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RHEOLOGICAL CHARACTERIZATION OF TEAR SUBSTITUTES

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ABSTRACT

The steady state viscosity as a function of shear rate was determined for eight commercial tear substitutes and compared with data for high molecular weight sodium hyaluronate solutions. The zero shear viscosity at steady state varied more than 100-fold from about 2 to 300 cP. Many samples were Newtonian, while some samples exhibited a varying degree of shear thinning (pseudoplastic) behaviour. The results are discussed in relation to the rheological behaviour of normal tears and mucin.

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INTRODUCTION

Polymer solutions are commonly employed as emollients, vehicles for ophthalmic medicaments, wetting solutions for contact lenses and as tear substitutes to replace natural secretions in cases of dry eye syndromes. Both natural (dextran, gelatin), semisynthetic (substituted cellulose) and synthetic (polyvinylalcohol, polyvinylpyrrolidone, polyoxyethylene) polymers are used. In addition, almost all products contain preservatives of various kinds, often in combination.

The purpose of tear substitutes used for treatment of dry eyes is to obtain a product that mimics the physical properties of normal tears[1-3]. These properties are mainly determined by tear mucin. This high molecular weight glycoprotein consists of a very long linear peptide chain to which a large number of oligosaccharide chains are bound. The sugar content is typically 70-90%[4,5]. Tear mucin has been suggested to be involved in the formation of the tear film and the hydration of corneal epithelial cells, thereby preventing the corneal surface from drying[3,6,7].

Sodium hyaluronate is a high molecular weight, hydrophilic, linear polysaccharide that is present in the extracellular matrix. In eye compartments, sodium hyaluronate is found both in the vitreous (100-400 μg/ml)[8] and the aqueous humour (0.5-6 μg/ml)[9]. A high molecular weight non-inflammatory preparation of sodium hyaluronate at a concentration of 1% (Healon) was introduced as a surgical aid in ophthalmic surgery in the early 1980's[8,10].

The chemical, structural, and rheological similarities between sodium hyaluronate and mucin, suggest that high molecular weight sodium hyaluronate solutions may be used to replace the natural tears in cases of dry eye syndromes[3]. A non-inflammatory preparation of high molecular weight sodium hyaluronate at concentrations of 0.1-0.2% has been tested and found to be beneficial in some cases when tested in severe dry eye syndromes[11-15].

Whereas the 1% solution of high molecular weight sodium hyaluronate used as a viscosurgical tool in ophthalmic surgery has a viscosity of about 200 000 cP, the viscosity decreases 10 000-fold to about 20 cP upon a 10-fold dilution to 0.1%. Another sodium hyaluronate preparation has been tested alone and in combination with chondroitin sulphate[16]. The chemical quality and biological purity of this material was not given.

In general, the rheological properties of polymer solutions are a composite effect of concentration and molecular size of the polymer(s). The molecular size is dependent on the molecular weight and the conformation of the polymer. Provided that the molecular size is high enough, the viscosity will be dependent of the shear rate. At low shear rates the viscosity will level off towards a constant value - the zero shear viscosity. At high shear rates the viscosity will decrease as the flexible macromolecules deform and align in the streamlines of flow; i.e. the solution will exhibit a shear thinning (pseudoplastic) behaviour. In contrast, solutions containing low molecular weight substances will exhibit a Newtonian behaviour where the viscosity will be independent of shear rate.

The rheological properties of tear substitutes have been examined since the introduction of such products in the early 1940's[17-29]. We have performed a comparative study on the rheological behaviour of 8 commercial tear substitutes and solutions containing high molecular weight, non-inflammatory sodium hyaluronate.

MATERIALS AND METHODS

The compositions of the tear substitutes tested are given in Table 1. Solutions containing non-inflammatory, high molecular weight sodium hyaluronate with concentrations 0.1% and 0.2% were obtained from Pharmacia AB, Uppsala, Sweden. The weight average molecular weight of sodium hyaluronate was $3\text{-}4 \times 10^6$, and 0.145 M NaCl in 0.002 M Na-phosphate with pH 7.3 and no preservative was used as solvent.

The experiments were performed in a computer controlled Couette viscometer (Bohlin VOR Rheometer, Bohlin Reologi AB, Lund, Sweden) at $25 \pm 0.1^{\circ}C$. The tear substitutes were tested at increasing shear rates from 0.1 to 1 000 s^{-1}. Measurement systems C25 and C14 with dimensions according to DIN-standard were used in combination with sensitive torsion wires. Measurement of viscosity was made at steady state conditions by application of a delay time before reading the stress signal.

RESULTS

Viscosity as a function of shear rate for all samples is plotted in Figure 1. The zero shear viscosity and viscosity at high shear rate (1000 s^{-1}) are given in Table 2.

The viscosity values are expressed in units of cP. A large difference in the rheological behaviour between the samples was evident. The zero shear viscosity varied more than 100-fold between the samples, from 2.2 cP for Hypotears up to 310 cP for sodium hyaluronate 0.2%.

TABLE 1:

Composition of Samples Investigated.

Sample	Polymer	Preservatives
Adsorbotear	Polyvinylpyrrolidone Polyoxyethylene Hydroxyethyl-cellulose 0.36%	Thimerosal 0.004% Edetate disodium 0.1%
Hypotears	Polyvinylalcohol 1% Polyethyleneglycol	Benzalkonium chloride Edetate disodium
Lacril	Hydroxypropylmethyl-cellulose 0.5% Gelatin A	Chlorobutanol
Liquifilm Forte	Polyvinylalcohol 3%	Thimerosal Edetate disodium
Liquifilm Tears	Polyvinylalcohol 1.4%	Chlorobutanol 0.5%
Neo-Tears	Polyvinylalcohol 0.1%	Thimerosal 0.004% Edetate disodium 0.02%
Tears Naturale (Duasorb)	Dextran 70 0.1% Hydroxypropylmethyl-cellulose 0.3%	Benzalkonium chloride 0.01%
Tears Plus	Polyvinylalcohol 1.4% Polyoxyethylene	Chlorobutanol
HA 0.1%	Na-hyaluronate 0.1%	None
HA 0.2%	Na-hyaluronate 0.2%	None

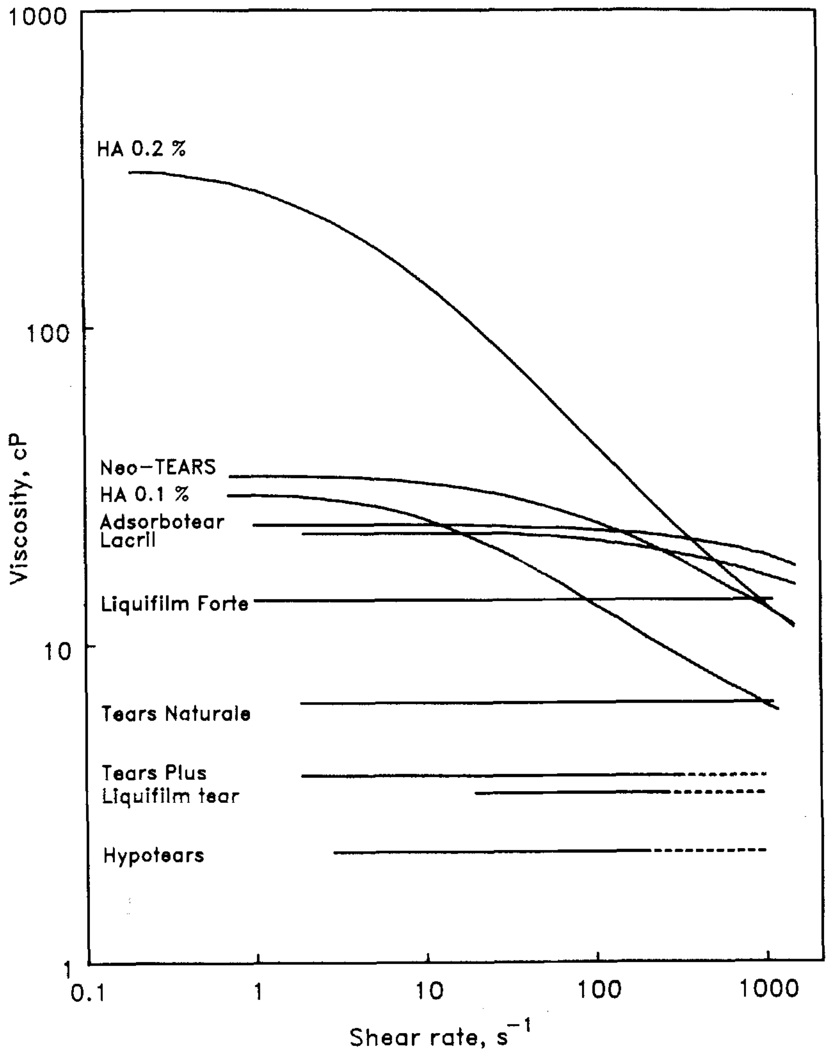

FIGURE 1:

Viscosity as a function of shear rate for all samples. The dashed lines denote extrapolation of Newtonian behaviour (see text). HA denotes sodium hyaluronate.

TABLE 2:

Zero Shear Viscosity and Viscosity at High Shear Rate (1000 s^{-1}) Sorted According to Decreasing Zero Shear Viscosity.

Sample	Zero shear viscosity (cP)	Viscosity at high shear rate (cP)
Na-hyaluronate 0.2%	310.	11.
Neo-Tears	34.	13.
Na-hyaluronate 0.1%	30.	6.5
Adsorbotear	24.	19.
Lacril	22.	17.
Liquifilm Forte	14.	14.
Tears Naturale	6.5	6.5
Tears Plus	3.9	3.9
Liquifilm Tears	3.4	3.4
Hypotears	2.2	2.2

The viscosity was independent of shear rate for 5 products (Liquifilm Forte, Tears Naturale, Tears Plus, Liquifilm Tears and Hypotears). These tear substitutes thus behaved as Newtonian fluids. A tendency of increasing viscosity at high shear rates for the solutions with very low viscosity was most probably due to the onset of turbulence at the highest shear rates. We have therefore assumed that the Newtonian behaviour of these solutions can be extrapolated to the highest shear rates (dashed lines in Figure 1).

Adsorbotear and Lacril exhibited some shear thinning behaviour as the viscosity decreased slightly at high shear rates. A higher degree of shear thinning behaviour was noted for Neo-Tears and the two sodium hyaluronate solutions. The difference between zero shear viscosity and viscosity at high shear rate (1000 s^{-1}) was 3-fold for Neo-Tears, 5-fold for the 0.1% sodium hyaluronate and 30-fold for the 0.2% sodium hyaluronate solution.

DISCUSSION

In a recent review article, Bron[3] presented data on the rheological properties of tears, certain tear substitutes and a sodium hyaluronate solution. The zero shear viscosity for Tears Naturale, Liquifilm Tears and sodium hyaluronate 0.1% determined by Kaura and presented by Bron[3] are in close agreement with our values.

All commercial tear substitutes listed in Table 1 contain one or more biological, chemically modified, or synthetic polymers. The products invariably contain one or more preservatives. An exception being the sodium hyaluronate solutions, which are single component solutions based on a biological polysaccharide with no preservative. The composition of the commercial tear substitutes seems to be poorly defined, and the lack of information on molecular weight is noteworthy. Moreover, the composition of the product may vary. Thus, Tears Naturale, investigated by Holly[30-32], contained dextran and polyvinylalcohol, while the composition reported by Holly and Esquivel[33] contained dextran and hydroxypropylmethylcellulose. Similarily, Neo-Tears contained both hydroxyethylcellulose and polyvinylalcohol when tested by Holly[1,30,33], while the product Neo-Tears tested by us contained polyvinylalcohol only.

The poor description of the tear substitutes prevents a detailed interpretation of the rheological results. Nevertheless, we suggest that the most obvious interpretation is valid. That is, the rheological behaviour of the solutions studied is in agreement with the expected behaviour of polymer solutions: low concentration of low molecular weight polymers results in a very low zero shear viscosity and a Newtonian behaviour, high concentration of low molecular weight polymers results in a higher zero shear viscosity but still a Newtonian behaviour, whereas the combination of low concentration and high molecular weight of polymer results in a high zero shear viscosity and shear thinning (pseudoplastic) behaviour.

Tears are normally subjected to varying shear rates. Between blinks the tears are essentially at rest and the shear rate is close to zero. The shear rate during a blink may be calculated from the speed of the eye lid and the thickness of the tear film, by dividing the former by the latter. The blinking velocity is about 10 cm/s[34,35], and the thickness of the tear film is about 7 μm[3]. Thus, we arrive at an average shear rate of more than 10 000 s^{-1}. Consequently, very high shear rates are prevalent during the movement of the eye lid over the thin tear film[3]. This conclusion is contradictory to a recent statement that low shear rates are found during blinking[16].

The fact that tears are subjected to shear rates varying from almost zero between blinking up to about 10 000 s^{-1}, during a blink, is important when discussing the rheological properties of tears and tear substitutes[3]. A degree of shear thinning will give a low viscosity at high shear rates, and, therefore, favour an even distribution of the solution and lubrication during high shear blinking. At low shear rates the solution should have a significant viscosity to prevent the solution from immediately flowing off the corneal epithelium. The viscosity should, however, not be so high that it results in blurred vision. If the viscosity is too high both at low and high shear rates (Newtonian or slightly shear thinning behaviour), the solution will most probably cause blurred vision. We, therefore, anticipate that an optimum composition should have rheological properties similar to normal tears: i.e. high viscosity at rest and low viscosity at high shear rates, as seen in the behaviour of a solution containing a high molecular weight polymer at low concentration.

The zero shear viscosity of tears has been reported to be 1-6 cP[36], while Kaura, according to Bron[3], found a relative viscosity of 2.8±0.2 cP (at a shear rate of less than 4 s^{-1}). Interestingly, it has also been reported[3] that normal tears exhibit a shear thinning behaviour, and that this shear thinning behaviour is most probably due to the presence of tear mucin. Tears are probably very similar to other secretions containing mucin in that the rheological properties (and the shear thinning behaviour) are dependent on the high molecular weight mucins.

The molecular weight of both sodium hyaluronate and mucin is very high (above 10^6) and the molecules adopt a conformation of highly extended random coils in solution. Even at very low concentrations these flexible randomly coiled molecules will entangle, resulting in a molecular network. Consequently, solutions containing mucin or sodium hyaluronate will exhibit similar rheological properties[3,37,38]. Both macromolecules form flexible molecular networks that result in a viscoelastic response, and shear thinning behaviour. Note that these properties are extremely dependent on the molecular size of the substances, and that even a modest degradation of the macromolecules will result in a substantial change in rheological properties[3,37,38]. A shear thinning behaviour of tears and native tear mucin has been reported(3), while mucin of bovine origin, often used in studies of the physical properties of mucins, exhibited Newtonian behaviour, suggesting degradation of the mucin macromolecule. Similarily, the effect of molecular weight on other rheological properties has been demonstrated(38).

CONCLUSIONS

In conclusion, our data confirm those previously presented(3,38). Many tear substitutes are Newtonian solutions, whereas Neo-Tears and sodium hyaluronate solutions exhibit substantial shear thinning behaviour. Based on the rheological conditions of the tears, very low shear rates between blinking and very high shear rate during blinking, we propose in agreement with Bron(3) that an optimal tear substitute should exhibit shear thinning behaviour similar to that of normal tears and mucin solutions. The large similarity in chemical structure, hydrophilicity, molecular weight and conformation, and rheological behaviour between mucin and sodium hyaluronate suggests that sodium hyaluronate may be used as a mucomimetic polymer as proposed and discussed previously(3,7).

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