Effect of pH on the Behavior of Hyaluronic Acid in Dilute and Semidilute Aqueous Solutions

Atoosa Maleki, Anna-Lena Kjøniksen, Bo Nyström*

Summary: Viscosity, asymmetric flow field-flow fractionation (AFFFF) methods, and dynamic light scattering (DLS) experiments were used to characterize the effect of pH on the behavior of dilute and semidilute aqueous buffered solutions of hyaluronic acid (HA). It is shown that degradation of HA occurs at $pH < 4$ and $pH > 11$, and in the domain $4 < pH < 11$ virtually no disruption of the HA chains occurs. The pH-induced scission of HA is attributed to the cleavage of glycosidic bonds. In dilute solutions, intramolecular rupture of HA chains occurs and in the semidilute concentration regime network-fragmentation is observed at low and high pH values. The degree of degradation of HA is most marked at high pH. From the molecular weight and radius of gyration obtained from AFFFF at different pH values, it is clear that the degradation of HA starts at early times after preparation of the solution, and continues for a couple of days. The kinetics of degradation of HA is fastest at high pH.

Keywords: degradation; dynamic light scattering; effect of pH; hyaluronic acid; rheology

Introduction

Hyaluronic acid (HA) is a biodegradable, biocompatible, and natural linear anionic polysaccharide consisting of alternating units of β-D-glucuronic acid and *N*-acetyl-β-*D*-glucosamine linked at 1,3 and 1,4 position, respectively.[1] HA can be found in compartments like umbilical cords, vitreous body, skin, and in synovial fluid. Because of its high molecular weight and special viscoelastic features,[2] HA has been extensively investigated and used for the treatment of arthritis,[3] ophthalmic surgery,[4] drug delivery,[5–8] and tissue engineering.[9] The rheological behavior is of decisive importance for almost all applications and since the value of pH differs depending on the type of application, it is vital to gain insight into the effect of pH on the physicochemical properties of solutions of HA. It has been reported[10] that HA is degraded at pH values below 1.5 and above 11 in the presence of salt. In a recent paper,[11] a slight degradation of HA was observed at acidic conditions ($pH = 1.6$) and in strongly basic medium ($pH = 12.6$) from molecular weight distribution analysis, but the rheological properties were only slightly influenced by pH. In chemically cross-linked gels of HA, the rate of degradation of the gels was observed[12] to be affected by the value of pH. In the light of these findings, we decided to investigate the influence of pH on dynamical and rheological features in solutions of HA.

In the present work, a dilute and a semidilute aqueous solution of HA were prepared in buffers at different values of pH in the range 1–13. These systems were investigated with the aid of asymmetric flow field-flow fractionation (AFFFF), dynamic light scattering (DLS) and rheology. The aim of this study is to survey the role of the pH on the dynamical and rheological behavior of HA in the dilute and semidilute concentration regimes. The AFFFF technique is utilized to establish the degree of degradation of the sample in the course of time at different values of pH. This knowledge is important to tailor-made HA

Department of Chemistry, University of Oslo,
PO Box 1033, Blindern, N-0315 Oslo, Norway
E-mail: atoosam@kjemi.uio.no

systems for special operations and applications where pH is altered.

Experimental Part

Materials

The HA sample (sodium hyaluronate, Pharma grade 150) was obtained from NovaMatrix, FMC BioPolymer, Drammen, Norway. The polymer was characterized by means of an asymmetric flow field-flow fractionation instrumentation AFFFF and the results showed[2] that the polymer 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 of $M_w/M_n = 1.1$. According to the manufacturer, the protein contents of the polymer are less than 0.1%.

The chemicals H_3PO_4, NaH_2PO_4, Na_2HPO_4, Na_3PO_4 were all purchased from Sigma-Aldrich, and sodium hydroxide was supplied by Fluka. These chemicals were used without any further purification. Double distilled Millipore water was utilized in this work.

HA was dissolved in 0.1 M phosphate buffers of different pH values (1, 3, 5, 7, 9, 11, 12, and 13); the buffers were prepared by mixing appropriate portions of H_3PO_4 and its salts (NaH_2PO_4, Na_2HPO_4 or Na_3PO_4) to attain the desired pH value. At the highest pH, a few drops of concentrated NaOH was added to the buffered solution to reach $pH = 13$. In the kinetical experiments with AFFFF, the samples were homogenized as fast as possible before commencement of the measurements. The DLS and shear viscosity experiments were carried out on both dilute (0.05 wt %) and semidilute (0.5 wt %) solutions of HA at buffered conditions. At a pH of 5 and an ionic strength of ca. 0.1 M, the overlap concentration was estimated to be 0.1 wt %.

As will be demonstrated and discussed below, degradation of HA occurs at low pH ($pH < 4$) and high pH ($pH > 11$) and since the degradation proceeds over time (cf. the AFFFF measurements below), all the buffered systems were allowed to stand on stirring for the same time (1 day) prior to the commencement of the viscosity and DLS measurements. By this procedure, the history of the samples was the same, and this facilitates a direct comparison of the results from the different buffered samples. The pH values of the buffered solutions were determined by using a Thermo Orion Model 720Aplus pH meter at room temperature.

Asymmetric Flow Field-Flow Fractionation (AFFFF)

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 were measured using a 350 μm spacer, a regenerated cellulose membrane with a cut-off of 10000, and an injection volume of 20 μL. The experiments were performed according to a scheme described previously.[2] The degradation of HA (change of molecular weight and radius of gyration) in the buffered solutions of different pH values was monitored at different times during the degradation process with the AFFFF instrument. For the AFFFF measurements, HA samples of different pH were examined at different times and the molecular weight and polydispersity index were registered. It should be noted that although the concentration of the injected sample was around 0.3 wt %, it is highly diluted (ca. 100 times) in the course of the experiment and the results refer to a solution with a low concentration.

Rheology

Shear viscosity experiments were conducted in a Paar-Physica MCR 300 rheometer using a cone-and-plate geometry, with a cone angle of 1° and diameter of 75 mm. The samples were carefully applied on the plate, and a thin layer of low-viscosity silicone oil was used to cover the free surface of the solution to prevent evaporation of solvent. No effect of the silicone oil on the physical properties of the samples could be detected, and the viscoelastic response of the sample is virtually not affected by this layer. The measurements were carried out over a broad shear rate domain. The measuring device is equipped with a temperature unit (Peltier plate) that provides a good temperature control ($25 \pm 0.05\,^{\circ}$C) over extended time.

Dynamic Light Scattering Measurements (DLS)

The setup for the intensity and dynamic light scattering experiments is an ALV/CGS-8F multi-detector version compact goniometer system, with 8 off fiber-optical detection units, from ALV-GmbH., Langen, Germany. The light source is a Uniphase cylindrical 22 mW HeNe-laser operating at a wavelength of 632.8 nm with vertically polarized light. The beam was focused on the sample cell (10-mm NMR tubes, Wilmad Glass Co., of highest quality) through a temperature-controlled cylindrical quartz container (with 2 plane-parallel windows), vat (the temperature constancy being controlled to within $\pm 0.01\,^{\circ}$C with a heating/cooling circulator), which is filled with a refractive index matching liquid (*cis*-decalin). The sample solutions were filtered in an atmosphere of filtered air through, depending on the viscosity of the solution, 0.45-, 0.8- or 5 -μm filters (Millipore) directly into precleaned NMR tubes.

From the DLS experiments we can define a wave vector $q = (4\pi n/\lambda)\ \sin(\theta/2)$, where λ is the wavelength of the incident light in a vacuum, θ is the scattering angle, and n is the refractive index of the medium. The refractive index (at $25\,^{\circ}$C) was measured with an automatic refractometer (model PTR 46) purchased from Index Instruments Ltd., England. The temperature of the instrument is controlled electronically to a high stability by using a Peltier cell.

Intensity autocorrelation functions were measured at 8 scattering angles simultaneously in the range 22–141° with four ALV 5000/E multiple-τ digital correlators. If the scattered field obeys Gaussian statistics, the measured correlation function $g^2(q,t)$ can be related to the theoretically amenable first-order electric field correlation function $g^1(q,t)$ by the Siegert relationship[13] $g^2(q,t) = 1 + B|g^1(q,t)|^2$, where B is usually treated as an empirical factor. For the systems considered in this work, no signs of non-ergodicity were found.

For all polymer solutions, except the dilute solution at the highest pH (where only a single relaxation mode (stretched exponential) was observed; see the discussion below), the decays of the correlation functions can be described initially by a single exponential, followed at longer times by a stretched exponential

$$g^{(1)}(t) = A_f\ \exp(-t/\tau_f) + A_s\ \exp[-(t/\tau_{se})^{\beta}] \tag{1}$$

with $A_f + A_s = 1$. The parameters A_f and A_s are the amplitudes for the fast and slow relaxation modes, respectively. This type of bimodal relaxation process has recently been reported[14–17] for other complex polymer systems. Analyses of the time correlation functions in the domain $qL < 1$, where L refers to the hydrodynamic radius R_h (dilute solution) or the hydrodynamic correlation length ξ_h (semidilute solution) have revealed that the first term (short-time behavior) on the right-hand side of Equation 1 is associated with diffusion ($\tau_f^{-1} = Dq^2$), where D is the mutual diffusion coefficient of molecularly dispersed molecules or small clusters of molecules (dilute solutions), or D is the cooperative diffusion coefficient in the semidilute concentration regime. The second term (long-time behavior) in Equation 1 is related to the relaxation of large moieties (dilute solutions) or is associated with disengagement relaxation of individual chains or clusters.[18] The parameter τ_{se} in Equation 1 is some effective slow relaxation time, and β ($0 < \beta \leq 1$) is a measure of the width of the distribution of relaxation times. The mean relaxation time for the slow mode is given by

$$\tau_s \equiv \int_0^\infty \exp\left[-\left(\frac{t}{\tau_{se}}\right)^\beta\right] dt = \frac{\tau_{se}}{\beta}\Gamma\left(\frac{1}{\beta}\right) \quad (2)$$

where $\Gamma(\beta^{-1})$ is the gamma function.

In the analysis of the correlation function data, a nonlinear fitting algorithm was utilized to best-fit values of the parameters A_f, τ_f, τ_{se}, and β appearing on the right-hand side of Equation 1.

Results and Discussion

Viscosity Properties

We will first present and discuss the viscosity features of the buffered samples in the dilute and semidilute concentration regime at diverse pH. Figure 1 shows the shear rate dependence of the viscosity for dilute (0.05 wt %) and semidilute (0.5 wt %) solutions at different pH values. The viscosity behavior for the solutions in the dilute regime is virtually Newtonian at all pH values; the modest upturn of the viscosity for the sample at pH = 13 at high shear rates is attributed to turbulence at these shear rates. As demonstrated below, the effect of pH on the viscosity is related to intramolecular rupture of the polymer chains at low and high pH. This effect is more dramatic in the semidilute concentration regime (Figure 1b), where the viscosity exhibits a drastic alteration at low and high pH, especially at pH = 13 where the viscosity is only approximately three times the viscosity of water. At intermediate pH values, shear thinning is observed at high shear rates, which probably can be ascribed to shear-induced disruption of entanglements and intermolecular interactions such as hydrogen bonds between adjacent chains.[2] The drop of the viscosity at low and high pH at low shear rates is attributed to disintegration of the network because of pH-induced cleavage of glycosidic bonds. At pH = 13, a practically Newtonian viscosity behavior is detected over the considered shear rate range, and this feature suggests that the connectivity of the network is lost and the network is highly fragmented and conformation of the chains may have changed.

Effects of pH on the zero-shear viscosity for dilute and semidilute solutions of HA are depicted in Figure 2a. For the dilute systems, the zero-shear viscosity is lower at low and high pH values, but the effect is much less pronounced than for the corresponding semidilute solutions. This behavior can be rationalized in the following framework. In dilute solutions, scission of chains occurs essentially intramolecularly,

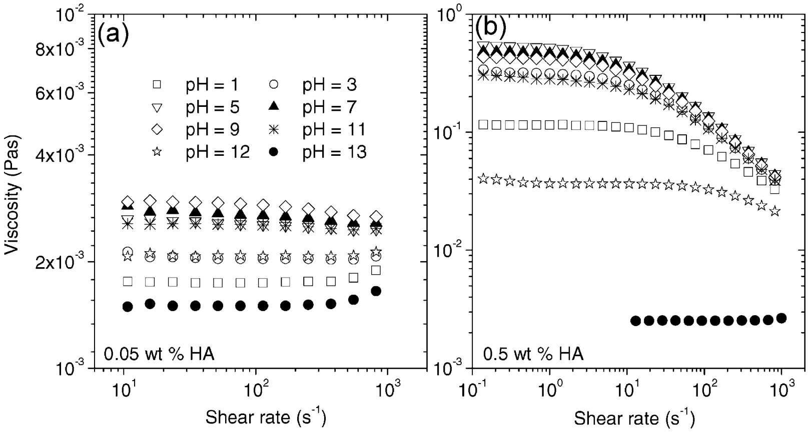

Figure 1.
Viscosity (every second point is shown) as a function of shear rate for dilute and semidilute buffered systems of hyaluronic acid at the pH values indicated.

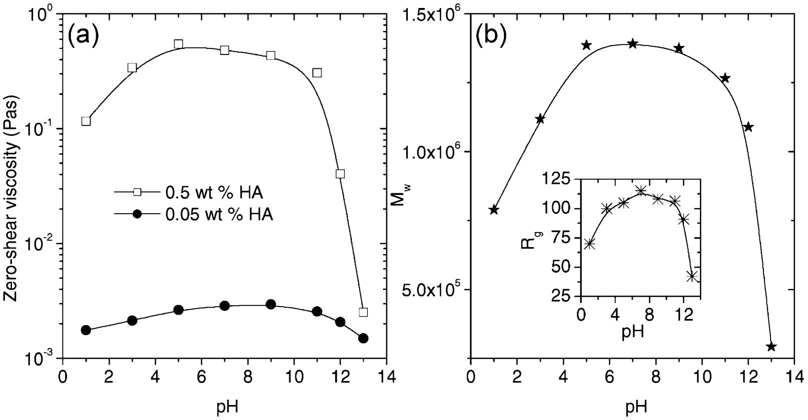

Figure 2.
Effects of pH on the zero-shear viscosity for dilute and semidilute samples and on the weight-average molecular weight in dilute HA solutions (AFFFF results). The pH values refer to the conditions at which the samples were exposed to. (see the main text for details). The measurements were carried out 1 day after the preparation of the buffered samples.

whereas in the semidilute concentration regime intermolecular cleavage of chains results in disintegration of the polymer network, and this happening has a larger impact on the viscosity feature than in the dilute regime where the entities act as separate units.

Several years ago, it was reported[19] for dilute solutions of HA that intramolecular cleavage (hydrolysis) of glycosidic bonds occurs at strongly basic conditions. In a recent rheological investigation[11] on the effect of pH in semidilute solutions, a slight degradation of the polymer was found in the domain $1.6 < \mathrm{pH} < 12$ from molecular weight distribution analysis, but the rheological behavior remained nearly unchanged. At $\mathrm{pH} > 12$ the viscosity decreased; this phenomenon was ascribed to reduced stiffness of the polymeric backbone under alkaline conditions because of partial breakage of the H-bond network. In contrast to this study, it has clearly been established from the present work that at a very low pH ($\mathrm{pH} = 1$) or very high pH ($\mathrm{pH} = 13$) the irreversible degradation of the polymer is found.

In Figure 2b, the weight average molecular weights (M_w) obtained from the AFFFF measurements are plotted as a function of pH for dilute solutions of HA. All the samples were measured 1 day after they were prepared, which is the same time as elapsed for the solutions employed in the rheology and DLS experiments. Prior to injection, the pH values were adjusted to neutral since very acidic or basic solutions destroy the membrane of the instrument. However, it is evident that the value of M_w decreases markedly when the samples had been exposed to strong acidic or basic conditions, whereas the molecular weight is practically constant at intermediate pH values. This trend strongly suggests cleavage of the HA chains at low and high pH values. The radius of gyration shows a similar trend (see the inset), which is expected since the molecular weight of HA decreases at low and high pH values. These findings are compatible with the rheological features and the DLS results presented and discussed below.

To elucidate the kinetics of degradation of HA, AFFFF measurements are conducted at various times on dilute solutions of different pH, and the time evolutions of M_w and the radius of gyration R_g are recorded and displayed in Figure 3. The results disclose that the degradation of the polymer is much faster at $\mathrm{pH} = 13$ than at $\mathrm{pH} = 1$, whereas at $\mathrm{pH} = 7$ the rupture of chains is virtually absent. As we expect,

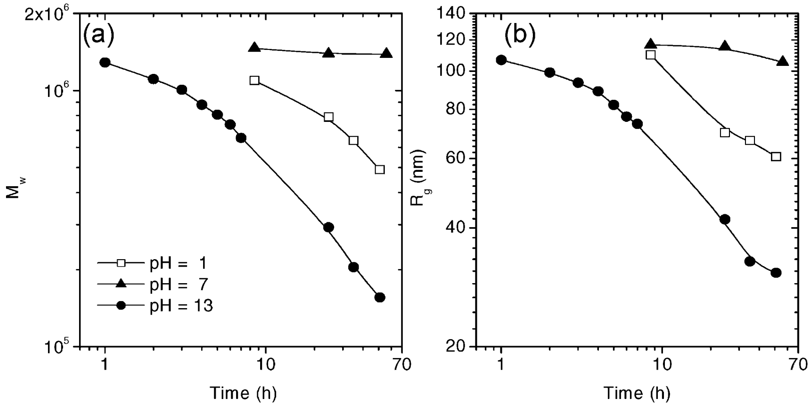

Figure 3.
Time evaluation of the degradation of dilute solutions of HA in terms of molecular weight and radius of gyration from AFFFF at the indicated values of pH.

scission of HA chains leads to a reduction of the molecular weight and the radius of gyration of the molecular species. After more than 50 h, R_g levels out. These findings clearly show that at low and high pH values the degradation of HA continues over a long time, and it is more prominent at a high value of pH.

Dynamic Light Scattering (DLS)

To characterize the effect of pH on the dynamical properties at the quiescent state, we have performed dynamic light scattering measurements on dilute and semidilute solutions at different pH values. Figure 4 shows time correlation function data (at a scattering angle of 90°) for dilute and semidilute concentrations of HA at different pH values, together with some curves fitted with the aid of Equation 1. The time correlation function is bimodal (a fast and a slow mode) at all conditions for both polymer concentrations, except at pH = 13 for the dilute solution where the correlation function can be described by a single stretched exponential. We note that

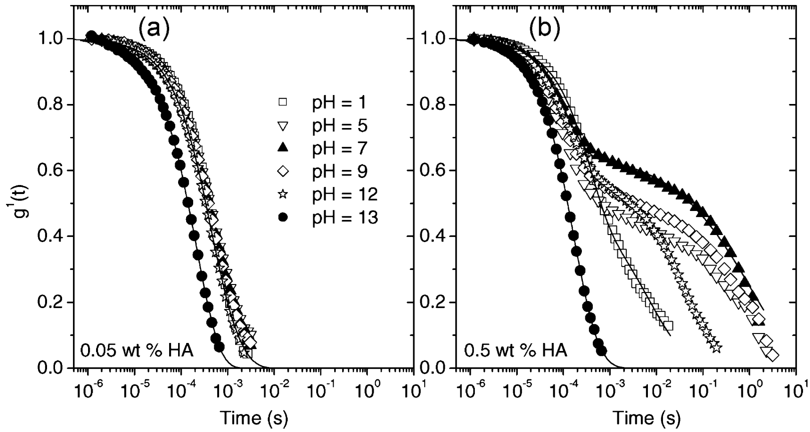

Figure 4.
Plot of the first-order electric field correlation function versus time (every second/fourth point is shown) for dilute and semidilute solutions of HA at the pH values indicated. The curves are fitted with the aid of Equation 1 (see text). The scattering angle $\theta = 90$ °.

the decay of the correlation function is fastest at pH = 13, both for the dilute and semidilute solution and this trend is consistent with the observation above, namely that the degree of degradation is strongest at this high pH. A characteristic feature of the semidilute systems at intermediate pH values is the "plateau-like" region of the correlation function visible at intermediate times. This behavior strengths the bimodal feature of the relaxation processes, and usually this type of attribute is a sign of formation of large association structures[20–23] and/or entanglements. At conditions of strong degradation of HA (pH = 1 and pH = 13), the intensity of association and entanglements of the polymer is weakened and the plateau-like region is not longer visible in the correlation function at pH = 13.

Effects of pH on the sizes of the moieties in dilute solutions and on the fast (τ_f) and the slow (τ_s) relaxation times of semidilute solutions of HA are given in Figure 5. The values of τ_f and τ_s are determined with the aid of the model relationship (Equation 1) in combination with Equation 2. In the dilute concentration regime (where both the fast and slow modes are diffusive; q^2-dependent), the fast mode represents single molecules, whereas the slow mode portrays the dynamics of non-degraded associated clusters of molecules. Via the Stokes-Einstein relationship $D = k_B T / 6\pi\eta_0 R_h$, where k_B is Boltzmann's constant, T is the absolute temperature, and η_0 is the solvent viscosity, the apparent hydrodynamic radii ($R_{h,f}$ and $R_{h,s}$), representing the fast and slow relaxation modes, can be estimated (see Figure 5a). The value of $R_{h,f}$ is almost constant, whereas $R_{h,s}$ passes through a broad maximum and the hydrodynamic radius drops strongly at pH = 13. This behavior reflects the intramolecular scission of the polymer chains at low and high pH, leading shorter chains and a decrease of the average size of the individual species. At pH = 13, the decay of the correlation function is well described by a single stretched exponential, and the value of the hydrodynamic radius falls off to a very low value, which suggests that most of the molecules are strongly degraded. In the pH range 1–13, the values of β are in the range 0.8–0.9 indicating a rather narrow distribution of relaxation times. Cleavage of glycosidic bonds in aqueous solutions of a polysaccharide has recently been reported[24] from a DLS study on alginate solutions in the presence of the photosensitizer riboflavin. Upon light irradiation of riboflavin, a photochemical degradation of alginate was observed.

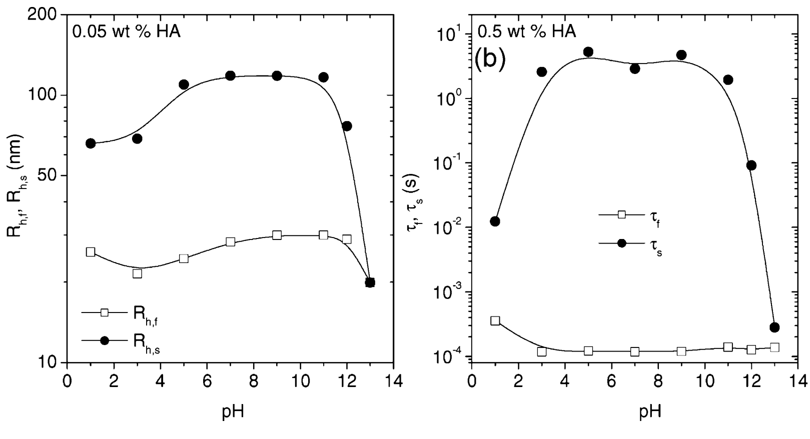

Figure 5.
Effects of pH on the apparent hydrodynamic radii for the dilute HA solutions, and on the fast (τ_f) and the slow (τ_s) relaxation times of semidilute HA solutions. The values of τ_f and τ_s have been obtained from a fitting procedure of Equation 1, together with Equation 2 (see text).

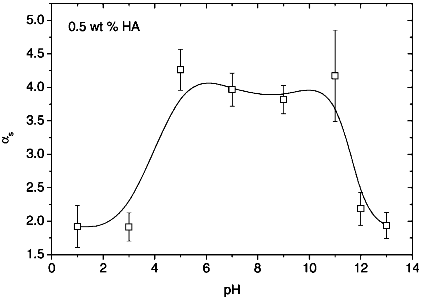

Figure 6.
Effect of pH on the power-law exponent α_s, illustrating the q dependence of the slow inverse relaxation time for HA solutions in the semidilute regime.

In the semidilute concentration regime, the fast relaxation mode is diffusive; yielding a cooperative diffusion coefficient that is only slightly pH-dependent. We may note that in the above cited DLS investigation[24] on aqueous solutions of alginate-riboflavin, no effect of photochemical degradation was found for the fast relaxation time in the semidilute concentration regime. These findings seem to indicate that the cooperative diffusion coefficient, which reflects a concerted motion of polymer chains relative to the solvent, is not very sensitive to the cutting of the polymer network. This may be related to the fact that the cooperative diffusion coefficient is independent of the molecular weight of the polymer as long as the solution is in the semidilute concentration regime.

In the case of the slow relaxation time drastic changes occur at low and high pH values (Figure 5b), and the profile of the pH dependence of τ_s is quite similar to that observed for the viscosity (cf. the discussion above). Again, the pH-induced cleavage of polymer chains lead to disintegration of the polymer network, favoring disengagement of chains and a faster relaxation process. These results support the finding that the fragmentation of the network is most marked at $pH = 13$.

A q^2-dependence is always observed for the fast mode and of the slow mode for the dilute solutions (the results are not shown here), which is the hallmark of a diffusive process. In the case of the semidilute concentration regime, the q dependence of the inverse slow relaxation time may be expressed as $\tau_s^{-1} \propto q^{\alpha}$s, and the pH dependence of α_s for 0.5 wt % solutions of HA is illustrated in Figure 6. At intermediate pH values, α_s is close to 4 and this value indicates internal motions with screened hydrodynamic interactions (Rouse-like behavior). This finding may be interpreted as arising from large clusters or the presence of large-scale "heterogeneities".[20,25,26] A number of DLS studies[12,16,22,24,27,28] on associating polymer systems have revealed that the q dependence of the slow mode is stronger than that of the fast mode. The strong angular dependence of the slow inverse relaxation time may be interpreted in the framework of the phenomenological coupling model of Ngai,[18] dealing with the problem of how the relaxation of a specific chain or cluster is slowed due to the coupling to complex surroundings. This coupling model predicts an enhanced q dependence of the slow mode for augmented coupling strength. At pH values around 1 and 13, α_s is close to 2 and this indicates a weaker coupling strength in the systems and a faster disengagement relaxation.

Conclusion

In this work, rheology, asymmetric flow field-flow fractionation (AFFFF) methods, and dynamic light scattering have been utilized to examine the effect of pH on dilute and semidilute buffered aqueous solutions of hyaluronic acid. It is clearly demonstrated that at $pH < 4$ and $pH > 11$, degradation of HA occurs. This irreversible chemical degradation of HA has been previously reported.[29] In the range $4 < pH < 11$ virtually no disruption of the HA chains takes place. In dilute solutions of HA, intramolecular degradation is observed at low and high pH, while network fragmentation is found in semidilute solutions of HA (see Figure 7). A higher degree of degradation of HA is observed at

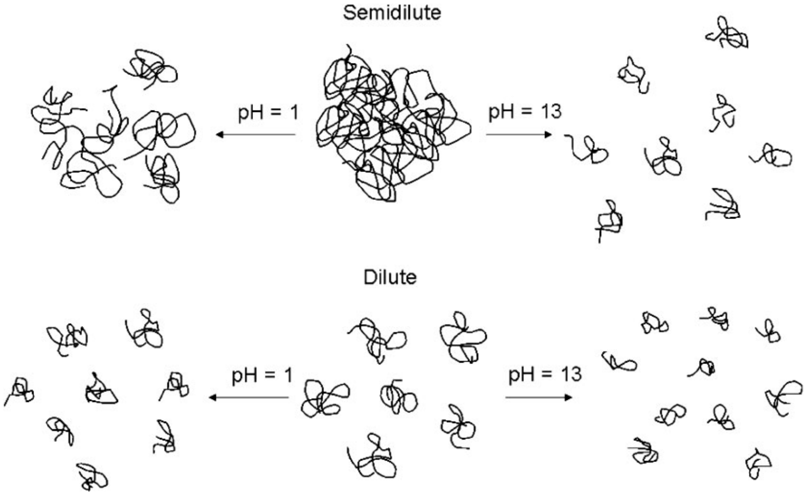

Figure 7.
Illustration of the pH-induced degradation of dilute and semidilute solutions of HA. In dilute solutions intramolecular cleavage of HA takes place, and in the semidilute concentration regime the polymer network is disintegrated. The degradation effect is more pronounced at pH = 13.

pH = 13 than at pH = 1. These effects at low and high pH can probably be ascribed to cleavage of glycosidic bonds. The pH dependences of the slow relaxation time from DLS and the shear viscosity are quite similar, and no effect of shear flow could be established.

The kinetics of the degradation process at different pH values was investigated with the aid of AFFFF on dilute solutions of HA, and the molecular weight and radius of gyration results disclosed that the scission of polymer chains at pH = 1 and pH = 13 commenced at early times and continued over a couple of days. It was shown that the degradation of HA is faster at pH = 13 than at pH = 1.

Acknowledgements: A.M. and B.N. gratefully acknowledge financial support provided by a FUNMAT Project (Novel functional polymer materials for drug delivery applications). We thank Dr. Trond Vidar Hansen for helpful comments concerning the preparation of buffers. We are grateful to Prof. Ramazani for a successful meeting and for his great hospitality in connection with our participation in the 8th International Seminar on Polymer Science and Technology at the Sharif University of Technology in Teheran 2007.

[1] T. C. Laurent, *Adv. Microcirc.* **1987**, *13*, 15.
[2] A. Maleki, A.-L. Kjøniksen, B. Nyström, *Polym. Bull.* **2007**, *59*, 217.
[3] E. A. Balazs, J. L. Denlinger, *J. Rheumatol.* **1993**, *20*, 3.
[4] E. A. Balazs, in: "*Healon (Sodium Hyaluronate). A. Guide to its Use in Ophthalmic Surgery*", D. Miller, R. Stegmann, Eds., Wiley, New York **1983**, p. 5.
[5] S. K. Hahn, S. J. Kim, M. J. kim, C. H. Chung, D. B. Moon, Y. P. Lee, *J. Controlled Release* **2005**, *104*, 323.
[6] S. K. Hahn, S. Jelacic, R. V. Maier, P. S. Stayton, A. S. Hoffman, *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 1111.
[7] S. K. Hahn, E. J. Oh, H. Miyamoto, T. Shimobouji, *Int. J. Pharm.* **2006**, *322*, 44.
[8] K. Motokawa, S. K. Hahn, T. Nakamura, H. Miyamoto, T. Shimobouji, *J. Biomed. Mater. Res.* **2006**, *78A*, 459.
[9] R. Ohri, S. K. Hahn, P. S. Stayton, A. S. Hoffman, M. Giachelli, *J. Biomed. Mater. Res.* **2004**, *70A*, 159.
[10] E. Gura, M. Hückel, P. J. Müller, *Polymer Degradation and Stability* **1998**, *59*, 297.
[11] J. Gatej, M. Popa, M. Rinaudo, *Biomacromolecules* **2005**, *6*, 61.
[12] A. Maleki, A.-L. Kjøniksen, B. Nyström, *Carbohydr. Res.* **2007**, *342*, 2776.
[13] A. J. F. Siegert, Massachusetts Institute of Technology Rad. Lab. Rep. No. 456, **1943**.

[14] J. E. Martin, J. Wilcoxon, J. Odinek, *Phys. Rev. A* **1991**, *43*, 858.

[15] P. Lang, W. Burchard, *Macromolecules* **1991**, *24*, 814.

[16] B. Nyström, B. Lindman, *Macromolecules* **1995**, *28*, 967.

[17] A. Maleki, A.-L. Kjøniksen, K. D. Knudsen, B. Nyström, *Polym. Int.* **2006**, *55*, 365.

[18] K. L. Ngai, *Adv. Colloid Interface Sci.* **1996**, *64*, 1.

[19] S. Ghosh, I. Kobal, D. Zanette, W. F. Reed, *Macromolecules* **1993**, *26*, 4685.

[20] W. Richtering, R. Löfler, W. Burchard, *Macromolecules* **1992**, *25*, 3642.

[21] M. Richtering, W. Gleim, W. Burchard, *Macromolecules* **1992**, *25*, 3795.

[22] B. Nyström, K. Thuresson, B. Lindman, *Langmuir* **1995**, *11*, 1994.

[23] N. Beheshti, G. T. M. Nguyen, A.-L. Kjøniksen, K. D. Knudsen, B. Nyström, *Colloids and Surfaces A* **2006**, *279*, 40.

[24] A.-L. Kjøniksen, S. G. Baldursdóttir, B. Nyström, *Macromol. Biosci.* **2004**, *4*, 76.

[25] J. T. Koberstein, C. Picot, H. Benoit, *Polymer* **1985**, *28*, 673.

[26] J. Bodycomb, M. Hara, *Macromolecules* **1995**, *28*, 8190.

[27] B. Nyström, H. Walderhaug, F. K. Hansen, *J. Phys. Chem.* **1993**, *97*, 7743.

[28] S. Z. Ren, C. M. Sorensen, *Phys. Rev. Lett.* **1993**, *70*, 1727.

[29] Y. Tokita, A. Okamoto, *Polymer Degradation and Stability* **1995**, *48*, 269.