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Shear-Rate Dependence of the Intrinsic Viscosity of Sodium Hyaluronate in 0.2 M Sodium Chloride Solution

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The dependence of the intrinsic viscosities ([η]) of seven well-characterized sodium hyaluronate (HA) samples in 0.2 M NaCl solution on shear rates ($\dot{\gamma}$) was investigated using four kinds of viscometers with $\dot{\gamma}$ values ranging from 0.1 to 2000 s^{-1}. Molecular weight distributions of these samples were checked by a gel permeation chromatograph connected to a low-angle laser light scattering photometer. The determination of [η] at zero shear rate ($[\eta]_0$) for high molecular weight HA had to be made using low shear viscometers with $\dot{\gamma} < 250$ s^{-1}, since [η] showed remarkable shear thinning behavior with increasing molecular weight. Double logarithmic plots of $[\eta]_0$ *vs.* M_w (the weight-average molecular weight) for HA in 0.2 M NaCl solution gave a relation expressed by $[\eta]_0 = 1.99 \times 10^{-4} \cdot M_w^{0.829}$ for $M_w \geqq 40 \times 10^4$, where $[\eta]_0$ was written in dl/g unit. The shear thinning behavior of [η] of HA in the solvent was analyzed by the Rouse–Zimm bead-spring model proposed by Fixman. Consequently, the $\dot{\gamma}$-dependence of HA in 0.2 M NaCl solution could be well described by this model of large viscosity expansion factors, and suggested that an HA chain in the solvent is a fairly expanded random coil by excluded volume effects.

Keywords shear-rate dependence; intrinsic viscosity; sodium hyaluronate; bead-spring model; random coil; excluded volume effect

Sodium hyaluronate (HA) is a major component of biopolymers found in cartilage, eye vitreous humor, and synovial fluid, and plays a very important role owing to its viscoelasticity.[1-6] Even its dilute solution exhibits non-Newtonian flow, that is, shear thinning behavior.[7-9] In fact, Cleland *et al.*[8] reported that the intrinsic viscosity ([η]) of HA in 0.2 M NaCl solution is dependent on shear rates ($\dot{\gamma}$).

The shear thinning behavior of dilute polymer solutions can be qualitatively explained by two typical models: the non-deformable highly elongated ellipsoid model[10,11] and the Rouse–Zimm bead-spring model[12-17] deformable under high shear rates. With respect to the bead-spring model, many attempts to modify the model have been made to explain the non-Newtonian behavior of [η] by adopting some assumptions. The assumptions are, for example, the concept of imperfect flexibility[13] of polymer coils, the idea of anisotropic hydrodynamic interactions,[14,15] and the excluded volume effect.[16,17] Among them, Fixman's theory[12] is the most successful and agrees remarkably well with observations of flexible polymers.[18,19]

In this paper, we investigate [η] of HA in 0.2 M NaCl solution under a wide range of $\dot{\gamma}$ containing ultra-low shear rate regions using a series of HA samples with different molecular weights, and we evaluated whether the Fixman's bead-spring model sufficiently describes the non-Newtonian behavior of [η] of HA in 0.2 M NaCl solution.

Experimental

Materials and Molecular Weight Determination Six HA samples prepared for another purpose,[2] C-04, P-0203, C-17b, C-17f, C-17g, and C-17i, and one pharmaceutical HA sample, Healon (Kabi Pharmacia), were investigated in this study. Each sample, except Healon, contained HA of >99.9%, protein of <0.1%, trace ashes, but no other mucopolysaccharides. The determination of the number- (M_n) and the weight-average molecular weights (M_w) of these samples were carried out in a Tosoh HLC-802UR high performance GPC (gel permeation chromatograph) connected in a series to a Tosoh LALLS (low-angle laser light scattering) photometer with a flow cell. An HA sample was so dissolved in 0.2 M NaCl solution that the product of the polymer concentration (c) and an estimated molecular weight gave a value of about 3×10^3 g/ml, and it was then subjected to GPC-LALLS measurements. Scattered intensities were measured at a scattering angle of 5° with the laser beam wavelength of 633 nm. The pressure in a GPC column (Type G-6000PW, Tosoh) was less than 50 kg/cm^2, and the flow rate was 0.5 ml/min. The effects of the second virial coefficient and the scattering angle on molecular weight determination were neglected.

The M_n and M_w values of HA samples, determined by the GPC-LALLS method,[20] are listed in Table 1. The polydispersity index M_w/M_n ranged from 1.31 for sample C-17g to 1.54 for sample Healon.

Viscometry A sodium chloride solution of 0.2 M was used as the solvent, and measurements were made at 25 °C. An HA sample was dissolved in 0.2 M NaCl solution so that the relative viscosity (η_r) was kept below 2, and the HA concentration of the solution was determined by the Bitter–Muir method[21] with glucuronolactone as a standard. Next, the solution was subjected to viscosity measurements. In order to measure [η] at $\dot{\gamma}$ ranging from 0.1 to 2000 s^{-1}, the following four kinds of viscometers were used. No correction for kinetic energy was made.

(i) Zimm–Crothers Ultra-low Shear Rotational Viscometer[22]: This viscometer was constructed in our laboratory and used for [η] measurements when the $\dot{\gamma}$ value for 0.2 M NaCl solution calculated from the following equation was less than 10 s^{-1}.

$$\dot{\gamma} = (8\pi N/60)\{R_o^2 R_i^2/(R_o - R_i)^{-2}(R_o + R_i)^2\} \ln(R_o/R_i) \qquad (1)$$

Here, R_i and R_o are the radii of the inner and the outer cylinders, respectively. N is the rotation speed of the inner cylinder. Measurements were made using the viscometer with $R_i = 0.52$ cm and $R_o = 0.60$ cm.

(ii) Four-bulb Spiral Capillary Viscometer[23]: For non-Newtonian fluids, the following Rabinowitsch equation[24] gives the true shear rate at the capillary wall.

$$\dot{\gamma} = (3 + b)Q(\pi r^3 \theta)^{-1} \qquad (2)$$

Here, Q is the volume of each bulb, r the radius of the capillary, and θ the efflux time. The correction term b is the slope of $\log \dot{\gamma}$ *vs.* $\log \tau$ where τ is the shear stress, and is the exponent in the typical power law equation for τ *vs.* $\dot{\gamma}$ behavior. At the dilute polymer concentration used in this study, b was very close to unity so that the Rabinowitsch correction could be substantially neglected. This viscometer was used when $\dot{\gamma}$ for 0.2 M NaCl solution ranged from 10 to 250 s^{-1} with $r = 0.0527$ cm and $Q = 2.6$, 1.9, 1.2, and 0.74 cm^3 for each bulb.

(iii) Five-bulb Spiral Capillary Viscometer: This viscometer was the same type as the four-bulb viscometer mentioned above, and covered $\dot{\gamma}$ values of 250—2000 s^{-1} with $r = 0.0733$ cm and $Q = 2.7$, 2.1, 1.6, 1.0, and 0.70 cm^3 for each bulb.

(iv) Conventional Capillary Viscometer of the Ubbelohde Type: A capillary viscometer with $r=0.0188\,\text{cm}$ and $Q=3.5\,\text{cm}^3$ was used. The $\dot{\gamma}$ value of this viscometer for the solvent was $1580\,\text{s}^{-1}$.

Data Analysis The data analysis was made by the Huggins' equation[25]:

$$\eta_{\text{sp}}/c=[\eta]+k'[\eta]^2c \tag{3}$$

the Kraemer's equation[26]:

$$(\ln\eta_{\text{r}})/c=[\eta]+(k'-0.5)[\eta]^2c \tag{4}$$

and the following equation derived by eliminating k' from Eqs. 3 and 4 and writing ϕ for $[\eta]$:

$$[2\{\eta_{\text{sp}}/c-(\ln\eta_{\text{r}})/c\}/c]^{1/2}=\phi \tag{5}$$

where k' and η_{sp} are the Huggins' constant and the specific viscosity, respectively. The $[\eta]$ value at a given $\dot{\gamma}$ was determined as a common intercept obtained by extrapolating the curves of Eqs. 3, 4, and 5 to infinite dilution (see Fig. 1).

Equation 5 has no special physical meaning, but gives the most accurate $[\eta]$ among Eqs. 3, 4, and 5 because of its extremely small slope.

Results and Discussion

Figure 1 shows examples of plots of η_{sp}/c, $(\ln\eta_{\text{r}})/c$, or ϕ *vs.* c for HA in 0.2 M NaCl solution, measured at 25 °C in a conventional Ubbelohde capillary viscometer of $\dot{\gamma}=1580\,\text{s}^{-1}$. Data points for all the samples fall on a straight line, and each line of η_{sp}/c, $(\ln\eta_{\text{r}})/c$, and ϕ results in a common intercept by simple extrapolation. Similar behavior was observed for all the samples at different $\dot{\gamma}$. The results suggest that Eqs. 3, 4, and 5 well describe the viscosity behavior of HA solutions within $\eta_{\text{r}}<2$, and give $[\eta]$ at a given $\dot{\gamma}$ easily by linear extrapolation to infinite dilution.

Figure 2 depicts the $\dot{\gamma}$-dependence of $[\eta]$ for HA samples tested in 0.2 M NaCl solution at 25 °C. With increasing molecular weight, $[\eta]$ depends more strongly upon $\dot{\gamma}$. For instance, $[\eta]$ for the lowest molecular weight sample, C-17i, stays almost constant over the range of $\dot{\gamma}$ studied, while $[\eta]$ for the highest molecular weight sample, Healon, exhibits a Newtonian plateau only when $\dot{\gamma}\leqq 3\,\text{s}^{-1}$. The values of $[\eta]$ at zero shear ($[\eta]_0$) of the HA samples, determined from the Newtonian plateau, are listed in the fifth column of Table I. Comparing $[\eta]_0$ with $[\eta]$ at $1580\,\text{s}^{-1}$ ($[\eta]_{1580}$), the former is only 2% larger than the latter for sample C-17i, whereas the former is about 30% larger than the latter for sample Healon. This shows that the determination of $[\eta]_0$ for high molecular weight HA samples should be made in ultra-low shear viscometers such as the Zimm–Crothers type.[22] However, it is also obvious from Fig. 2 that $[\eta]_0$ read from the Newtonian plateau is in good agreement with 'apparent' $[\eta]_0$ estimated by extrapolating $[\eta]$ at $\dot{\gamma}=10$—$250\,\text{s}^{-1}$ to $\dot{\gamma}\to 0$. Thus, it can be said that such a multi-bulb spiral capillary viscometer covering $\dot{\gamma}=10$ to $250\,\text{s}^{-1}$, as used in this study, is also available for the determination of $[\eta]_0$ of high molecular weight HA samples.

Figure 3 indicates the dependence of k' at $\dot{\gamma}=0.6$ and $1580\,\text{s}^{-1}$ on $[\eta]_0$ for HA in 0.2 M NaCl solution at 25 °C. It is clearly observed that k' at $\dot{\gamma}=0.6\,\text{s}^{-1}$ scatters around 0.35 over the entire range of $[\eta]_0$ studied, while k' at $1580\,\text{s}^{-1}$ seems to increase with increasing $[\eta]_0$. This behavior is similar to the fact that $[\eta]$ of HA depends not only on molecular weight but also $\dot{\gamma}$. This result may be reasonable, because the shear stress deforms dissolved HA chains, and k' would reflect the molecular deformation. However, we cannot give a more concrete explanation for the result at present.

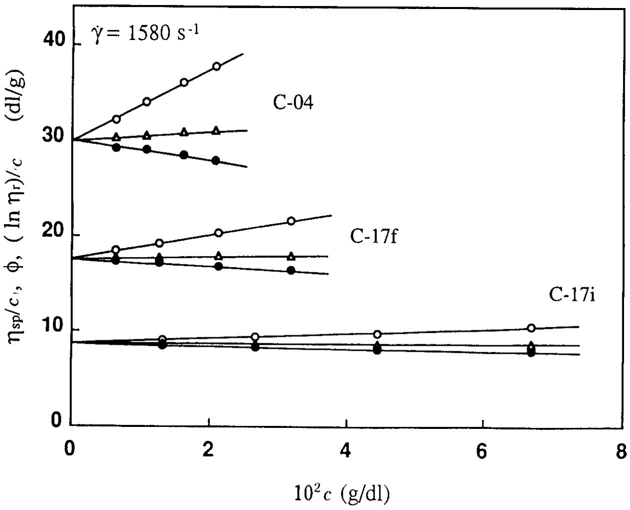

Fig. 1. Examples of Plots of η_{sp}/c (○), $(\ln\eta_{\text{r}})/c$ (●), or ϕ (△) *vs.* c for HA in 0.2 M NaCl Solution, Measured at 25 °C in an Ubbelohde Capillary Viscometer of $\dot{\gamma}=1580\,\text{s}^{-1}$

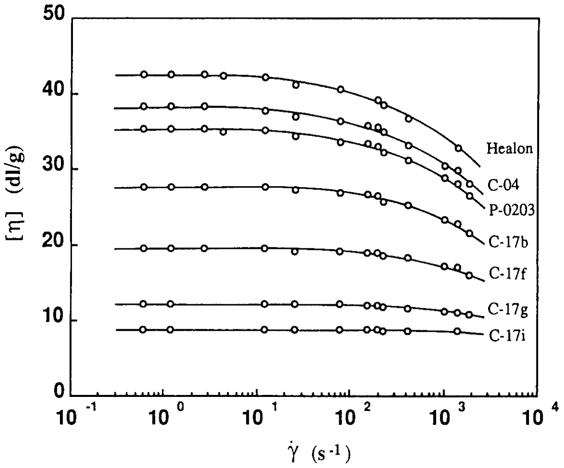

Fig. 2. Shear Rate-Dependence of $[\eta]$ in 0.2 M NaCl Solution at 25 °C for HA Samples Indicated

Table I. Molecular Characteristics of HA Samples Used

Code	$M_{\text{w}}\times 10^{-4}$	$M_{\text{n}}\times 10^{-4}$	$M_{\text{w}}/M_{\text{n}}$	$[\eta]_0$ (dl/g)
Healon	266	173	1.54	42.6
C-04	245	163	1.50	38.4
P-0203	215	150	1.43	35.4
C-17b	154	115	1.34	27.7
C-17f	107	77.5	1.38	19.6
C-17g	62.2	47.5	1.31	12.2
C-17i	40.1	28.6	1.40	8.80

Figure 4 shows the relation between $[\eta]_0$ and M_{w} for HA in 0.2 M NaCl solution at 25 °C. Data points can be well fitted by a straight line expressed by the equation: $[\eta]_0=1.99\times 10^{-4}\cdot M_{\text{w}}^{0.829}$, where $[\eta]_0$ is written in the conventional unit of dl/g. For HA solutions, Mark–Houwink exponents have been determined previously by several researchers.[8,27-32] In most cases, 0.2 M NaCl

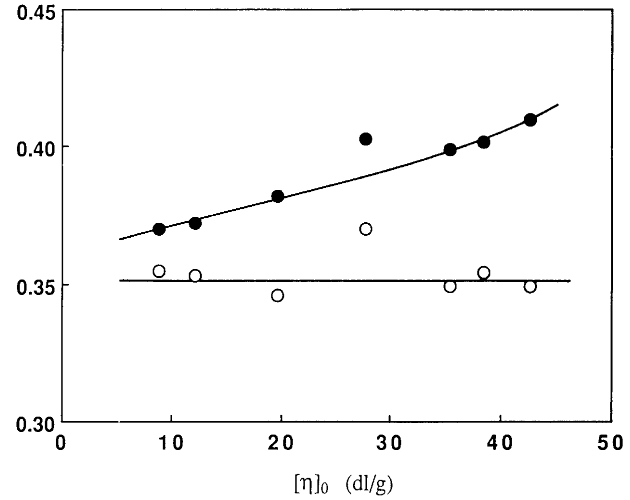

Fig. 3. Dependence of k' at $\dot{\gamma}=0.6$ (○) and $1580\,\mathrm{s}^{-1}$ (●) on $[\eta]_0$ for HA in 0.2 M NaCl Solution at 25 °C

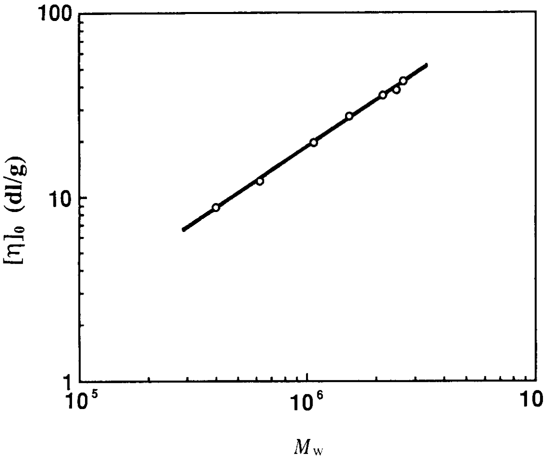

Fig. 4. Double Logarithmic Plots of $[\eta]_0$ *vs.* M_w for HA in 0.2 M NaCl Solution at 25°C

solution close to physiological condition was used as a solvent. The exponent reported so far in the solvent ranges from 0.76 to 0.82. Our value, 0.829, is consistent with these values, supporting the suggestion that an HA molecule acts as a strongly expanded random coil in the solvent by excluded volume effects.

Fixman[12)] gives the non-Newtonian $[\eta]$ value for a flexible chain (bead-spring) model at various values of the viscosity expansion factor (α_η), which is a measure of excluded volume effects, as a function of the generalized shear rate (K_η). This theory is an extension of the Rouse–Zimm bead-spring model[11,13–17)] but includes excluded volume effects. This dimensionless parameter K_η incorporates the effects of molecular weight and chain stiffness, and can be expressed by[12)]

$$K_\eta = 1.71\,\dot{\gamma}([\eta]_0 M_w \eta_0/RT) \qquad (6)$$

where η_0 is the solvent viscosity and other symbols have their usual meaning.

Figure 5 exhibits the theoretical curves of $[\eta]/[\eta]_0$ *vs.* K_η for the Fixman's bead-spring model[12)] with $\alpha_\eta = 1.0$ (for unperturbed chains), 1.1, 1.3, 1.7, and 2.5. This figure also shows typical experimental values of $[\eta]/[\eta]_0$ for samples Healon, C-17b, and C-17g in 0.2 M NaCl solution, plotted against K_η calculated from Eq. 6. Data points for each sample seem to fall along a theoretical curve, suggesting that an HA chain in 0.2 M NaCl solution can be described qualitatively by Fixman's model[12)] within the range of M_w studied. Estimating an α_η value which matches a theoretical curve to data points, we get $\alpha_\eta \sim 1.3$ for sample C-17g of $M_w = 62.2 \times 10^4$, $1.3 < \alpha_\eta < 1.7$ for sample C-17b of $M_w = 154 \times 10^4$, and $\alpha_\eta \sim 1.7$ for sample Healon of $M_w = 266 \times 10^4$. Clearly, α_η increases as M_w increases. This behavior is consistent with the characteristic of expanded Gaussian chains by excluded volume effects.[33)] Also on this point, Fixman's bead-spring model is successful in describing HA chains within the M_w range studied here. Very recently, Fouissas *et al.*[32)] have reported on the expansion factor (α_s) obtained from light scattering. Their values are smaller than ours if compared at the same molecular weight and the same ionic strength. Considering that generally $\alpha_\eta \leqq \alpha_s$,[34)] the values of α_η based on the bead-spring model may be estimated to be rather large. However, a more important point is that an HA chain outlined by Fixman's bead-spring model supports a consensus that this polymer is dissolved as a fairly expanded random coil[7,27,35–37)] by excluded volume effects and, more microscopically, the coil is locally stiffened by hydrogen bonding.[6,38–42)]

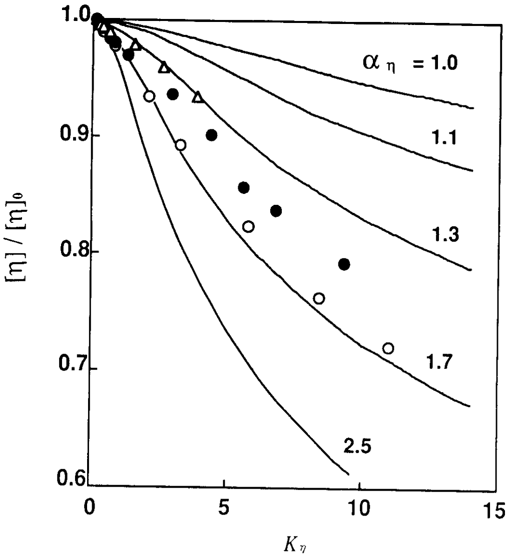

Fig. 5. Typical Experimental Plots of $[\eta]/[\eta]_0$ *vs.* K_η for Samples Healon (○), C-17b (●), and C-17g (△) in 0.2 M NaCl Solution, together with Theoretical Curves of $[\eta]/[\eta]_0$ *vs.* K_η for Fixman's Bead-Spring Model of $\alpha_\eta = 1.0$ (for Unperturbed Chains), 1.1, 1.3, 1.7, and 2.5

Recently, Norisuye *et al.*[43)] have examined the effects of chain stiffness on excluded volume effects in dilute polymer solutions using the Kratky–Porod worm-like chain.[44)] They pointed out that generally the excluded volume effects occur when the contour length of the chain is longer than 100 times as long as the persistence length, regardless of the chain stiffness. On the other hand, Cleland *et al.*[31,36)] determined a persistence length in the range of 4.0—5.3 nm for HA in 0.2 M NaCl solution using small-angle X-rays and light scattering. Assuming that the molecular mass per unit length of HA chains is 400 dalton/nm[31)] and the persistence length of HA in the solvent is 4.0 nm, the value of 16×10^4 is obtained as the critical molecular weight at which the excluded volume effects start to appear. This means that the M_w of HA samples used are high enough for an HA chain to act as a Gaussian chain. Thus, our HA samples can be treated as an expanded Gaussian chain (random coil) by volume effects.

From these results, the following conclusions can be derived within the range of M_w studied: (1) the determination of $[\eta]_0$ for high molecular weight HA in 0.2 M NaCl solution should be made at $\dot{\gamma} < 250\,\mathrm{s}^{-1}$ using low shear viscometers; (2) double logarithmic plots of $[\eta]_0$ *vs.* M_w for HA in 0.2 M NaCl solution give a relation expressed by $[\eta]_0 = 1.99 \times 10^{-4} \cdot M_w^{0.829}$, and the Mark–Houwink exponent indicates that an HA chain in the solvent behaves like an expanded random coil by excluded volume effects; (3) $\dot{\gamma}$-dependence of $[\eta]$ for HA in 0.2 M NaCl solution can be explained qualitatively by Fixman's bead-spring model of large α_η, which is compatible with the Mark–Houwink exponent obtained in this study, 0.829.

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