

Anomalous Viscosity Behavior in Aqueous Solutions of Hyaluronic Acid

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Summary

Effects of steady shear flows on intermolecular interactions in dilute and semidilute aqueous solutions of hyaluronic acid (HA) are reported. Pronounced shear thinning behavior is observed for solutions of HA at high shear rates, and no hysteresis effects are detected upon the subsequent return to low shear rates. With the aid of the asymmetric flow field-flow fractionation (AFFFF) technique, it is shown that mechanical degradation of the polymer does not take place in these shear viscosity experiments, even at high shear rates. The low shear rate viscosity of a semidilute HA solution decreases by approximately 40 % when the temperature is increased from 10 °C to 45 °C. It is shown that when a dilute HA solution is exposed to a low fixed shear rate (0.001 s^{-1}), a marked viscosification occurs in the course of time and prominent intermolecular complexes are formed. It is argued that shear-induced alignment and stretching of polymer chains promote the evolution of hydrogen-bonded structures, where cooperative zipping of stretched chains generates a network. At a higher constant shear rate (0.1 s^{-1}), the viscosity decreases as time goes because of the alignment of the polymer chains, but the higher shear flow perturbation prevents the chains in dilute solutions from building up association complexes. The viscosity of an entangled HA solution is not changed in the considered time window at this shear rate, but the network structures breakdown at the highest shear rate (1000 s^{-1}), and then they are restored upon return to a low shear rate.

Keywords

Biopolymers, hyaluronic acid, rheology, shear-induced viscosification, aqueous solutions

Introduction

Sodium hyaluronate, also called hyaluronan, is referred to as hyaluronic acid (HA) in this study. HA is a linear anionic polysaccharide, composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine (see Figure 1), with a molecular weight in the range from 10^4 up to about 10^7 . This polymer is a basic constituent of numerous tissues in the organism. It can be found in compartments like

umbilical cords, vitreous body, skin, and in synovial fluid. The high viscosity of HA solutions and the important role of the polymer in synovial fluid as well as in many medical applications have stimulated studies of molecular interactions in aqueous HA solutions by means of rheological methods. In the semidilute concentration regime, entanglements are formed in solutions of HA and the systems exhibit viscoelastic properties [1-4]. Previous studies have disclosed that with increasing shear rate, even at fairly low shear rates and moderate concentrations, HA solutions exhibit shear thinning behavior [3-8].

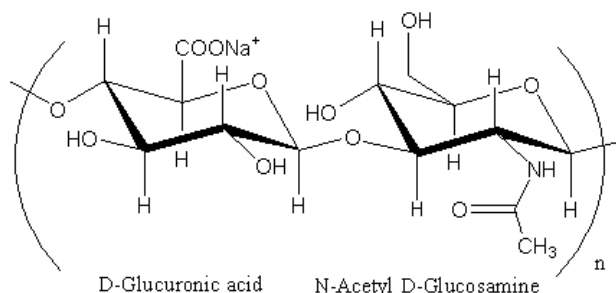


Figure 1. A schematic illustration of the principal structure of HA.

In the present investigation, a time-induced viscosification of HA solutions of low concentrations has been observed at low shear rates, whereas at higher shear rates the viscosity decreases in the course of time. These are novel findings that, to the best of our knowledge, have not been reported before. In this work, we have carried out shear viscosity measurements at mainly 25 °C and 37 °C (body temperature) on HA solutions of various concentrations and at different shear rates. The aim of this study is to provide a better insight into the rheological behavior of HA solutions at different conditions of shear rate, temperature, and polymer concentration. The molecular weight of HA will be characterized by the asymmetric flow field-flow fractionation technique, both before and after the sample has been exposed to high shear rate, and mechanical degradation of the polymer will be addressed. This is an issue that has attracted a great deal of interest in recent years.

Experimental

Materials and solution preparation

The HA sample (sodium hyaluronate pharma grade 150) utilized in this work was purchased from NovaMatrix, FMC BioPolymer, Drammen, Norway. The HA polymer was characterized with the aid of an asymmetric flow field-flow fractionation (AFFFF) instrumentation. The results on dilute aqueous HA solutions showed that the sample has a weight-average molecular weight of $M_w = 1.9 \times 10^6$ and a narrow molecular weight distribution with a polydispersity index $M_w/M_n=1.1$ and a radius of gyration of approximately 165 nm (more information about the technique and results are given below). According to the manufacturer, the protein contents of the polymer are less than 0.1 %. The HA sample was dissolved in water and was allowed to swell for 1 h, followed by very slow stirring (6 h) with a magnetic stirrer; thereafter the

solution was allowed to rest for 17 h prior to commencement of the experiments. Fresh solutions were used for all experiments, and the solutions were at all times prepared according to this procedure to obtain reproducible results on samples with the same history.

Asymmetric flow field-flow fractionation

The AFFFF experiments were conducted on an AF2000 FOCUS system (Postnova Analytics, Landsberg, Germany) equipped with an RI detector (PN3140, Postnova) and a multiangle (7 detectors in the range 35 – 145°) light scattering detector (PN3070, $\lambda = 635$ nm, Postnova). The HA samples (0.1 wt% in 0.01 M NaCl) were measured using a 350 μm spacer, a regenerated cellulose membrane with a cut-off of 5000, and an injection volume of 20 μL . The measurements were performed by employing a constant detector flow rate of 1 mL/min. The focusing time was 6 min at a cross-flow of 2 mL/min. Thereafter, the cross-flow was reduced exponentially (exponent of 0.2) to 0.1 mL/min during a 5 min period. The cross-flow was then linearly reduced to zero during a period of time of 10 min.

Processing of the measured data was achieved by the Postnova software (AF2000 Control, version 1.1.011). The molecular weight and root-mean radius of gyration of the dilute solutions were determined using this software with a random coil fit, and a refractive index increment (dn/dc) of 0.134 (determined by using the RI-detector at 32 °C).

Rheology experiments

Shear viscosity measurements on HA solutions of different concentrations were carried out in a Paar-Physica MCR 300 rheometer using a cone-and-plate-geometry, with a cone angle of 1° and a diameter of 75 mm. The samples were introduced onto the plate with great care to avoid shear effects on the solutions. To prevent evaporation of the solvent, the free surface of the sample was always covered with a thin layer of low-viscosity silicone oil (the viscoelastic response of the samples is virtually not affected by this layer). The measuring device is equipped with a temperature unit (Peltier element) that provides a good temperature control over an extended time at the temperatures considered in this study. The viscosity measurements were conducted over an extended shear rate range.

Results and discussion

Figure 2 shows plots of the shear viscosity versus shear rate at 25 °C and 37 °C (body temperature) for HA solutions of different concentrations. The shear rate dependence of the viscosity is similar at both temperatures, and at the lower two concentrations (0.1 and 0.2 wt %) shear thinning is visible at low shear rates. This may indicate that the flow field align the stiff HA chains, and thereby reduces the viscosity. At higher polymer concentrations (in the semidilute regime) Newtonian behavior is visible at low shear rates, followed by shear thinning higher shear rates.

The progressive decrease in viscosity as the shear rate rises for semidilute HA solutions is ascribed to the breakdown of the network junctions; that is, the rate of network disruption exceeds the rate at which associations and entanglements are reformed. This type of behavior for HA solutions has been reported in previous

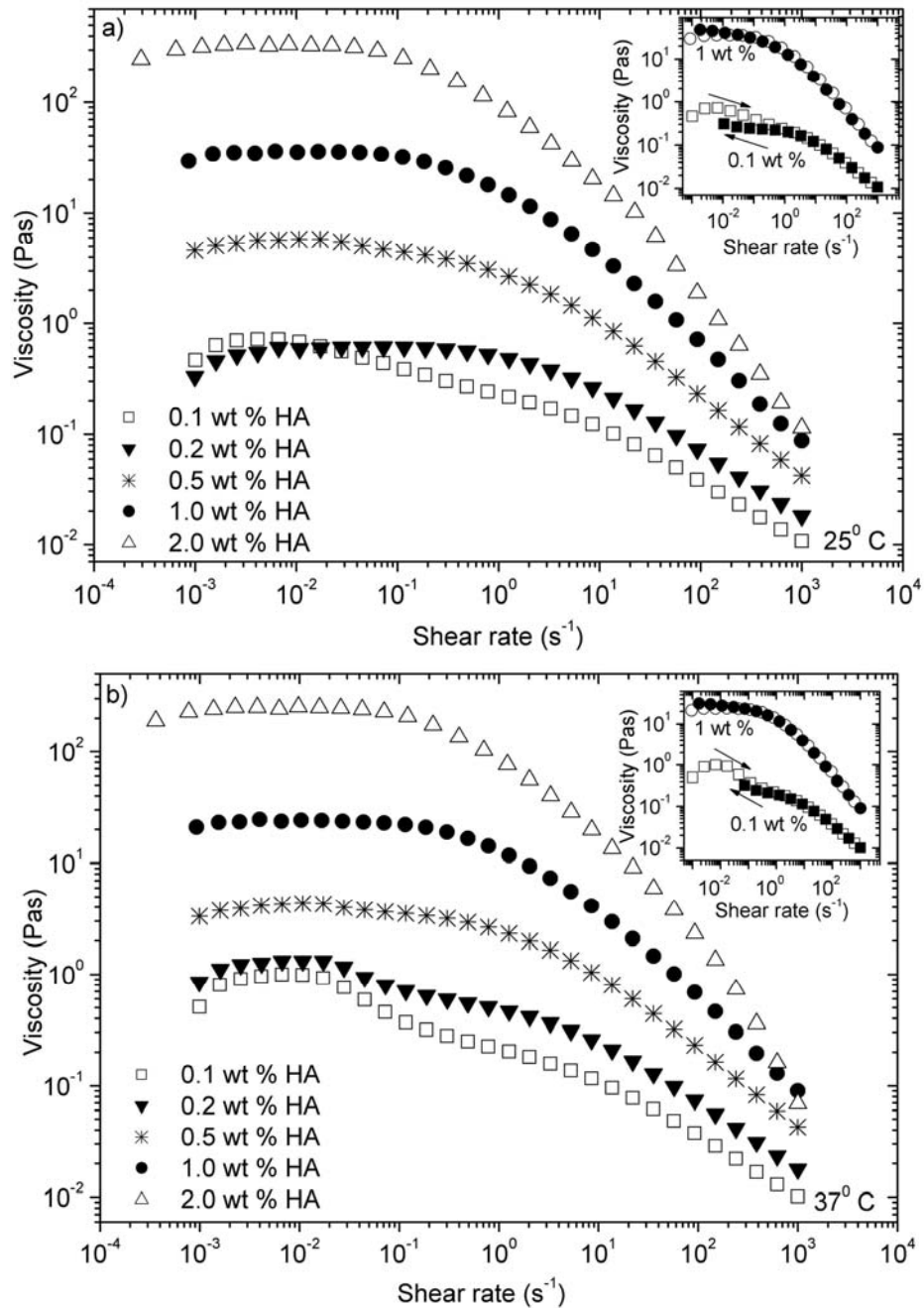


Figure 2. Shear rate dependencies of the viscosity at 25 °C (a) and 37 °C (b) for HA solutions of various concentrations. The insets show data obtained under increasing (open symbols) and decreasing (filled symbols) shear rates for HA solutions of concentrations of 0.1 wt % and 1.0 wt %. (Every 2nd point is shown in the inset plots.)

studies [3-8]. It has been argued [9] that the major contribution to the stiffness of HA and the related non-Newtonian viscoelastic features are due to intrachain hydrogen bonding between adjacent carbohydrate units.

The inset plots demonstrate that there is practically no hysteresis effect on the viscosity for a dilute and semidilute solution when the shear rate is increased (upramp curve) and subsequently decreased (downramp curve). The curves at both temperatures virtually collapse onto each other. This performance is further consolidated by the AFFFF results below.

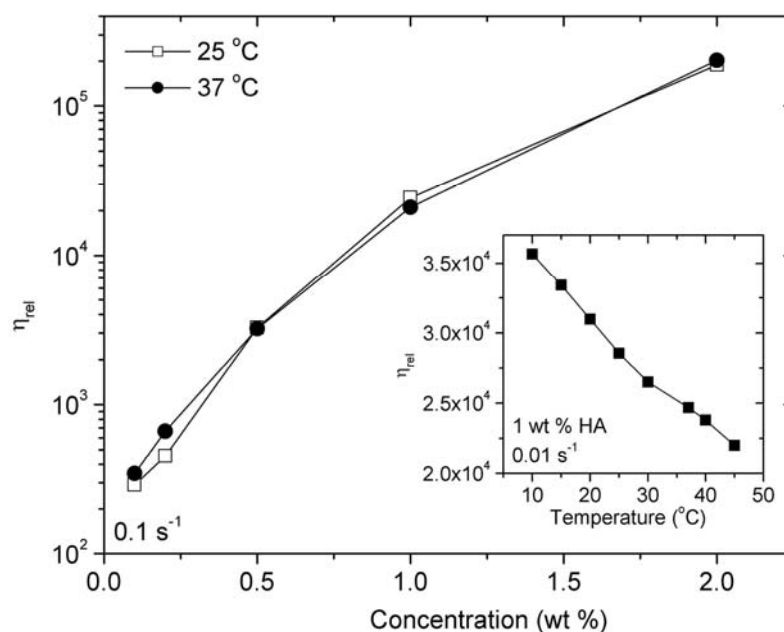


Figure 3. Concentration dependence of the relative viscosity at a low shear rate for HA at the temperatures indicated. The inset shows the temperature dependence (over a broad temperature interval) of the relative viscosity at a low shear rate for a 1 wt % HA solution.

Temperature is a variable that in many cases affects the viscoelasticity of interacting polymer systems, and must be considered in medical applications, such as ophthalmic surgery. To take into account trivial changes of solvent viscosity with temperature, the viscosity results are presented in terms of the relative viscosity η_{rel} ($\eta_{rel} \equiv \eta/\eta_{solvent}$, where $\eta_{solvent}$ is the viscosity of water). Figure 3 illustrates the effects of temperature and polymer concentration on the relative viscosity at a low shear rate. We observe a strong rise of η_{rel} with increasing HA concentration, which reflects the impact of entanglements as the concentration increases. A moderate temperature rise (from 25 °C to 37 °C) has virtually no visual influence on the relative viscosity over this limited temperature range. To scrutinize the temperature dependence of η_{rel} , a broader temperature interval (10 °C up to 45 °C) is examined in the inset plot for a 1 wt % HA solution. This illustration discloses a monotonous decrease of η_{rel} with increasing temperature. This may be related to breakage of hydrogen bond interactions at elevated temperatures. However, the stiffness of HA characterized by the intrinsic

persistence length was shown [10] by molecular modeling to decrease as the temperature increases. This effect and the enhanced mobility of the chains at elevated temperatures can weaken the entanglement couplings, and thereby give rise to a drop in the viscosity.

Mechanical degradation of HA has been observed [7,11,12] in shear viscosity experiments, and to check whether this constitute a problem in the present measurements, we have used the AFFFF technique to determine the molecular weight of a HA sample that has been exposed to a high shear rate (1000 s^{-1}) for different time intervals (see Figure 4). We note that within the experimental accuracy, the molecular weight is constant and it is not possible to detect any degradation of the polymer. This indicates that if the HA sample is not subjected to high shear rates over long times, the effect of mechanical degradation of the sample can be ignored. This is consistent with the viscosity results presented above (see the insets of Figure 2). It should be mentioned that the AFFFF measurements were carried out on a dilute solution (0.1 wt %) of HA in the presence of 0.01 M NaCl to avoid association of molecules or entanglements. To obtain a good resolution, it is necessary to conduct the AFFFF experiments at low salinity, but there is no reason to believe that this salt addition would affect the molecular weight or molecular weight distribution of the sample.

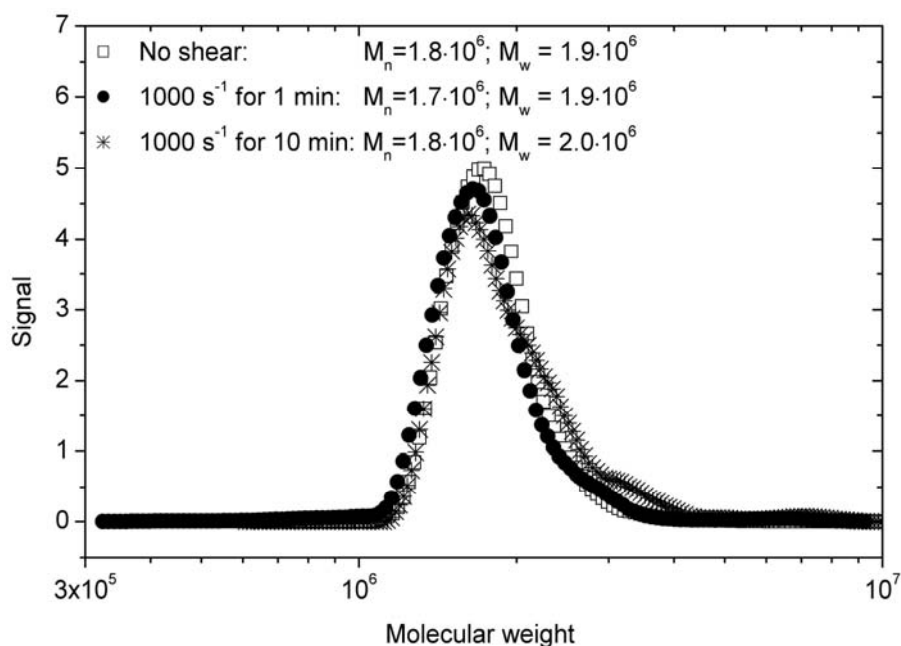


Figure 4. Molecular weight distribution curves on a 0.1 wt % HA sample that has been in a quiescent state and exposed to a shear rate of 1000 s^{-1} for 1 min and 10 min. The measurements were carried out in 0.01 M NaCl. The experimental errors of the molecular weights are about $\pm 0.1 \times 10^6$.

Figure 5 shows the time evolution of the shear viscosity at a fixed shear rate (0.001 s^{-1} or 0.1 s^{-1}) for different concentrations of HA at $25 \text{ }^\circ\text{C}$ and $37 \text{ }^\circ\text{C}$. Let us first discuss the viscosity behavior at the lowest shear rate. The most conspicuous feature is the time

evolved viscosification of the dilute HA solution at the low shear rate. This novel finding is observed at both temperatures, and may be attributed to shear-induced alignment of the semirigid chains and it is also possible that additional stretching of the chains occurs [13]. Both stretching and alignment of the chains may result in more sites that become available for intermolecular junctions through hydrogen bonds. As an outcome of this process the chains hydrogen-bond to each other to form an interconnected three-dimensional network. It has been suggested [14] that this type of network arrangement is in the form of staggered ladderlike structures. The basic ingredients in the formation of such structure are cooperative mobility, orientational effects, and cooperative zipping of stretched chains through hydrogen bonds. Shear-induced viscosification, or even gelation, in the course of time has recently been reported [15,16] for aqueous solutions of a polydisperse low molecular weight pectin. This effect was ascribed to the growth of hydrogen-bonded structures. At a HA concentration of 0.5 wt % only a modest enhancement of the viscosity in the course of time is registered, whereas no effect is observed for the highest polymer concentration at 25 °C. This suggests that as the polymer concentration increases, the growth of entanglements creates a strong network that is not substantially deformed or reorganized in this weak flow field. At 37 °C the overall picture is similar, but for the highest concentration the viscosity exhibits a moderate increase with time. This can probably be traced to enhanced chain mobility at elevated temperature, which can facilitate network deformation and the formation of stronger interpolymer associations. At a constant shear rate of 0.1 s⁻¹ a different picture emerges (Figure 5c,d). In this case, the viscosity of the lowest polymer concentration (at both temperatures) falls off

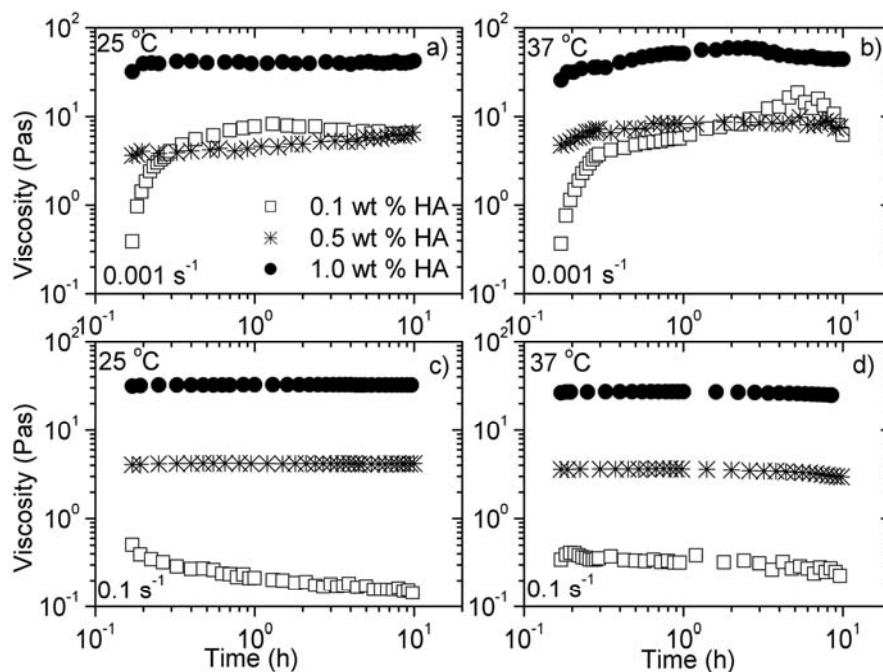


Figure 5. Time evolution of the shear viscosity at a fixed shear rate (0.001 s⁻¹ or 0.1 s⁻¹) for different HA concentrations at 25 °C and 37 °C.

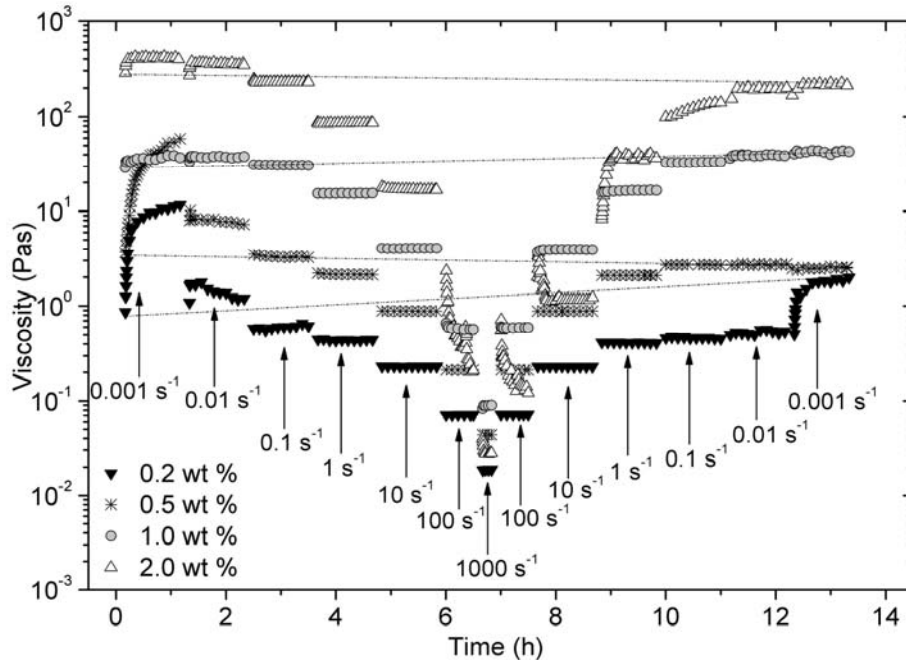


Figure 6. Viscosity as a function of shear time, where the shear rate is periodically increased up to 1000 s^{-1} , followed by a subsequent decrease of the shear rate with the same periodicity as in the upramp curve for the HA concentrations indicated.

in the course of time. This announces that the intensity of the flow field is too high to promote evolution of a connected network, but rather the polymer chains are gradually aligned in the flow field to decrease the viscosity. At higher concentrations, the viscosity is virtually independent of time because at this fairly low shear rate the entanglements obstruct a reorganization of the network and the shear flow is too weak to disrupt the network.

Effects of progressively increasing transitory shear rate perturbations and the return to the lowest shear rate with the same periodicity are depicted in Figure 6 for the HA concentrations indicated. The most striking feature is the initial growth of the viscosity for the lower two concentrations at the lowest shear rate, which is attributed to shear-induced alignment and stretching of HA chains and the formation of hydrogen-bonded network structures, where cooperative zipping of stretched chains play an important role. This type of behavior is not observed for the higher polymer concentrations. As the transitory shear rate perturbations become stronger, the viscosities for all the systems gradually fall off as the shear rate approaches 1000 s^{-1} . Upon return to the lowest shear rate (0.001 s^{-1}), the viscosities of the higher two polymer concentrations (1 and 2 wt %) are almost restored. This means that the network structures are broken down by high shear rate, but they are easily build-up without any hysteresis effect when the shear rate declines. In the case of the lower two concentrations, the return from the highest to the lowest shear rate yields viscosity values that are almost the same as the start values. However, the high viscosity values evolved initially in the presence of the lowest shear rate are not reached, which may be associated with the strong transitory shear rate perturbations that the samples have

been subjected to. As a consequence the chains have not relaxed back to the positions giving optimal conditions for the growth of intermolecular interactions. This demonstrates that the building up of shear-induced association structures in time is a delicate process. We note the pronounced minimum in the viscosity at the highest shear rate for all the considered HA concentrations, which signals that even if the network is strongly entangled it is disrupted by this high shear rate perturbation.

Conclusions

In this work, we have reported some novel findings about the influence of steady shear flows on intermolecular association in dilute and semidilute aqueous solutions of hyaluronic acid. The main results can be summarized in the following way: (1) A pronounced shear thinning behavior is found for semidilute solutions of HA at high shear rates, and no hysteresis effects are observed upon the subsequent return to low shear rates. In the case of dilute solutions, the shear-induced decrease of the viscosity may be related to the alignment of polymer chains. (2) The results from the AFFF experiments show no tendency of mechanical degradation of HA, even if the sample is exposed to a high shear rate (1000 s^{-1}) for 10 min. (3) When the temperature is changed from $10 \text{ }^{\circ}\text{C}$ to $45 \text{ }^{\circ}\text{C}$ for a 1 wt % solution, the low shear rate viscosity decreased by ca. 40 %. (4) When a low fixed shear rate (0.001 s^{-1}) was applied on a dilute HA solution, a significant viscosification occurred in the course of time. The growth of stronger association structures at lower HA concentrations is probably due to an easier reorganization of the chains. The conjecture is that the shear-induced alignment and stretching of polymer chains favor the formation of hydrogen-bonded structures, where cooperative zipping of stretched chains yields an interconnected network. (5) At a higher constant shear rate (0.1 s^{-1}), the polymer chains in a dilute solution are aligned and the viscosity decreases as time goes. In this case, the higher shear flow perturbation obstructs the chains from building up association complexes. (6) When HA solutions are exposed to increasing shear rate perturbations during a short period of time the network structures breakdown, but they are restored upon return to the low shear rate.

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