Molar Mass—Intrinsic Viscosity Relationship of High-Molar-Mass Hyaluronans: Involvement of Shear Rate

[a]D. LATH, [a]K. CSOMOROVÁ, [a]G. KOLLÁRIKOVÁ, [b]M. STANKOVSKÁ, and [b]L. ŠOLTÉS*

[a]*Polymer Institute, Slovak Academy of Sciences, SK-842 36 Bratislava*

[b]*Institute of Experimental Pharmacology, Slovak Academy of Sciences, SK-841 04 Bratislava*
e-mail: ladislav.soltes@savba.sk

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Empirical Kuhn—Mark—Houwink—Sakurada (KMHS) relationships for high-molar-mass hyaluronans, exhibiting non-Newtonian behaviour, are presented in 0.2 mol dm^{-3} NaCl at 25 °C as a function of shear rate. Viscometric determination of the molar mass in conventional Ubbelohde viscometer of constant shear rate requires the proper use of KMHS relationship and concentration correction for moisture content of the hyaluronan sample characterized.

Ultrapure viscous hyaluronan solutions have been introduced for various medicinal purposes – viscosurgery, viscosupplementation, *etc.* – where knowledge of the sample molar mass is decisive for their application. The flow properties of hyaluronates in solution are interesting and important due to their potential applications. Up to now few rheological studies have been reported, where the range of molar mass was limited [1—3]. In 1994 *Yanaki* and *Yamaguchi* [4] published a shear-rate dependence of the intrinsic viscosity $[\eta]$ of high-molar-mass sodium hyaluronate (HA) samples in 0.2 mol dm^{-3} aqueous NaCl solution. The authors used four kinds of viscometers for the measurement of viscosity values, namely i) Zimm—Crothers ultra-low shear rotational viscometer, ii) four-bulb spiral capillary viscometer, iii) five-bulb spiral capillary viscometer, and iv) conventional capillary viscometer of the Ubbelohde type. For seven HA samples (M_m value ranging between 401 kg mol^{-1} and 2660 kg mol^{-1}) a strong shear-rate dependence of the determined $[\eta]$ values has been observed. The intrinsic viscosity value at the extrapolated zero shear rate ($[\eta]_0$) reported by Japanese authors [4] can be used for the calculation of the $[\eta]_0 = f(M_m)$ relationship in the Kuhn—Mark—Houwink—Sakurada (KMHS) power law form ($[\eta] = K \cdot \{M_m\}^a$)

$$[\eta]_0 = 1.99 \times 10^{-4}\{M_m\}^{0.829} \qquad (1)$$

where $[\eta]_0$ is written in the conventional unit of 100 cm^3 g^{-1}. As stated by the Japanese authors, eqn (*1*) can be safely applied for calculations of the molar mass of a HA sample from its $[\eta]$ value if determined at zero shear rate.

For such solutions the non-Newtonian behaviour cannot be ignored in the evaluation of the $[\eta]$ values and the empirical relationship between molar mass and $[\eta]$ is inevitably dependent on shear rate. It seems advisable to establish the empirical molar mass—$[\eta]$ relationship as a function of shear rate applicable within the range of shear rates prevailing in the usual viscosity measurements since the extrapolation method for obtaining $[\eta]$ at zero shear rate is too laborious for routine molar mass determinations.

The graphical dependences published by *Yanaki* and *Yamaguchi* [4] were converted to the pairs of the KMHS parameters K and a at a particular shear-rate value. In such a way one can estimate several sets of the K and a values (Table 1) valid at different shear rates. Moreover, for the determination of the molar mass values of a HA biopolymer even a capillary viscometer of the Ubbelohde type can be used – with the shear rate equaling several hundreds up to thousand(s) reciprocal seconds.

To test such a potential, we selected an Ubbelohde viscometer with relatively very high shear-rate value, of actually 2050 reciprocal seconds. Then we determined the $[\eta]_{2050}$ values for nine HA samples and calculated the viscometric, M_v, values exploiting the KMHS parameters, valid for 2000 s^{-1}, as anticipated in the paper of *Yanaki* and *Yamaguchi* (Table 1). Finally we compared the values of M_v derived from capillary viscometry with the molar mass values determined by using the SEC-MALS method.

The aim of this contribution is thus the presentation of the results found on determining the M_v value for nine high-molar-mass hyaluronans in 0.2 mol dm^{-3}

*The author to whom the correspondence should be addressed.

Table 1. KMHS Parameters Anticipated for the System HA—0.2 mol dm^{-3} NaCl at 25 °C and Different Shear-Rate Values (Adapted from Ref. [4])

Shear rate/s^{-1}	K/(100 cm^3 g^{-1})	a
1	1.72×10^{-4}	0.839
10	1.74×10^{-4}	0.838
100	2.61×10^{-4}	0.808
250	3.44×10^{-4}	0.786
500	4.55×10^{-4}	0.763
750	6.37×10^{-4}	0.737
1000	6.79×10^{-4}	0.731
1250	7.37×10^{-4}	0.724
1500	8.02×10^{-4}	0.716
1750	8.78×10^{-4}	0.709
2000	8.32×10^{-4}	0.711

NaCl solutions at 25 °C on applying a conventional capillary viscometer of the Ubbelohde type with a relatively high shear-rate value.

EXPERIMENTAL

Nine HA samples – sodium hyaluronates – characterized earlier by SEC-MALS analysis [5] (*cf.* Table 2) were used in this study. Water was of Milli-Q quality (Millipore, Bedford, USA) and all other chemicals were of anal. grade.

The content of water in the hyaluronan samples was determined using the Mettler—Toledo TGA/SDTA/851^e thermobalance apparatus. The samples (1—2 mg each) were heated under nitrogen flow (30 cm^3 min^{-1}) up to 200 °C at a heating rate of 5 °C min^{-1}.

The capillary viscometric measurements were performed at (25 ± 0.05) °C using an Ubbelohde viscometer (Schott Glass, Mainz, Germany). Sodium chloride solution of 0.2 mol dm^{-3} was used as the sample solvent/diluent. The diameter of the viscometer capillary was 0.53 mm and the flow-through time of the diluent (η_0) was 85.1 s. The flow-through times of the diluent and of the investigated HA solutions (η_i) were measured with a precision of 0.1 s for the run. During the measurements the relative viscosity (η_{rel}) of the solutions was kept below 2.

The data analyses were made by the *Huggins* [6] and by the *Kraemer* [7] equation

$$\{\eta_{sp}\}/c = [\eta] + k_H[\eta]^2 c$$
$$\ln \eta_{rel}/c = [\eta] + (k_H - 0.5)[\eta]^2 c$$

where η_{sp} is the HA sample specific viscosity, k_H is the Huggins constant and c is the HA sample concentration in 100 g cm^{-3}.

The Rabinowitsch equation [8]

$$G = (3 + b)Q(\pi r^3 \theta)^{-1}$$

where G is the shear rate, r the radius of the capillary, Q the volume of the measuring bulb, and θ the efflux time. The correction term b at the dilute polymer concentration used in this study is very close to unity.

The Rabinowitsch equation was exploited to calculate the shear rate of the Ubbelohde viscometer used. For the solvent applied the value was found to be 2050 s^{-1}.

RESULTS AND DISCUSSION

The HA polymer chain can degrade easily under the action of various physical and chemical factors, like mechanical stress, heat, radiation, acidic or alkaline hydrolysis, oxidation, and enzymatic digestion [9, 10]. For quick determination of changes in molar mass a simple and exact viscometric method should be available for different applications of the studied materials. The determination of intrinsic viscosities of HA samples, as of typical hygroscopic polyelectrolytes, depends on many parameters, such as the hyaluronic acid neutralization degree, ionic strength of the solvent, presence of impurities (proteins, metals), proper concentration range, and water content. As most of these influences can be standardized, for correct determination of the molar mass in capillary viscometers of constant shear rate two parameters are decisive – shear-rate dependence and water content in HA samples.

The water content in HA samples is relatively high and variable ranging between 5—15 %. The decomposition temperature of HA samples is, as observed, slightly over 200 °C. The measured $[\eta]$ values for nine HA samples were corrected for water content and the results of the molar mass characterization and capillary viscometry of the HA samples studied are summarized in Table 2.

The corrected intrinsic viscosity values measured in an Ubbelohde viscometer were used for the calculation of the KMHS relationship and the following equation was obtained

$$[\eta]_{corr} = 6.01 \times 10^{-4}\{M\}^{0.722} \qquad (2)$$

This equation is close to the empirical relation $[\eta]$—shear rate (Table 1) valid for shear-rate ranges applicable in routine viscosity measurements and confirms the strong shear-rate dependence of the HA samples studied.

To test the potential of the viscometric method for molar mass determination using an Ubbelohde viscometer of constant and high shear rate, we calculated the viscometric molar mass at zero shear rate M_0 (eqn (*1*)) and at shear rate 2000 s^{-1} M_{2000} (from Table 1) and the results are summarized in Table 2. We can see that the M_0 values are strongly underestimated, while the M_{2000} values present more realistic data.

Table 2. Characteristics of the HA Samples

Sample code	SEC-MALS		Capillary viscometry			
	M_m	M_N	$[\eta]_{\mathrm{meas}}$	$[\eta]_{\mathrm{corr}}$	M_0	M_{2000}
	kg mol^{-1}	kg mol^{-1}	100 cm^3 g^{-1}	100 cm^3 g^{-1}	kg mol^{-1}	kg mol^{-1}
ALTISSIMO	1553	830.5	16.00	17.76	937	1228
F 1750762	1378	855.9	13.31	14.77	750	947
GENZYME B22157	1340	893.3	14.32	15.90	820	1051
OFTALMICO	1292	792.6	14.10	15.65	804	1028
LIFECORE P 9710-2	1215	678.8	13.97	15.51	796	1015
SIGMA 35H0439	1017	558.8	9.32	10.35	489	574
CPN	659.4	350.7	10.26	11.39	548	657
LIFECORE 1-9100-1	426.2	232.9	7.31	8.11	364	408
GENZYME HYLUMED	90.2	50.1	1.86	2.06	70	59

Thus for the viscometric molar mass determination of samples exhibiting non-Newtonian behaviour the application of proper KMHS parameters and the concentration correction due to the HA sample moisture content is necessary.

CONCLUSION

The choice of proper KMHS parameters valid for the applied capillary viscometer and application of concentration correction according to the moisture content in the HA sample are primary prerequisites for correct determination of the molar mass of hyaluronan samples by viscometry.

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REFERENCES

1. Gibbs, D. A., Merrill, E. W., Smith, K. A., and Balazs, E. A., *Biopolymers 6*, 777 (1968).
2. Ribitsch, G., Schurz, J., and Ribitsch, V., *Colloid Polym. Sci. 258*, 1322 (1980).
3. Reed, C. E., Li, X., and Reed, W. F., *Biopolymers 28*, 1981 (1989).
4. Yanaki, T. and Yamaguchi, M., *Chem. Pharm. Bull. 42*, 1651 (1994).
5. Stankovská, M., Šoltés, L., Vikartovská, A., Mendichi, R., Lath, D., Molnárová, M., and Gemeiner, P., *Chem. Pap. 58,* 348 (2004).
6. Huggins, M. L., *J. Am. Chem. Soc. 64*, 2716 (1942).
7. Kraemer, E. O., *Ind. Eng. Chem. 30*, 1200 (1938).
8. Rabinowitsch, B., *Z. Phys. Chem.* (Leipzig) *145A*, 1 (1929).
9. Reháková, M., Bakoš, D., Soldán, M., and Vizárová, K., *Int. J. Biol. Macromol. 16*, 121 (1994).
10. Orviský, E., Šoltés, L., Chabreček, P., Novák, I., and Stančíková, M., *Chromatographia 37*, 20 (1993).