Rheological Methods to Characterize Sodium Hyaluronate Eyedrops

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Purpose

Sodium hyaluronate (HLA)-containing eyedrops are often used as a first-line treatment for dry eye syndrome. The rheological testing of eyedrops typically involves viscometric characterization using rheometers with a parallel plate geometry, where the shear rate is inherently non-uniform in the fluid gap. This can lead to challenges in interpreting viscoelastic data and prediction of on eye performance. This study was carried out to identify parameters to improve eyedrop rheology characterization using steady state flow and small amplitude oscillatory shear (SAOS) experiments.

Methods

Three HLA-containing eyedrops were tested that contained the demulcent glycerin. All the eyedrops contained high molecular weight HA's which is typically defined as being greater than 1000kDa.[1,2] HLA -1, HLA-2 and HLA-3 had concentrations of HLA of 0.15%, 0.3% and 0.2%, respectively. The steady shear viscosity and viscoelastic properties of the eyedrops were characterized with an Anton Paar MCR 502 rotational rheometer using a cone (50mm DIA1° cone angle) and plate geometry set at room temperature. The steady state shear viscosity measurements were performed over a shear rate range of 1-5000 s-1. SAOS measurements were used for characterizing viscoelastic behavior over an applied frequency range of 0.01-500 rad/s.

Test Protocol Experimental

Anton Paar MCR 502 rotational rheometer with vapor trap

- CP50-1 Fixture: 50mm Dia., 1° Cone Angle, Truncated 0.102 mm
  - AOS Frequency Sweep: 0.1-500 rad/s @ 10% Strain Amplitude
  - Steady Shear Rate Sweep: 1-5000 s^{-1}
- PTD200: Peltier temperature controlled base plate
  - Temperature: 25°C

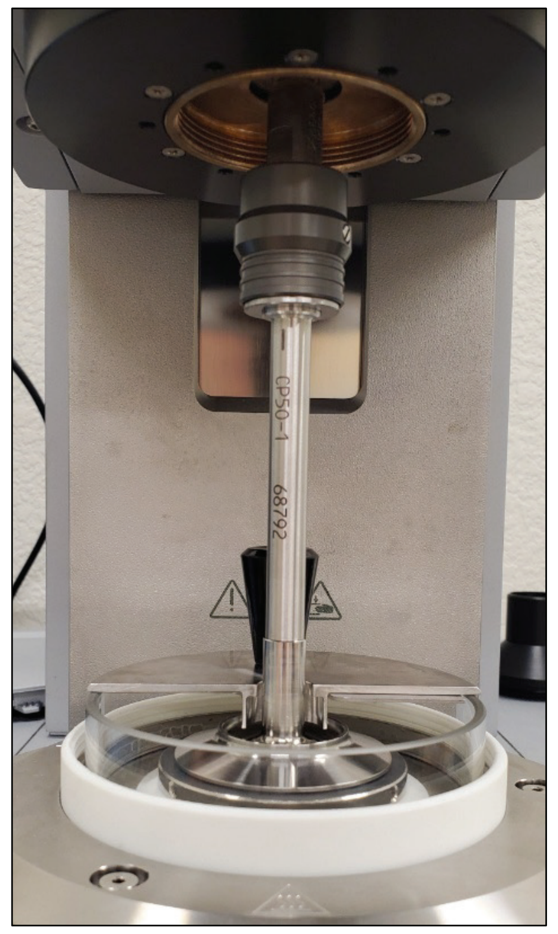

Figure 1. Anton Paar MCR 502 rotational rheometer with vapor trap

Results

The rheological testing of the HLA polymer solutions commonly involves only viscometric characterization (steady shear viscosity vs. steady shear rate measurements) using a parallel plate geometry, where the shear rate is inherently non-uniform in the fluid gap. For a fluid confined in a parallel plate geometry, the nominal applied shear rate (the "reported" shear rate) occurs only along the edge of the plate and decreases to a shear rate value of zero at the center of the plate. Considering that reported viscosity data claims have achieved nominal applied shear rate values as high as 50,000 s-1 (although the influence of edge effects appear to have been neglected), this means that the shear rates witnessed by the fluid in the gap between the parallel plates were highly non-uniform for the polymer solutions being tested

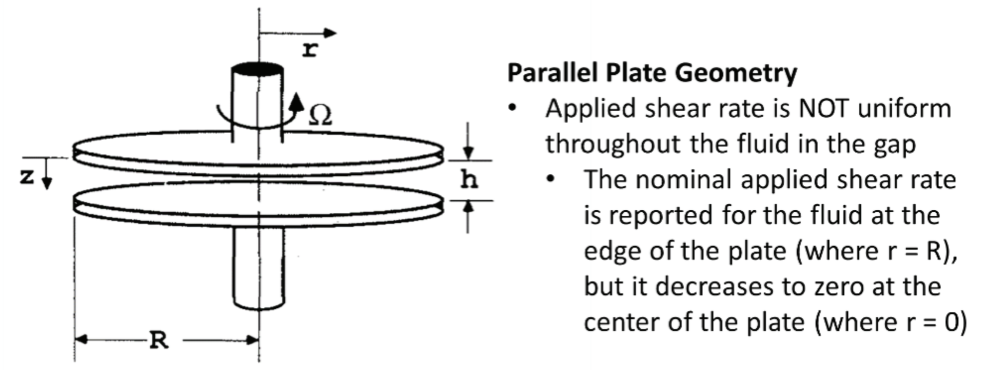

Figure 2. Parallel Plate Geometry showing applied shear rate nonuniformity in the gap

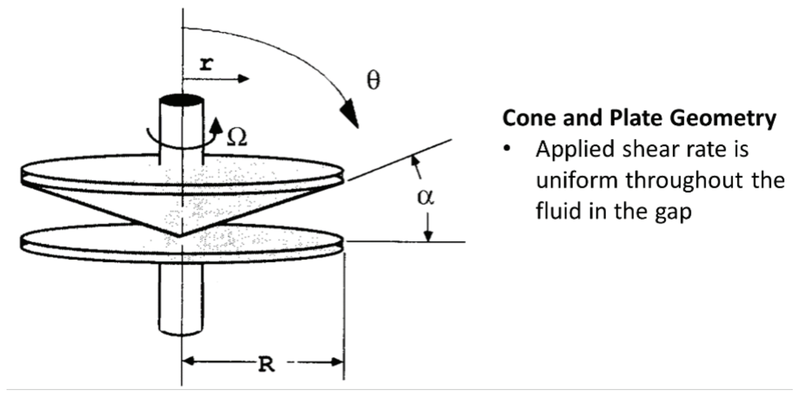

Figure 3. Parallel Plate Geometry showing applied shear rate nonuniformity in the gap

As a result of the gap non-uniformity, when characterizing the shear rheology of complex fluids such as HLA eyedrop formulations, it is preferable to use a more suitable geometry such as a cone-and-plate, where the applied shear rate is uniform throughout the entire fluid gap between the cone and flat plate.

Although useful in characterizing the viscous flow properties of simple fluids comprised of small molecules, viscometric measurements aren't as discriminating in characterizing the macrostructural features of more complex large molecule polymeric fluids which are inherently viscoelastic (possessing both viscous and elastic properties). Small amplitude oscillatory shear (SAOS) measurements are more appropriate for characterizing the viscoelastic behavior of large molecule polymeric solutions and as such are better suited for probing the molecular weight and molecular weight distribution characteristics of long chain molecules. The effects of molecular weight and solution concentration on the dynamic shear rheological behavior of similar large molecule HLA polymer solutions have been reported elsewhere in the literature.[3-6]

Based on this information the development of an experimental testing protocol to characterize the steady shear viscosity as well as the viscoelastic properties of exemplar HLA polymer solutions was carried out at room temperature.

The steady shear sweep data is shown in **Figure 4** where the steady shear viscosity measurements were done at a shear rate range: 1-5000 s-1.

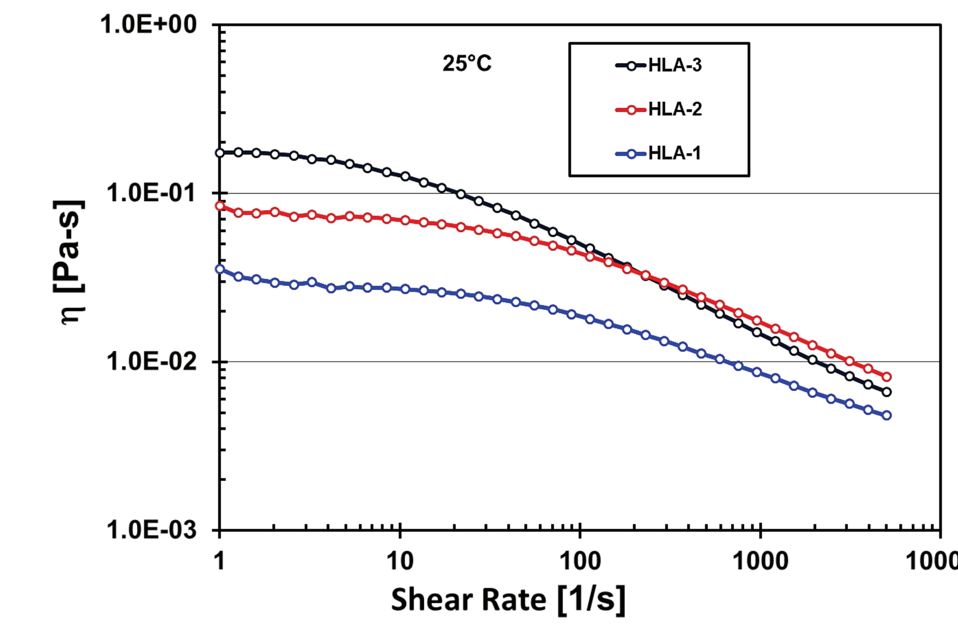

Figure 4. Steady shear viscosity measurements for HLA -1, HLA-2, and HLA -3 at 0.15%, 0.3% and 0.2%, respectively.

As shown in **Figure 4** at the lowest steady shear rate range, the eyedrops exhibited zero-shear viscosities ranging from 30 cPs to 175 cPs in order of increasing MW. When fit with a Carreau fluid model, the shear thinning power index ranged from 0.7 to 0.54 in order of increasing MW. These data indicated all the HLA solutions displayed non-Newtonian behavior. The HLA-3 eyedrop displayed the strongest pseudoplastic behavior.

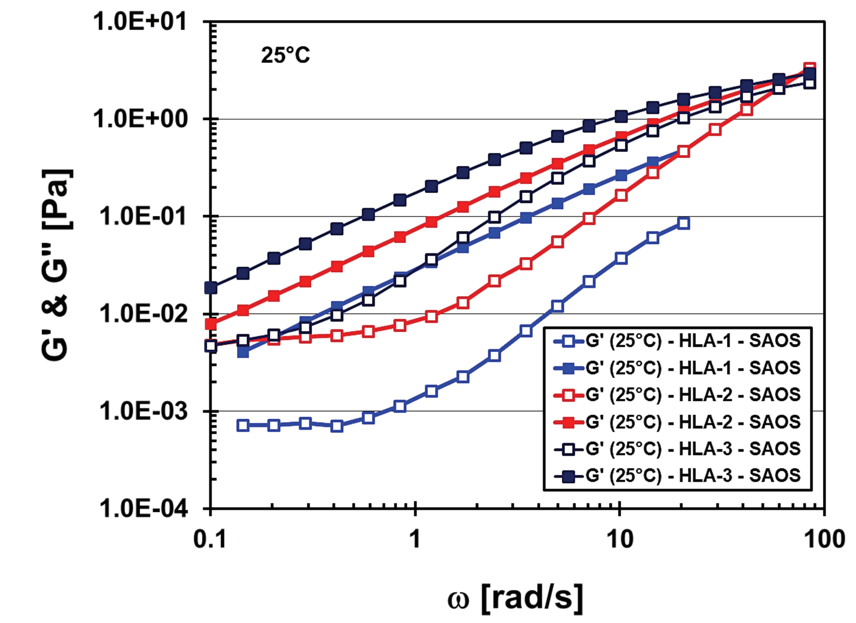

Figure 5. Small Amplitude Oscillatory Shear (SAOS) – Frequency Sweeps. Viscous and Elastic Moduli as a function of angular frequency for HLA-1, HLA-2, and HLA-3 at 0.15%, 0.3%, and 0.2%, respectively.

General observation from the SAOS characterization in **Figure 5** showed the following:

- Above a certain applied angular frequency, the dynamic shear data became irregular with sudden changes particularly with the storage modulus (G') data.
- It is believed that fluid inertia effects are no longer negligible above a certain angular frequency which is dependent on the fluid properties, more specifically dependent upon the polymer concentration in the Newtonian matrix and the molecular weight of the polymer itself.
- More pronounced at lower polymer concentrations and polymer MW.
- In general, frequencies above 100 rad/s yielded anomalous rheological behavior.

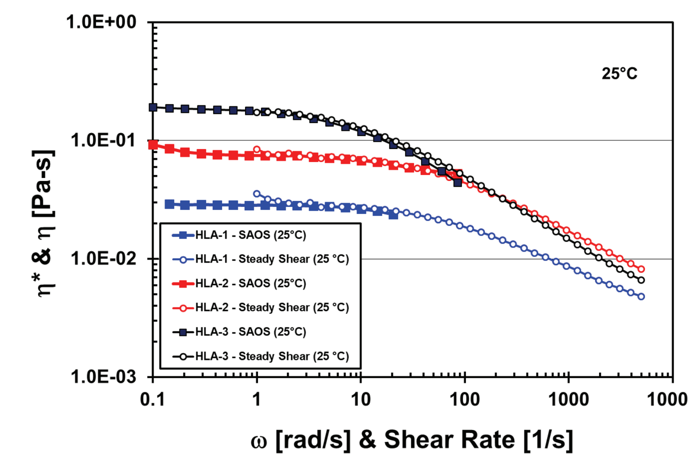

Figure 6. Complex viscosity vs. frequency data superposed with the steady shear viscosity vs. shear rate data for HLA -1, HLA-2, and HLA-3 at 0.15%, 0.3% and 0.2%, respectively.

Conclusion

The HLA-based eyedrops exhibited quasi-Newtonian behavior at low rates of deformation, along with shear thinning behavior at high rates of deformation, which scaled with polymer concentration and MW. The superposition of the complex and steady shear viscosity (as shown in Figure 6) data revealed the expected dynamic rheological behavior of these eyedrops at high frequencies. The experimental approach is believed to be generally applicable and valuable for most eyedrops where dilute polymer concentrations are used, and the viscosity depends on several experimentally challenging factors.

References

1. Tavianatou, A.G., Caon, I., Franchi, M., Piperigkou, Z., Galesso, D. and Karamanos, N.K.(2019), Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. FEBS J, 286: 2883-2908
2. Fouda MM, Abdel-Mohsen AM, Ebaid H, Hassan I, Al-Tamimi J, Abdel-Rahman RM, Metwalli A, Alhazza I, Rady A, El-Faham A, Jancar J. Wound healing of different molecular weight of hyaluronan; in-vivo study. Int J Biol Macromol. 2016 Aug;89:582-91
3. Kobayashi, Y. et al. Viscoelasticity of hyaluronic acid with different molecular weights. Biorheology, 31 (3), pp. 235-244 (1994)
4. Ambrosio, L et al. Rheological study on hyaluronic acid and its derivative solutions. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, A36(7&8), pp. 991-1000 (1999)
5. Cowman MK, Schmidt TA, Raghavan P, Stecco A. Viscoelastic Properties of Hyaluronan in Physiological Conditions. F1000Res. 2015 Aug 25;4:622
6. Snetkov, P. et al. Hyaluronic Acid: The Influence of Molecular Weight on Structural, Physical, Physico-Chemical, and Degradable Properties of Biopolymer. Polymers, 12, 1800 (2020)

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