 wt% PVA-1 wt% borax (PVAb) solution shows a Newtonian plateau at low frequencies and becomes thinning with increasing frequency as shown in Fig. 3(b). Since the relaxation time of a material can roughly be estimated as the inverse of crossover frequency, the relaxation time of the HA solution is larger than that of the PVAb solution. As a consequence, it reflects that the molecular weight distribution of the PVAb solution is narrower than that of the HA solution, and the relaxation spectrum is also narrower 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.

In Fig. 4, the complex viscosities of the HA/PVAb mixtures at 1 rad/s are compared with those of 1 wt% HA/10 wt% PVA mixtures without borax as a function of HA composition. Although the PVA concentration of the mixtures without borax is 5 times higher than that of HA/PVAb, the rheological properties are lower and the tendency depending on HA composition differs significantly. Two types of curves are clearly seen: the one follows a linear additive mixing rule over the entire composition range and the other shows a strong positive deviation at 20 wt% of HA composition. The positive deviation from the linear additive mixing rule means that there exists a strong interaction between HA and PVA-borax complex. The rheological properties of HA/PVAb (20/80) mixture which

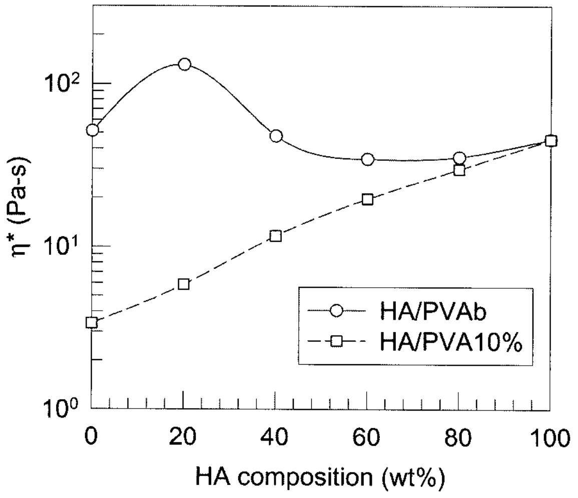


Fig. 4. Comparison of complex viscosity in HA/PVAb mixture and 1% HA/10% PVA mixture as a function of HA composition at 1 rad/s.

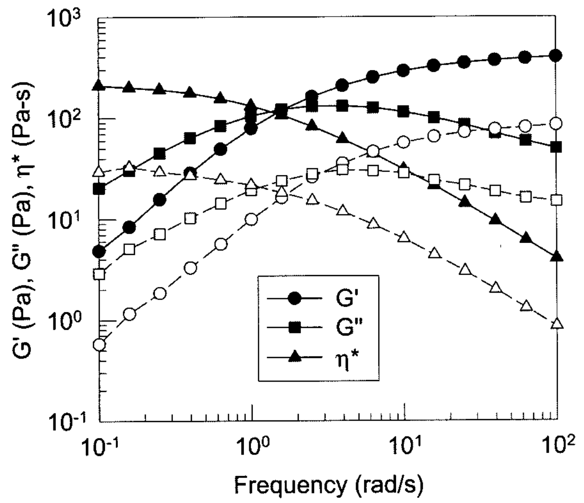


Fig. 5. Rheological properties of HA/PVAb (20/80) mixture (filled symbol) and HA/PVA2b (20/80) mixture (open symbol) as a function of frequency at 10% strain.

shows a maximum value of complex viscosity are represented in Fig. 5 with filled symbols.

3.2. Effect of degree of hydrolysis

The hydrogen bonding between HA and PVA is an important factor for the attractive interaction between them. The rheological properties of HA/PVAb mixtures decrease when the degree of hydrolysis of PVA is low as it reduces the number of active sites of PVA. As the hydroxyl groups in PVA molecule participate in hydrogen bonding with the carboxyl groups in HA molecule, the extent of interaction, consequently rheological behavior, can be evaluated by using another PVA having equal molecular weight but different degree of hydrolysis. To see the effect of the degree of hydrolysis, PVA2 (87-89% hydrolyzed) was used for the purpose of comparison. The rheological properties of HA/PVA2b (20/80) mixture are shown in Fig. 5 with open symbols. The effect of the degree of hydrolysis is clearly seen from two sets of rheological properties, which shows that the interaction between HA and PVA-borax association decreases as the number of hydroxyl sites of PVA decreases. When the degree of hydrolysis of PVA is changed, the difference of complex viscosity between HA/PVAb and HA/PVA2b is much smaller than that between PVAb and PVA2b, as seen in Fig. 6. Without the interaction between HA and PVA-borax, the difference of complex viscosity might be as large as that of PVAb and PVA2b.

As the rheological properties of HA/PVAb mixtures depend on the degree of hydrolysis of PVA, it is expected that the microstructure of the mixtures HA/PVAb (20/80) and HA/PVA2b (20/80) would be different. The morphology of the two mixtures was investigated in film states and

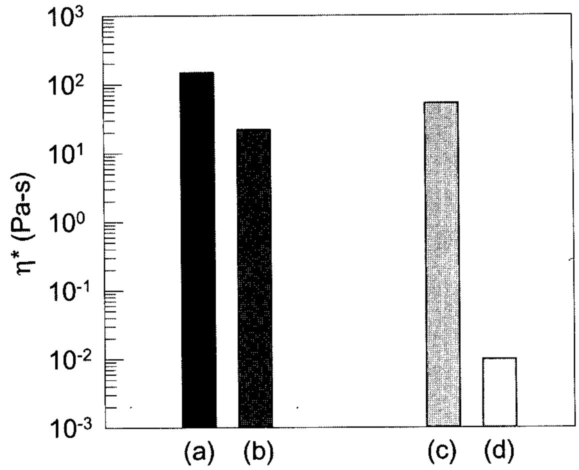
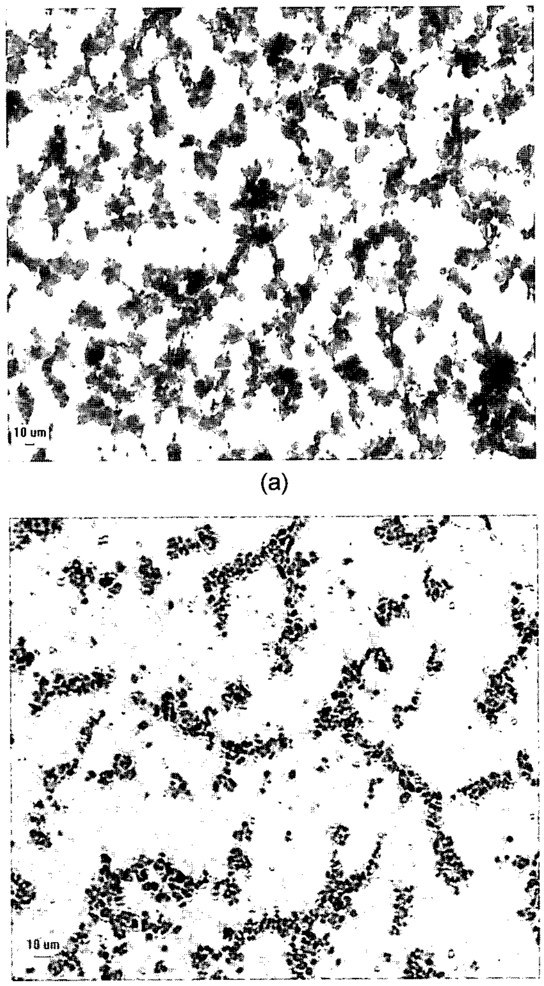


Fig. 6. Comparison of complex viscosity in HA/PVAb mixtures and PVAb solutions: (a) HA/PVAb (20/80) mixture, (b) HA/PVA2b (20/80) mixture, (c) PVAb solution and (d) PVA2b solution.



(a)

(b)

Fig. 7. Comparison of morphology depending on degree of hydrolysis: (a) HA/PVAb (20/80) mixture and (b) HA/PVA2b (20/80) mixture.

the results are shown in Fig. 7. Here, the dispersed phase is HA that is stained by the Alcian blue method, and the matrix is PVA-borax complex. The morphology shows that HA forms aggregates of the micrometer scale and the attractive interaction between aggregates exists, following that HA easily forms aggregates due to hydrogen bonding and the aggregates tend to flock together as well. The chaining phenomenon of aggregates is distinctly observed in the mixture of HA/PVAb (20/80) as shown in Fig. 7(a). The degree of network formation of HA in HA/PVAb is stronger than that in HA/PVA2b due to the difference of attractive interaction between HA and PVA-borax complex. It seems that HA aggregates form a network structure or a chaining structure by hydrogen bonding with PVA chains through PVA-borax crosslinking as a bridge, and the network structure is different with the degree of hydrolysis of PVA. Thus, it is conjectured that 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.

3.3. Effect of borax concentration

The effect of borax concentration of HA/PVAb mixture on the rheological properties were investigated. The composition ratio of HA and PVA-borax complex was chosen as 20/80, exhibiting maximum rheological properties as already shown in Fig. 4, and the concentration of borax was varied from 0.1 wt% to 1.0 wt%. Two peculiar results have to be pointed out by judging from the rheological behavior shown in Fig. 8. One is a big jump of complex viscosity at 0.3 wt% and the other is a change of the large amplitude oscillatory shear pattern from strain thinning to strain hardening. For the case of 0.1 wt% borax concentration, the complex viscosity shows strain thinning behavior at large strains after a Newtonian plateau at small strains. In this borax concentration, the strain thinning behavior of HA and PVA mixture in itself seems to be dominant on the mixture behavior. The complex viscosity significantly increases at 0.3 wt% borax concentration, above which it does not increase considerably. The increase of complex viscosity is caused by the crosslinking reaction between PVA and borax as schematically shown in Fig. 2, but this effect gradually diminishes as the borax concentration increases. Since the increase of viscosity from 0.5-1.0 wt% borax concentration is very small, it seems that additional borax over 1.0 wt% hardly contributes to the crosslinking reaction. Deviations of the large amplitude oscillatory shear behavior are found at more than 0.5 wt% borax concentration. Strain thinning at large strain region is maintained up to 0.3 wt%. When the borax concentration is 0.5 wt%, the complex viscosity is almost constant throughout entire strain range. Over the concentration of 0.5 wt%, the strain hardening behavior starts to appear at large strain region. The degree of strain hardening tends to increase as the borax concentration increases. From the different behavior observed at the large amplitude oscillatory shear test, it is confirmed that the borax complex resists to flow and the mixture shows shear dependent structure.

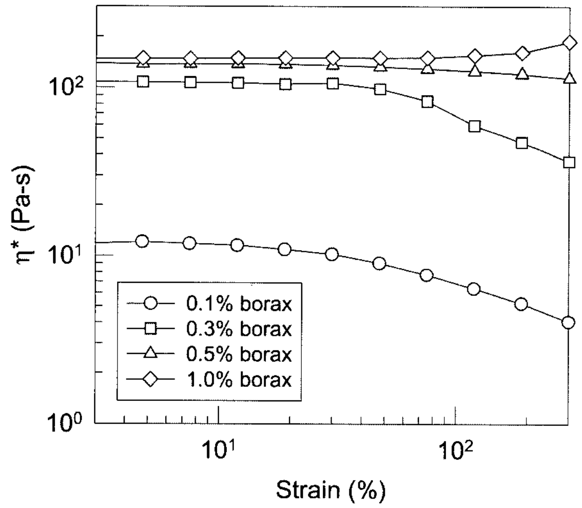


Fig. 8. Complex viscosity of HA/PVAb (20/80) mixtures depending on borax concentration at 1 rad/s.

3.4. Rheological behavior of HA/PVA hydrogels

Hydrogels are hydrophilic polymer networks which may absorb from 10-20% up to thousands of times their dry weights in water. Hydrogels based on both natural and synthetic polymers have continued to be of interest for encapsulation of cells and most recently such hydrogels have been especially attractive to the new field of tissue engineering as matrices for repairing and regenerating a wide variety of tissues and organs (Hoffman, 2002). In order to obtain a longer effect of HA in the action site and to reduce the risk of infection due to numerous injections, the formation of a hydrogel would be one of the ultimate goals for the development of water-insoluble biomaterials (Barbucci *et al.*, 2002). Here, physical hydrogels made of HA/PVA mixtures without borax were chosen to study its potential applications in biomedical fields. Mixtures of 1 wt% HA solution and 8 wt% PVA solution with different HA composition were prepared for hydrogels and their rheological properties were analyzed. Oscillatory shear tests were conducted for HA/PVA mixtures as solution state before freeze-thaw process. As gel structures were not formed, the storage modulus and complex viscosity of the mixtures increased with increasing HA composition. The mixture after freeze-thaw process showed different behavior due to the formation of a gel, following the opinion that PVA hydrogels can be considered as complex systems whose structure and macroscopic properties depend on several phenomena, such as crystallization, hydrogen

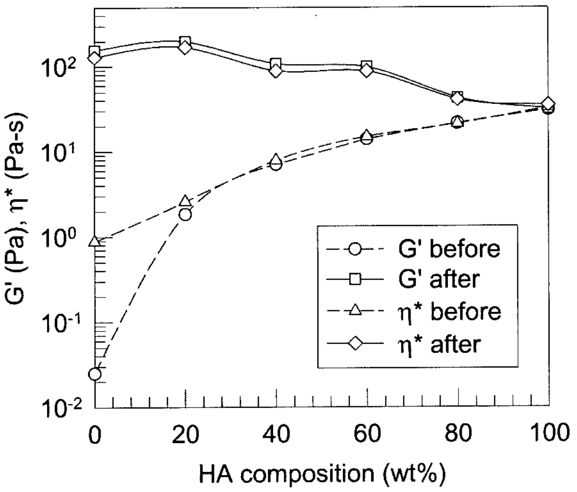


Fig. 9. Comparison of rheological properties between HA/PVA mixtures before and after freeze-thaw process as a function of HA composition at 1 rad/s.

bonding and phase separation, occurring during gelation (Ricciardi *et al.*, 2003). Comparison of rheological properties between HA/PVA mixtures before and after freeze-thaw process as a function of HA composition is shown in Fig. 9. The sample of PVA solution without HA and the resultant hydrogel show totally different rheological properties. The storage modulus of the gel after freeze-thaw process is roughly 5000 times higher than that of the solution before the process. On the contrary, the HA solution itself without PVA does not form a hydrogel by freeze-thaw process. Thus, the gel properties of HA/PVA mixtures show slightly decreasing tendency as the content of PVA decreases. In the limited experiments performed here, the maximum value of rheological properties is found at the gels comprised of 0-20 wt% of HA composition.

4. Conclusions

Aqueous mixtures of HA solution and PVA-borax solution were prepared and their rheological properties were investigated. Preparation parameters of the mixtures were HA composition, the degree of hydrolysis of PVA and borax concentration. Depending on the HA composition, the complex viscosity showed a positive deviation from the linear additive mixing rule and a maximum was measured at the composition of HA/PVAb (20/80), while a linear mixing rule prevailed over the entire compositions when borax was not used. Depending on the degree of hydrolysis of PVA, the mixtures showed different rheological properties. The mixture having higher degree of hydrolysis of PVA showed higher rheological properties, indicative of strong attractive interaction. This confirms that the important factor of interaction is the hydrogen bonding between HA and PVAb. From the morphological measurements, HA/PVAb (20/80) showed the chaining and networking structure of HA aggregates, whereas HA/PVA2b (20/80) showed rather well-dispersed state of HA aggregates in matrix, which proves that the difference in rheological behavior is attributed to morphological difference. The crosslinking reaction between PVA and borax increased but the effect diminished with increasing borax concentration. Borax concentration over 1.0 wt% hardly contributed to the crosslinking reaction. Physical hydrogels of HA and PVA mixture were prepared by freeze-thaw process and their rheological properties before and after freeze and thaw process were compared. The HA/PVA mixtures were able to form hydrogels and gel properties increased as the content of PVA increased.

Acknowledgement

The authors gratefully acknowledge the Korean Science and Engineering Foundation (KOSEF) for the financial support through the Applied Rheology Center, an official engineering research center (ERC) in Korea.

References

Ambrosio, L., A. Borzacchiello, P. A. Netti and L. Nicolais, 1999, Rheological study on hyaluronic acid and its derivative solutions, *J. Macromol. Sci., Part A: Pure Appl. Chem.* **36**, 991-1000.

Barbucci, R., S. Lamponi, A. Borzacchiello, L. Ambrosio, M. Fini, P. Torricelli and R. Giardino, 2002, Hyaluronic acid hydrogel in the treatment of osteoarthritis, *Biomaterials* **23**, 4503-4513.

Cascone, M. G., 1997, Dynamic-mechanical properties of bioartificial polymeric materials, *Polymer International* **43**, 55-69.

Clark, G., 1981, Staining Procedures, 274-275, Williams & Wilkins, Baltmore.

Dahl, L. B., I. M. Dahl, L. A. Enstrom and K. Granath, 1985, Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies, *Ann. Rheum. Dis.* **44**, 817-822.

Denlinger J. L., 1998, Hyaluronan and its derivatives as viscoelastics in medicine, in Chemistry, Biology and Medical Applications Hyaluronan and Its Derivatives, ed. by Laurent, T. C., 235-242, Portland Press, London.

Finch, C. A., 1973, Polyvinyl Alcohol: Properties and Applications, 41-43, Wiley, London.

Fouissac, E., M. Milas and M. Rinaudo, 1993, Shear-rate, concentration, molecular weight, and temperature viscosity dependences of hyaluronate, a wormlike polyelectrolyte, *Macromolecules* **26**, 6945-6951.

Hoffman, A. S., 2002, Hydrogels for biomedical applications, *Adv. Drug Delivery Rev.* **43**, 3-12.

Hynes, W. L., A. R. Dixon, S. L. Walton and L. J. Aridgides, 2000, The extracellular hyaluronidase gene (hylA) of streptococcus pyogenes, *FEMS Microbiol. Lett.* **184**, 109-112.

Kim, S. H., K. Hyun, T. S. Moon, T. Mitsumata, K. H. Ahn and S. J. Lee, 2005, Morphology-rheology relationship in hyaluronate/poly(vinyl alcohol)/borax polymer blends, *Polymer* (accepted for publication).

Koopal, L. K., 1981, Unperturbed conformation and interaction parameters of polyvinylalcohol in aqueous solutions - polydispersity effects, *Colloid Polym. Sci.* **259**, 490-498.

Lozinsky, V. I., E. S. Vainerman, L. V. Domotenko, A. M. Mamtsis, E. F. Titova, E. M. Belavtseva and S. V. Rogozhin, 1986, Study of cryostructurization of polymer systems. VII. Structure formation under freezing of poly(vinyl alcohol) aqueous solutions, *Colloid Polym. Sci.* **264**, 19-24.

Maerker, J. M. and S. W. Sinton, 1986, Rheology resulting from shear-induced structure in associating polymer solutions, *J. Rheol.* **30**, 77-99.

Oerther, S., A. C. Maurin, E. Payan, P. Hubert, F. Lapicque, N. Presle, J. Dexheimer, P. Netter and F. Lapicque, 2000, High interaction alginate-hyaluronate associations by hyaluronate deacetylation for the preparation of efficient biomaterials, *Biopolymers* **54**, 273-281.

Pelletier, S., P. Hubert, E. Payan, P. Marchal, L. Choplin and E. Dellacherie, 2001, Amphiphilic derivatives of sodium alginate and hyaluronate for cartilage repair: rheological properties, *J. Biomed. Mater. Res.* **54**, 102-108.

Pezron, E., A. Ricard, F. Lafuma and R. Audebert, 1988, Reversible gel formation induced by ion complexation. 1. Borax-galactomannan interactions, *Macromolecules* **21**, 1121-1125.

Pezron, E., L. Leibler, A. Ricard, F. Lafuma and R. Audebert, 1989, Complex formation in polymer-ion solutions. 1. Polymer concentration effects, *Macromolecules* **22**, 1169-1174.

Prokopova, E., P. Stern and O. Quadrat, 1985, Rheological investigation of aqueous solutions of poly(vinyl alcohol) during ageing. I. Changes in non-Newtonian viscosity and normal stress, *Colloid Polym. Sci.* **263**, 899-904.

Ricciardi, R., C. Gaillet, G. Ducouret, F. Lafuma and F. Laupretre, 2003, Investigation of the relationships between the chain organization and rheological properties of atactic poly(vinyl alcohol) hydrogels, *Polymer* **44**, 3375-3380.

Shibayama, M., M. Sato, Y. Kimura, H. Fujiwara and S. Nomura, 1988, ^{11}B n. m. r. study on the reaction poly(vinyl alcohol) with boric acid, *Polymer* **29**, 336-340.

Sinton, S. W., 1987, Complexation chemistry of sodium borate with poly(vinyl alcohol) and small diols. A ^{11}B NMR study, *Macromolecules* **20**, 2430-2441.

Weiss, C., 2000, Why viscoelasticity is important for the medical uses of hyaluronan and Hylans, in New Frontiers in Medical Sciences: Redefining Hyaluronan, ed. by Abatangelo, G. and P. H. Weigel, 89-103, Elsevier, Amsterdam.

Yamaura, K., N. Shindo and S. Matsuzawa, 1981, Mechanical denaturation of high polymers in solutions – 37. Effect of micro-gel on the flow-induced crystallization of poly(vinyl alcohol) from its aqueous solution, and the flow-induced crystallization of low molecular weight poly(vinyl alcohol), *Colloid Polym. Sci.* **259**, 1143-1146.