Biomacromolecules **2003,** *4,* 1805−1810

Evaluation of Radius of Gyration and Intrinsic Viscosity Molar Mass Dependence and Stiffness of Hyaluronan

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Received July 1, 2003; Revised Manuscript Received September 5, 2003

Nine hyaluronan (HA) samples were fractionated by size-exclusion chromatography, and molar mass (M), radius of gyration (R_g), and intrinsic viscosity ($[\eta]$) were measured in 0.15 M NaCl at 37 °C by on-line multiangle light scattering and viscometer detectors. Using such method, we investigated the R_g and $[\eta]$ molar mass dependence for HA over a very wide range of molar masses: M ranging from 4×10^4 to 5.5×10^6 g/mol. The R_g and the $[\eta]$ molar mass dependence found for HA showed a meaningful difference. The $R_g = f(M)$ power law was substantially linear in the whole range of molar masses explored with a constant slope of 0.6. In contrast, the $[\eta] = f(M)$ power law (Mark−Houwink−Sakurada plot) showed a marked curve shape, and a linear regression over the whole range of molar masses does not make sense. Also the persistence length (stiffness) for HA was estimated. The persistence length derived by using both the Odijk's model (7.5 nm from R_g vs M data) and the Bohdanecky's plot (6.8 nm from $[\eta]$ vs M data) were quite similar. These persistence length values are congruent with a semistiff conformation of HA macromolecules.

Introduction

A fundamental aspect in understanding the physical properties of a polymer involves determination of the dimension of the macromolecules in solution as a function of the molar mass. In such contest, an accurate determination of the root-mean-square radius, hereafter denoted in short as radius of gyration (R_g), vs molar mass power law and of the intrinsic viscosity ($[\eta]$) vs molar mass power law, generally known as Mark−Houwink−Sakurada (MHS) plot, are of fundamental importance. Theory of the $R_g = f(M)$ and $[\eta] = f(M)$ scaling functions assumes homogeneous monodisperse fractions in molar mass and dimension of the macromolecules. Hence, a preliminary step in such a study is the fractionation of the starting polydisperse polymer. Off-line fractionation of broad molar mass distribution (MMD) polymers is time-consuming and tedious, and more important, the final results are often not adequate, that is, the MMD of the final fractions is again relatively broad. Alternatively, the $R_g = f(M)$ and $[\eta] = f(M)$ power laws are determined using broad MMD polymers. Eventually, some authors used theoretical corrections for the polydispersity of the samples.[1] In general, however, the influence of the polydispersity of the samples on the final results is meaningful, and the validity of the theoretical corrections is often doubtful. Consequently, an accurate fractionation of the broad MMD samples should produce more accurate results.

The on-line fractionation of broad MMD samples by size-exclusion chromatography (SEC) is rapid and efficient and requires a small amount of the polymer. Recently, the use of molar mass and viscosity sensitive detectors coupled on-line to a SEC system has become relatively common in many laboratories.[2] Virtually, a multiangle laser light scattering (MALS) photometer, along with a viscometer, provides directly M_i, R_{gi}, and $[\eta]_i$ of each eluting fraction. Assuming ideal SEC fractionation, each fraction eluted from the chromatographic column could be considered homogeneous (monomolecular) in molar mass, and at least in principle, the $R_g = f(M)$ and $[\eta] = f(M)$ power laws can be obtained from one single broad MMD sample during one single SEC run.

Hyaluronan, which is the sodium salt of hyaluronic acid, is a linear biopolymer. HA is a regularly alternated polysaccharide composed of *N*-acetylglucosamine and D-glucuronic acid. HA, in origin either extractive or bacterial, is water-soluble, and in aqueous solutions, it is a negatively charged polyelectrolyte. The MMD of the native HA samples is relatively broad; the molar mass ranges from medium to high up to ultrahigh. There is large industrial interest for HA as attested by the extensive use in medicine, cosmetics, etc.[3] Furthermore, there is a large scientific interest for the HA properties in solution. In the past, many authors have investigated R_g and $[\eta]$ of HA as a function of the molar mass.[4−10] Several studies concerned also the HA macromolecule conformation in aqueous solution, and in particular, the stiffness of the macromolecules (persistence length).[11−20] Really, the study of R_g and $[\eta]$ as a function of the molar mass, ionic strength, etc. is a main concern for HA. To the best of our knowledge, almost all of the previous studies on the size and the conformation of HA macromolecules, with very few exceptions,[8] used off-line methods and unfractionated samples having broad MMD. In contrast, our goal

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10.1021/bm0342178 CCC: $25.00

presented here was to study R_g and $[\eta]$ molar mass dependence, together with the persistence length of HA, by exploiting simultaneously the SEC fractionating and MALS and viscosity on-line detecting capabilities.

Experimental Section

Materials. The study was performed using nine HA samples with the weight-average molar mass, M_w, ranging from 9.1×10^4 to 3.5×10^6 g/mol (Table 1). Sample sources were the following: HA_01 and HA_07 from Genzyme Corporation (Cambridge, MA); HA_02 and HA_03 from Pentapharm AG (Basel, Switzerland); HA_04 from CPN LTD (Ústí nad Orlici, Czech Republic); HA_05, HA_08, and HA_09 from Pharmacia & Upjohn (Nerviano, Milan, Italy); HA_06 from Lifecore Biomedical Inc. (Chaska, MN). Each HA sample contained typically less than 0.2% of proteins. Bovine serum albumin (BSA) was obtained from Sigma (Milan, Italy). Water was MilliQ grade (Millipore, Bedford, MA). All other chemicals were of analytical grade.

Chromatographic System. The study was performed with a multidetector SEC chromatographic system using three on-line detectors: a homemade single capillary viscometer (SCV), a MALS Dawn DSP-F photometer from Wyatt (Santa Barbara, CA), and a UV 996 from Waters (Milford, MA) as a concentration detector. This multidetector SEC system was described in detail previously.[2,21] The experimental conditions consisted of 0.15 M NaCl as mobile phase at 37 °C, 0.2 mL/min flow rate, and 200 μL injection volume. The column set was composed of two TSKgel PW (G6000 and G5000, 17 μm particle size) from TosoHaas (Montgomeryville, PA).

Light Scattering. The MALS photometer uses a vertically polarized He−Ne laser ($\lambda = 632.8$ nm) and simultaneously measures the intensity of the scattered light at 15 angular locations ranging in aqueous solvent from 14.5° to 158.3°. The calibration constant was calculated using toluene as standard assuming a Rayleigh factor of 1.406×10^{-5} cm^{-1}. The normalization of the photodiodes was performed by measuring the scattering intensity of BSA, a globular protein, assumed to act as an isotropic scatterer. Details of the used MALS photometer were described elsewhere.[22]

Viscometry. The original homemade SCV detector used was described in detail elsewhere.[2,21] The dimensions of the capillary tube were 0.02 in. of internal diameter and 20 in. of length. Because the signal of the on-line viscometer depends on the intrinsic viscosity ($[\eta]$) and on the concentration of the polymeric solution, to obtain constant signal-to-noise ratio, the concentration, c, of the HA samples was adjusted so that $[\eta]c = 0.1$.

Results

Table 1 summarizes more important data of the nine HA samples investigated. Some complementary data, together the experimental protocol, on the same HA samples determined by analogous methods were already published.[18,23,24] As evident from the data listed in Table 1, the MMD of the HA samples was relatively broad with the polydispersity index, $D = M_w/M_n$, ranging from 1.6 to 2.2 (where M_n denotes the numeric-average molar mass).

Table 1. Summary of More Relevant Data for Nine HA Samples

sample	M_w (kg/mol)	D	R_g (nm)	$[\eta]$ (mL/g)
HA_01	91.0	1.8	28.3	250.1
HA_02	236.0	2.2	55.0	526.0
HA_03	580.0	1.8	89.0	1060.9
HA_04	665.0	2.1	98.0	1173.2
HA_05	1060	1.8	126.1	1660.4
HA_06	1210	1.6	120.4	1796.8
HA_07	1400	1.7	132.9	1962.0
HA_08	1650	1.8	163.3	2165.1
HA_09	3500	1.6	257.7	3402.2

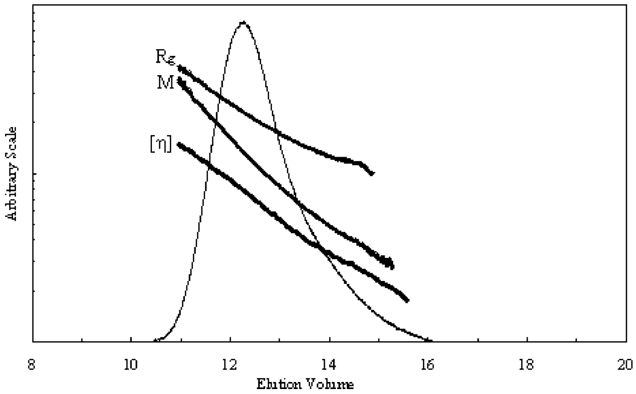

Figure 1. UV detector signal of the HA_05 sample and molar mass, radius of gyration, and intrinsic viscosity vs elution volume experimental functions.

Figure 1 shows, as a typical example, the normalized chromatogram obtained by using the UV detector (λ set at 206 nm) on analyzing the HA_05 sample. Figure 1 also shows three experimental functions: $M = f(V)$ and $R_g = f(V)$, obtained from MALS, and $[\eta] = f(V)$, obtained from SCV, where V denotes the elution volume. The $\log(M) = f(V)$ function represents the "classical SEC calibration curve" obtained, in this case, by running one single polymer sample having broad MMD and by combining the output signals of the two detectors applied, namely, UV and MALS.

The three-detector system (MALS−SCV−UV) exploited allows us to determine directly the two investigated relationships, namely, the $R_g = f(M)$ and $[\eta] = f(M)$ power law functions (shown, respectively, in Figures 2 and 3. Both plots, in double logarithmic scale, indicated relatively straight lines. Thus the parameters (intercept, slope) of both lines were calculated. Respectively, R_g was expressed in nanometers and $[\eta]$ in milliliters per gram.

$$R_g = 2.53 \times 10^{-2}(M^{0.601})$$

$$[\eta] = 3.21 \times 10^{-2}(M^{0.783})$$

It should be pointed however here, that the parameters calculated were determined simply by SEC analysis of the sample HA_05 alone. Analogously, by analyzing the all nine HA samples, one can generate nine pairs of relationships analogous to those described above. However, another and more meaningful analysis could be done by gathering together all nine sets of R_g vs M data. As evident from Figure 4, the superposition of all nine individual R_g vs M dependencies resulted in one single relationship valid for all nine analyzed samples. The $R_g = f(M)$ power law function in

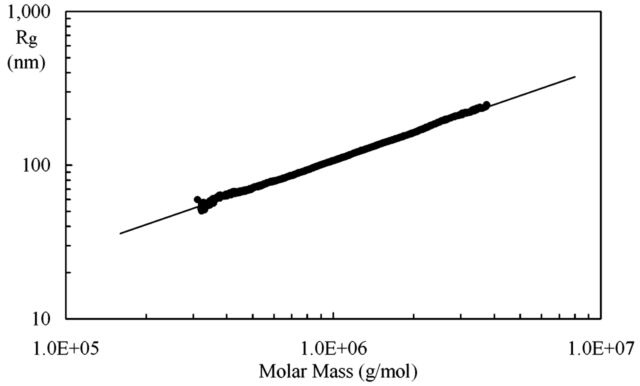

Figure 2. $R_g = f(M)$ power law for the HA_05 sample from on-line MALS detector, M_w = 1060 kg/mol.

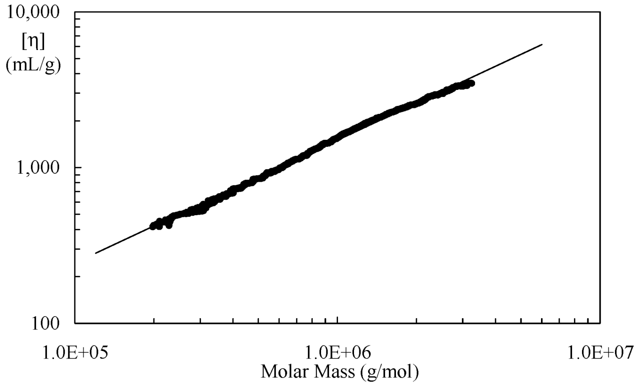

Figure 3. $[\eta] = f(M)$ power law for the HA_05 sample from the on-line viscometer, M_w = 1060 kg/mol.

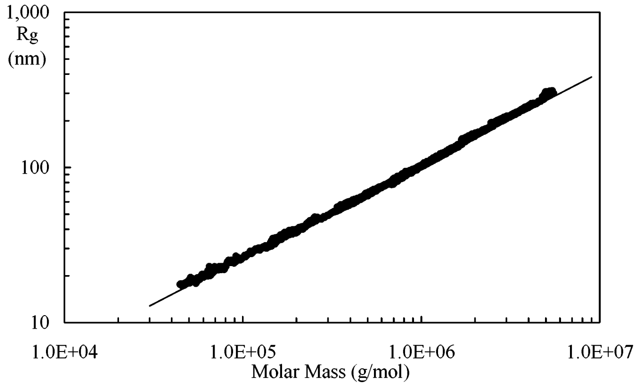

Figure 4. $R_g = f(M)$ power law constructed by gathering experimental data of the nine HA samples. Experimental data (●) and linear regression (—) over the whole molar mass range.

Figure 4 can be well described by one single equation:

$$R_g = 2.75 \times 10^{-2}(M^{0.596})$$

The $R_g = f(M)$ power law dependence estimated is a nice straight line approximately ranging in molar mass between 40 and 5500 kg/mol and in radius of gyration between 15 and 290 nm. It should be pointed out here that, as expected, the power law function $R_g = f(M)$ obtained by analyzing one single sample (HA_05) and that function generated by superpositioning all nine HA samples have a near identical slopes (∼0.6) and closely identical intercepts.

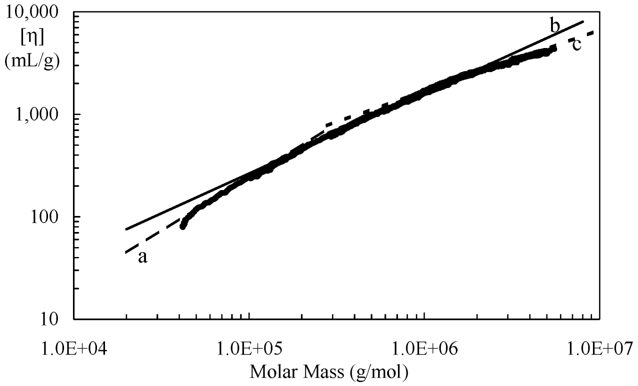

Figure 5. MHS plot constructed by gathering data of nine HA samples: (line a, – – –) M lower than 1 × 10^5 g/mol; (line b, —) linear regression over the molar mass range from 1 × 10^5 to 1 × 10^6 g/mol; (line c, ···) M higher than 1 × 10^6 g/mol.

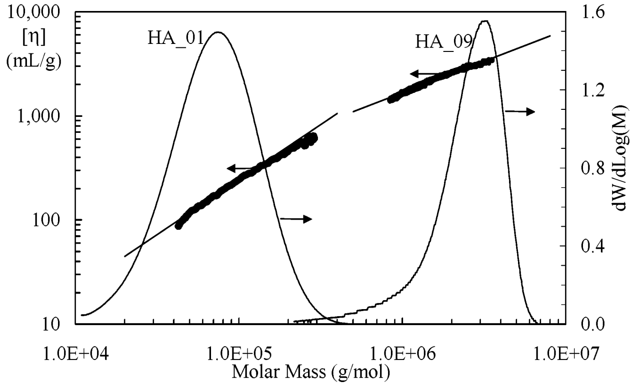

Figure 6. Differential MMD of the lower molar mass sample (HA_01) and higher molar mass sample (HA_09), along with the respective $[\eta] = f(M)$ power laws.

Figure 5 represents the results of the analogous procedure performed by superpositioning the nine individual $[\eta]$ vs M dependencies obtained by SEC analysis of the nine HA samples. In this plot, $[\eta]$ ranged approximately from 80 to 4330 mL/g. As evident, the relationship $[\eta]$ vs M indicated a marked nonlinearity. Consequently, a linear regression over the whole range of the $[\eta]-M$ data is evidently not acceptable. However, only for comparison with the MHS linear coefficients for HA published previously in the literature the whole range of the $[\eta]$ vs M data was divided into three subregions, namely, (a) $M < 10^5$ g/mol; (b) $10^5 < M < 10^6$ g/mol; (c) $M > 10^6$ g/mol. By such a way, three particular $[\eta] = f(M)$ power law functions were calculated:

$$[\eta] = 1.29 \times 10^{-3}(M^{1.056}) \qquad \{M < 10^5 \text{ g/mol}\} \qquad \text{(a)}$$

$$[\eta] = 3.39 \times 10^{-2}(M^{0.778}) \qquad \{10^5 < M < 10^6 \text{ g/mol}\} \qquad \text{(b)}$$

$$[\eta] = 3.95 \times 10^{-1}(M^{0.604}) \qquad \{M > 10^6 \text{ g/mol}\} \qquad \text{(c)}$$

Figure 5 shows the previous three MHS equations for HA. Figure 6 is just another presentation of the procedure described above. As evident, the two individual HA samples, namely, HA_01 and HA_09, that is, those having the lowest and the highest molar mass, resulted in two particular, not identical, $[\eta] = f(M)$ power law functions. However, the power law functions $R_g = f(M)$ for these two samples are identical (not shown).

Discussion

The on-line SEC−MALS−SCV system is a powerful tool to estimate the molecular parameters of the HA macromolecules. Really, this system allows the estimation of the $R_g = f(M)$ and $[\eta] = f(M)$ power laws from homogeneous or at least very narrow HA fractions. Obviously by “fraction”, we mean each slice eluted from the SEC column. The scaling functions, $R_g = f(M)$ and $[\eta] = f(M)$, are very important because they furnish relevant information on the stiffness of the macromolecules. HA stiffness in aqueous solution has been studied in the course of the years by many authors. In general, the hypothesis of semistiff conformation for HA found large consensus. Many MHS linear coefficients could be found in the literature,[7,10] as well as $R_g = f(M)$ coefficients and persistence length values.[11−20] Although not all of the published values for HA seem to converge, the previous assertion on the HA stiffness is accepted from the majority of authors. Usually $R_g = f(M)$ and MHS coefficients and persistence length values for HA have been estimated using several HA samples with different molar mass. Unfortunately, all of the HA samples present a relatively broad MMD. HA polydispersity influences to some extent the recovered value of the coefficients of the power laws and of the persistence length. Hence, our results obtained after SEC fractionation over a very wide range of molar mass is particularly meaningful.

$R_g = f(M)$ Power Law. The slope of the $R_g = f(M)$ power law calculated with a linear regression over the whole set of R_g-M data of the nine HA samples was 0.6. More in detail, the slopes of the individual $R_g = f(M)$ power laws of the nine HA samples were quasi-constant ranging from 0.592 to 0.604. The experimental data of the $R_g = f(M)$ plot approximately cover a R_g range from 15 to 290 nm. In this explored range, the $R_g = f(M)$ power law was substantially linear. However, the theoretical $R_g = f(M)$ power law calculated, for example, with the Odijk’s model, described in detail in the following text, predicts a curvature of the plot in the lower R_g range, but our experimental data does not cover this low R_g interval. Our finding on the slope of the $R_g = f(M)$ power law is a little higher than the value (0.57) obtained from Fouissac[14] in quasi-similar conditions (0.1 M NH_4NO_3). In addition, in a previous study[18] using unfractionated HA samples with broad MMD, we found a lower slope value (0.57). Evidently the polydispersity of the broad HA samples used in those studies thus influenced the recovered slope result.

$[\eta] = f(M)$ Power Law. Very interesting is the marked curvature of the MHS plot for HA. Although the curvature of the MHS plot is not an original result but is the normal behavior of a wormlike chain, the interest arises from the experimental evidence of this quite unusual behavior. We have anticipated in the Results section that we have divided the molar mass range into three subregions only for comparison with MHS coefficients for HA published previously in the literature. Evidently, this procedure is an artifice because the MHS plot for HA is not divided into three linear subregions but shows a continuous curvature. However, many MHS linear coefficients were published for HA in the course of the years, and a comparison in the corresponding molar mass range could be interesting. In brief, our findings are (1) $a \approx 0.78$ with M ranging from 100 to 1000 kg/mol, (2) $a \approx 0.6$ with M higher than 1000 kg/mol, and (3) $a \approx 1.06$ with M lower than 100 kg/mol. Different values of the slope of the MHS plot on the basis of different ranges of molar mass is not new for HA. Bothner[7] provided two sets of MHS coefficients. The first one was valid for M lower than 10^6 g/mol ($a = 0.779$); second one was valid for M higher than 10^6 g/mol ($a = 0.601$). Hence, also Bothner’s experimental results reflected a different slope of the MHS plot for HA in different ranges of molar mass. Furthermore, other authors[6,11] reported values of the slope of the MHS plot approximately 1 for HA with M lower than 10^5 g/mol. In particular, Cowman and Matsuoka[9] predict $a = 1.16$ for HA with M lower than 37.5 kg/mol. The nonlinearity of the MHS plot reflects the conformational change of the HA chain on basis of the chain length. Cleland[5] explained the conformational change of HA in terms of wormlike chain or non-Gaussian behavior of short chains at lower molar mass and flexible chains or Gaussian behavior of long chains at higher molar mass. Cowman and Matsuoka[9] assert that short HA chains act like free-draining chains and long HA chains act like non-free-draining coils. These considerations explain quite well the strong changes of the slope of the MHS plot for HA when a wide range of molar masses is explored.

Considering the coefficients of the central part of the MHS plot, $100 < M < 1000$ kg/mol, which are more meaningful from a practical point of view, we point out that they are in agreement with part of the published values for HA in similar conditions. Many MHS coefficients for HA have been published in the course of the years. Unfortunately, part of the published MHS coefficients disagree. Several differences could explain these discrepancies: (i) origin of the HA samples (extractive from varied source or fermentative); (ii) experimental conditions (shear rate, ionic strength, pH, temperature); (iii) molar mass range. In addition, $[\eta]$ measure is relatively complex in consequence of the non-Newtonian behavior of HA dilute solutions when the molar mass is high. However, despite the relative meaning of the linear analysis of the $[\eta]$ vs M data, we point out that our MHS coefficients are in quite good agreement with several previously published results.[4,7,8] Eventually, there is only a little discrepancy in the intercept k that could be explained with the higher temperature 37 °C used in our study against 25 °C of the Bothner study.[7]

An interesting point arises from the difference between R_g and $[\eta]$ molar dependence. Really, it is not immediate to understand why only the $[\eta]$ power law results in a nonlinear dependence. It is well-known that the R_g data are the results of equilibrium measurements while for $[\eta]$ data this is not the case. In addition, the “partial draining” nature of charged chains changes with their length, that is, their hydrodynamic volume changes with M. As a consequence, it is interesting to note that the so-called “universal constant” Φ, which relates $[\eta]$ and the dimension of the macromolecule, is not a constant.

Persistence Length. Using the molar mass dependence of the radius of gyration and of the intrinsic viscosity, one can estimate the stiffness of the HA chain via the persistence length, q. In the literature, q values recovered from R_g data or in alternative from $[\eta]$ data do not agree. Several different q values ranging from 2 to 20 nm were published.[11,15] Cleland[5,11] found $q = 4-5$ nm for HA in aqueous NaCl. A quite similar value ($q = 4.1-4.2$ nm) was found from

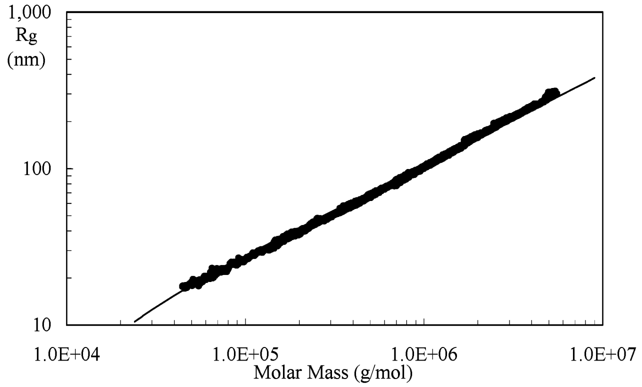

Figure 7. Comparison of the experimental R_g data (●) and of the theoretical $R_g = f(M)$ power law (—) calculated with the Odijk's model and a persistence length $q = 7.5$ nm.

Hayashi.[17] In contrast to Cleland's and Hayashi's results, Rinaudo[14,19] found a much larger q value of 7−8 nm. This large discrepancy in q value for HA is surprising, and the present results, obtained with SEC fractionated HA samples, will be useful in deepening this intriguing topic.

A convenient method to estimate q or the analogous parameter λ^{-1}, which is the Kuhn's statistical segment, $\lambda^{-1} = 2q$, from the molar mass dependence of R_g is the Odijk's model.[25] Such a model describes the dimensions of polyelectrolytes in solution as a function of the chain length and of the ionic strength. In the Odijk's model, the total persistence length, q_T, of wormlike polyelectrolyte chains is the sum of two contributions: $q_T = q_0 + q_e$. The first one, q_0, is the intrinsic persistence length corresponding to an equivalent neutral chain in which all of the electrostatic interactions are screened out. The second one, q_e, is the electrostatic contribution to the total persistence length due to the electrostatic short-range interactions, which depend on the ionic strength.

The unperturbed radius of gyration, $(R_g)_\theta$, corresponding to an infinite ionic strength, of a wormlike chain depends on two parameters: q_T (in θ-condition, $q_e = 0$, $q_T = q_0$) and the chain contour length, $L = M/M_L$, where M_L is the molar mass per unit of contour length or shift factor as defined by Yamakawa.[26] The relation among $(R_g)_\theta$, q_T, and L was found by Benoit and Doty.[27]

$$(R_g)_\theta{}^2 = (q_T L/3) - q_T{}^2 + (2q_T{}^3/L) - 2(q_T{}^4/L^2)[1 - \exp(-L/q_T)] \quad (1)$$

The effective dimensions of the HA macromolecules in the used ionic strength depend also on the long-range interactions, that is, the excluded volume. Consequently, R_g is given by

$$R_g = (R_g)_\theta \alpha_{S,T} \quad (2)$$

where $\alpha_{S,T}$ is the total expansion factor. Following Odijk, in the case of polyelectrolytes with large contour length ($L \gg q_T$), we have assumed that all of the long-range interactions are of electrostatic type. Consequently, $\alpha_{S,T}$ can be replaced with a good approximation with the electrostatic expansion factor, $\alpha_{S,El}$. It is interesting to note that in the Odijk's model the presence of ionic sites along the macromolecular chain influences the dimension of the macromolecules via two contributions: the electrostatic persistence length, q_e, and electrostatic expansion factor, $\alpha_{S,El}$.

The electrostatic persistence length, q_e, substantially depends on the ionic strength of the solvent. A method to calculate q_e for a specific ionic strength is reported in the Fouissac article.[14] Specifically, q_e for HA in 0.15 M NaCl is relatively low, 0.11 nm. For $\alpha_{S,El}$, Odijk adopted the Yamakawa−Tanaka[26] approximation, and the electrostatic expansion factor is given by

$$\alpha_{S,El} = 0.541 + 0.549(1 + 6.04 z_{El})^{0.46} \quad (3)$$

$$z_{El} = \left(\frac{3^{3/2}}{4\pi^{1/2}}\right) L^{1/2} \kappa^{-1} q_T{}^{-3/2} \quad (4)$$

$$\beta_{El} = 8\pi\kappa^{-1} q_T{}^2 \quad (5)$$

where z_{El} is the electrostatic excluded volume parameter, β_{El} is the electrostatic excluded volume between two rods of length λ^{-1}, and κ^{-1} is the Debye radius.

Using eqs 1−5, assuming[15] $M_L = 410$ nm^{-1}, we calculated the theoretical R_g molar mass dependence. The best superimposition between the experimental and the theoretical R_g vs M data was obtained with an intrinsic persistence length q_0 of 7.5 nm. Figure 7 shows the comparison of the experimental and theoretical R_g molar mass dependence for HA in 0.15 M NaCl calculated with the Odijk's model and $q_0 = 7.5$ nm.

Alternatively, the persistence length q may be derived using the molar mass dependence of the intrinsic viscosity and the Bohdanecky's procedure.[28] Bohdanecky showed that the Yamakawa−Fujii theory[26] for $[\eta]$ of wormlike macromolecules can be approximated with the following equation:

$$\left(\frac{M^2}{[\eta]_0}\right)^{1/3} = A_\eta + B_\eta M^{1/2} \quad (6)$$

A_η and B_η parameters can be easily estimated by a linear regression over the whole set of experimental data. A_η and B_η parameters are related to M_L and λ^{-1} by the following equations:

$$A_\eta = \Phi_{0,\infty}{}^{-1/3} A_0 M_L \quad (7)$$

$$B_\eta = \Phi_{0,\infty}{}^{-1/3} B_0 \left(\frac{\lambda^{-1}}{M_L}\right)^{-1/2} \quad (8)$$

where $\Phi_{0,\infty} = 2.86 \times 10^{-23}$ mol^{-1} is the limiting value of the Flory's constant Φ for nondraining coils in the limit of infinite molar mass. Both A_0 and B_0 are known functions of the reduced hydrodynamic diameter, $d_r = d/\lambda^{-1}$, and are tabulated in the Bohdanecky's article.[28] In essence, $[\eta]$ depends on three parameters: q, M_L, and the hydrodynamic diameter, d. In the estimation of these three parameters, we need an additional relation. Bohdanecky proposed the use of the following equation:

$$d = (4\nu M_L/(\pi N_A))^{1/2} \quad (9)$$

where ν is partial specific volume at infinite dilution ($\nu = 0.57$ cm^3/g for HA in NaCl salt[29]) and N_A is the Avogadro's

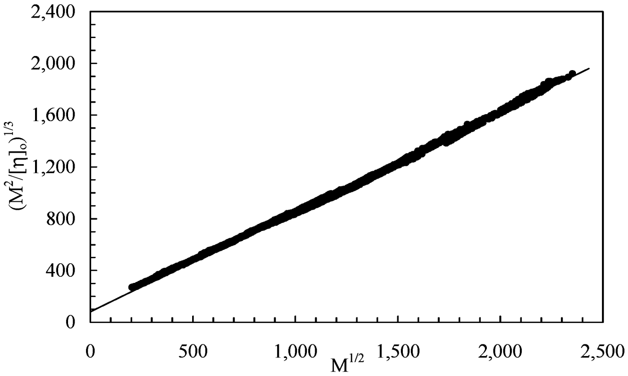

Figure 8. Bohdanecky's plot: $(M^2/[\eta]_0)^{1/3}$ vs $M^{1/2}$: experimental data (●) and linear regression (—) over the whole set of experimental data (nine HA samples).

number. Furthermore, in calculating the unperturbed $[\eta]_0$ values, one needs the expansion factor for the intrinsic viscosity, α_η. The expansion factor for the intrinsic viscosity α_η is defined in eq 10 [30] and is a complex function of the expansion factor for the radius of gyration, α_S. Weill and Cloizeaux[31] derived a semiempirical relation, eq 11, between α_η and α_S.

$$[\eta] = [\eta]_0 \alpha_\eta^{\ 3} \tag{10}$$

$$\alpha_\eta^{\ 3} = \alpha_S^{\ 2.43} \tag{11}$$

Figure 8 shows the Bohdanecky's plot of our experimental data. Very interestingly, the Bohdanecky's plot is linear over the whole set of HA experimental data, and the estimation of the intercept A_η and of the slope B_η was relatively simple. Using the A_η and B_η values and the eqs 6−11, we have obtained the following results: $q = 6.8$ nm, $d = 0.8$ nm, and $M_L = 480$ nm^{-1}. We point out that a hydrodynamic diameter $d = 0.8-1$ nm is a well-accepted value for HA. On the contrary, $M_L = 480$ nm^{-1} is a little bit higher of the accepted value (400−410 nm^{-1}). On the whole, the Bohdanecky's procedure applied to our $[\eta]-M$ experimental data has furnished congruent results. In addition, the agreement between the persistence length value recovered with the Odijk's model (7.5 nm from R_g), and the Bohdanecky procedure (6.8 nm from $[\eta]$) is quite good considering that the evaluation of the stiffness of a macromolecular chain through the persistence length is a very delicate task.

Conclusions

We have found that the $R_g = f(M)$ power law for HA is linear over the whole range of molar masses explored with a quite constant slope value of 0.6. In contrast, the $[\eta] = f(M)$ power law for HA shows a marked curvature, and a single linear regression over the whole range of molar masses explored does not make sense. It is well-known that such curvature is the normal behavior of wormlike chains. Only for comparison with previous published MHS coefficients for HA, we have also estimated three different sets of coefficients of the MHS plot. The slope of the MHS plot was approximately 1.06 for M lower than 100 kg/mol, 0.78 for M ranging from 100 to1000 kg/mol, and 0.6 for M higher than 1000 kg/mol. We have also found that the persistence length for HA was 7.5 nm using the Odijk's model and the R_g vs M data and 6.8 nm using the Bohdanecky's plot and the $[\eta]$ vs M data. We consider our findings particularly meaningful because they were obtained using SEC fractionated HA samples (theoretically monodisperse fractions) and absolute, MALS, and SCV on-line detectors. Our results are congruent with a semistiff conformation for HA.

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BM0342178