Chapter 6

VISCOSITY ENHANCEMENT OF HYALURONIC ACID SOLUTIONS

6.1 Introduction

During the replacement of a cataract with an intraocular lens (IOL), ophthalmic surgeons use solutions of high molecular weight polyelectrolytes aptly termed viscoelastics.[1-3] These viscoelastics are coated on the corneal epithelium prior to phacoemulsification (ultrasonic emulsification and removal) of the lens to protect these delicate cells and the surrounding ocular tissue. Viscoelastics are also injected into the lens capsule (the portion of the eye in which the lens was located) to maintain its shape during implantation of the IOL. Without this injection, the vitreous pushes the rear wall of the lens capsule foreword, which can distort the chamber. In the medical literature the desired mechanical properties of viscoelastics are qualitatively characterized by being both “cohesive” and “dispersive.” That is, they stay where the surgeon places them and flow when the surgeon wants to deform them.

Ophthalmic viscoelastics are solutions of hyaluronic acid (HA) or HA mixed with the structurally similar chondroitin sulfate. Unfortunately, HA is unstable.[4] Consequently, ophthalmic viscoelastic must be stored cold (2º-8ºC, recommended).[1] Even with cold storage, however, HA gradually degrades and causes a large variation in the mechanical properties of viscoelastics. The technical literature for DisCoVisc, one such viscoelastic,

lists the viscosity of the product as 75,000 ± 35,000 cP. This large variation in properties is especially inconvenient for surgeons, who desire consistency in the materials they use during medical procedures.

Degradation of HA can occur by both chemical and mechanical means. Miyazaki and coworkers have shown that degradation of HA by shear can occur in a cone-and-plate viscometer[5]. This degradation was confirmed by GPC traces of the HA samples and not just inferred from a drop in viscosity. The presence of salt was important in determining the rate of HA degradation: degradation increased as the concentration of sodium chloride was increased from 0.001 *M* to 1 *M*. Besides mechanical degradation, many authors have measured degradation of HA by chemical means[6-9]. Most of this chemical degradation is ascribed to attack by radicals since it can be reduced by the presence of radical scavengers. Hydroxide radicals, in particular, are theorized to be responsible for this degradation. Metal surfaces and metal ions are implicated, as well. Further studies by Miyazaki and coworkers, for example, showed that mere contact with the stainless steel components of the viscometer in the absence of shear could cause degradation of HA[10]. The same degradation was observed in the presence of stainless steal beads, but was absent when HA solutions were stored in glass. Also, several metal ions (Fe^{2+}, Cu^{2+}, Co^{2+} and Cr^{3+}) were found to cause degradation of HA. The authors believed that hydroxyl radicals were created by the metal surface or by the dissolved metal ions.

Since HA aids in healing,[11] completely eliminating it from viscoelastic formulations in favor of more stable, biocompatible polymers is undesirable. The possibility of adding other polymers to stabilize the mechanical properties of HA solutions, however, is a possibility. For example, HA alone under physiological conditions shows no indications of self-interaction or transient network formation.[12] In the presence of albumin, however, HA solutions become rheoplexic (shear stress increases with time at constant shear rate) due to formation of a transient network.[13] It is our desire to screen for other polymers that might alter HA rheology; our study will examine the change in viscosity of HA solutions with the addition of various hydrophilic polymers. The hydrophilic polymers chosen are intended to expose the effects of charge (anionic, cationic, and neutral), hydrogen

bonding (hydrogen bond accepters, mixed hydrogen bond accepters and donors), and flexibility. Both synthetic and natural polymers were investigated. After identifying polymers that enhance HA viscosity, the effects of temperature, pH, and concentration on this enhancement are probed. Finally, we present a hypothesis of the origin of the viscosity enhancement in these solutions.

6.2 Experimental

Materials. Hyaluronic acid was purchased from Lifecore Biomdeical. Carboxymethylcellulose (CMC), hydroxypropyl methylcellulose (HPMC), sodium alginate (NaAlg), and propylene glycol alginate (PG-Alg) were purchased from Spectrum. Polyethyleneglycol (PEG), polyvinylpyrrolidone (PVP), dextran, DEAE-dextran, and dextran sulfate were purchased from Sigma. DisCoVisc, ProVisc, and Viscoat were provided by SK Pharmaceuticals.

Sample Preparation. Samples were prepared in borate buffer, adjusted to pH 7.2 unless otherwise noted. All samples containing two different polymers were prepared by first making concentrated stock solutions of both polymers in buffer. Stock solutions were placed on a shaker until the polymers were fully dissolved and the samples homogenous. To make the final samples, the needed amount of each stock solution was measured out and extra buffer added to give the final desired concentration. These samples were again shaken until homogenous. Very viscous samples required equilibration overnight to allow bubbles to escape from the solutions.

Viscometry. A stress rheometer (model AR 1000, TA Instruments, New Castle, Delaware) was used for all measurements. With the exception of the surgical viscoelastic materials, the shear stress was adjusted to cover a range of shear rates from 20 to 160 sec^{-1}. A 60 mm, 1º cone was used for solutions with viscosity less than 0.5 Pa·s, and a 40 mm, 2º cone was used for solutions with viscosity greater than 0.5 Pa·s. The viscosity was measured by increasing and then decreasing the shear rates for all materials but the

surgical viscoelastics, which were only measured by increasing the shear rates. Measurements were performed at 25ºC unless otherwise noted.

Light Scattering. Static light scattering measurements were performed on a Wyatt Technology (Santa Barbara, CA) Dawn EOS light scattering instrument (Figure 6.1). A diode laser operating at 685nm was used for the scattering experiments. The MALS was operated in batch mode using scintillation vials. For each sample, the data was collected for one minute. Data from each detector was then averaged.

6.3 Results

For comparison with HA solutions and mixtures, the rheology of current viscoelastic materials was tested. DisCoVisc, ProVisc, and Viscoat all showed shear thinning behavior (Figure 6.2). The description of "cohesive" and "dispersive" properties of these materials in the medical literature corresponds well to shear thinning: cohesiveness being the solid-like behavior of the materials at low strain rates and dispersiveness being their tendency to flow at higher strain rates. ProVisc and Viscoat are designed to be used as a pair, with ProVisc being the more cohesive of the two materials and Viscoat being the more dispersive.[1, 2] Viscosity measurements match these qualitative descriptions, with Viscoast having a lower viscosity than ProVisc. DisCoVisc is designed to be used alone, combining both dispersive and cohesive properties adequately to be used in place of two separate solutions.[3] The viscosity of DisCoVisc and ProVisc are similar at low shear rate; surprisingly, DisCoVisc does not have character intermediate between those of Viscoat and ProVisc at any shear rate. .

To evaluate the ability of different hydrophilic polymers to alter HA rheology, the viscosity of solutions composed of 0.15% HA and 0.5% of the different additives was tested. Phase separation was observed when a cationic polymer (DEAE-dextran) was mixed with the anionic HA. A white solid formed at the bottom of the scintillation vial. The viscosity of this solution was therefore not tested, and no other cationic polymers were examined.

Several polymers showed no ability to enhance HA viscosity when present at a composition of 0.5% (Figure 6.3a-d). These polymers were both neutral (PEG, PVP, and dextran) and anionic (dextran sulfate). Two very different molecular weights of dextran sulfate were tested (40K and 500K), but the higher molecular weight polymer failed to increase the viscosity of the HA solution more than the lower molecular weight polymer.

Four polymers produced an enhancement in HA viscocity: CMC, HPMC, NaAlg, and PG-Alg (Figure 6.4a-d). All four of these polymers are polysaccharides; CMC, PG-Alg, and NaAlg are anionic, while HPMC is neutral. When a 0.5% solution of these polymers was tested without HA, the viscosity could be either greater than (e.g. HPMC) or less than (e.g. CMC) the viscosity of a 0.15% solution of HA. Either way, the viscosity of the binary solution was greater than either of the counterpart solutions with just one polymer. The ability of these polymers to act as viscosity enhancers were therefore not tied to their viscosity relative to that of HA.

To examine the dependence of this viscosity enhancement on the molecular weight of HA, solutions were prepared consisting of 0.5% CMC and 0.15% of different molecular weights of HA (Figure 6.5). Testing different molecular weights of HA simulated testing the same solution over time as HA degrades into fragments of decreasing molecular weight. The relative variation in viscosity of the HA solutions was greater than the variation in viscosity of the mixed HA and CMC solutions: at a shear rate of 100 sec^{-1}, for example, the HA solutions varied over approximately a decade in viscosity while the mixed HA and CMC solutions at the same strain rate varied over approximately a third of a decade (Figure 6.5).

To probe the effect of the concentration of the viscosity enhancer, four concentrations of HPMC and NaAlg (0.125%, 0.25%, 0.5% and 1.0%) were tested maintaining the HA concentration at 0.15%. The ratio of the viscosity of the binary solution to the sum of the viscosities of the two single component solutions shows a maximum enhancement when the concentration of the viscosity enhancer is 0.25% (Figure 6.6a and 6.6f). As a measure

of the enhancement of the viscosity, we normalize the viscosity of the mixture (η_{A+B}) by the sum of the individual components at their respective concentrations in the mixture ($\eta_A+\eta_B$). A simple additive effect would give $\eta_{A+B} / (\eta_A+\eta_B) = 1$. When these individual solutions have very different viscosities (as in Figures 6.6 b,d,e,g,i and j), this ratio corresponds to the ratio of the mixture viscosity to that of the more viscous single component system. In cases where the solutions have similar viscosity to each other (c and h), the denominator represents the additive result that one would obtain if both species were dilute and the species did not interact with each other. Thus, normalizing in this manner highlights the nonlinear enhancement of the viscosity (note values of $\eta_{A+B} / (\eta_A+\eta_B)$ substantially greater than 1 in Figures 6.6a and 6.6f, particularly at low shear rates).

Finally, changes in viscosity were measured as a function of temperature and pH. The viscosity of the solutions was robust with respect to temperature (Figure 6.7 and Figure 6.8) and pH (Figure 6.9). Solutions with a higher concentration of the viscosity enhancer (1.0%) showed a smaller dependence of the viscosity on temperature (Figure 6.8) than did solutions with a lower concentration of the enhancer (0.5%) (Figure 6.7). The change in viscosity with pH is consistent with the increased charge on HA at pH 7.2. The pKa of the polyelectrolyte is approximately 6.5.

Upon visual inspection, the solutions that showed viscosity enhancement also showed a change in appearance—while remaining transparent, the solutions gave a “speckled” appearance. These “speckles” were more subtle in the CMC sample and quite pronounced in the PG-Alg sample. In an attempt to quantify this observation, light scattering was measured for all the samples tested (Figure 6.10). In accord with visual observations, the binary solution of HA with PG-Alg, NaAlg, or HPMC scattered more strongly than the other solutions. The scattering was great enough that all three samples flooded the low angle detectors. The anomalous sample among those that showed enhanced viscosity was the CMC and HA; it showed no greater scattering than many of the samples that showed no viscosity enhancement.

6.4 Discussion and Conclusions

Although the four polymers that caused viscosity enhancement of HA solutions are all polysaccharides, this can not be the source of the enhancement since dextran and dextran sulfate failed to cause any increase in viscosity. The property that separates the polymers that cause the viscosity enhancement from the polymers that do not cause the viscosity enhancement is flexibility. All the polymers which increased the viscosity of the HA solutions have persistence lengths longer than HA, while all of the polymers that failed to increase the viscosity of HA have a persistence length less than that of HA (Table 6.1).

Polysaccharide flexibility is tied to the bonding between monosaccharide residues, termed glycosidic bonds.[14] The difference in flexibility between cellulose and dextran illustrates the importance of the glycosidic bond. Both polymers are formed from the same monosaccharide, D-glucopyranose. In cellulose, D-glucopyranose units are bonded together in β-1→4 linkages, which means the bond is located in an equatorial position on both residues. Consequently, rotation about this bond hardly changes the angle between the residues and the polymer has a linear conformation. This linear conformation gives cellulose derivatives large persistence lengths: 12-16 nm for cellulose.[15, 16] The majority of bonds in dextran, on the other hand, are α-1→6 such that the bond lies in an equatorial position on one residue but an axial position on the other residue. Rotation about this bond allows bending of the backbone and gives the polymer a persistence length of 1.5 to 1.8 nm.[17] Bonding in HA alternates between β-1→4 (the equatorial-equatorial bond that gives cellulose its rigidity) and β-1→3 glycosidic bonds (equatorial-axial),[12] which gives the polymer flexibility. Not surprisingly, the persistence length of HA lies between that of dextran and cellulose, at approximately 4nm.[18, 19]

The structure of alginates is more complex. Alginates are blocky copolymers of two monosaccharide residues, D-manuronic acid and L-gulunronic acid.[14] Within alginates, residues can be found that are bound equatorial-equatorial, axial-axial, and equatorial-axial.[20] The polymer thus has blocks of high flexibility and blocks of low flexibility. Blocks where L-guluronic acid residues are bound axial-axial have the ability to form

crosslinks between different chains bridged by divalent cations. Since the boric acid buffer used in these experiments contained both calcium and magnesium, not only the rigidity of the polymer, but crosslinking as well, might be important in explaining the increase in solution viscosity.

Mixtures of polymers with different persistence lengths can undergo phase separation due to depletion interactions.[21] Once the total concentration of polymer increases sufficiently, phase separation occurs to minimize the excluded volume of the system. Ordering of the rigid phase occurs to allow overlap of the excluded volume from neighboring rods. In biopolymers, this effect has been widely used to cause the crystallization of proteins for x-ray studies by the addition of PEG to protein solutions. Separation of hydrophilic polymers of mismatched flexibility has also been observed in mixtures of dextran and cellulose[22] and mixtures of tobacco mosaic virus and PEG or albumin.[21] At low polymer concentrations, phase separation in the TMV and PEG or TMV and albumin system did not show precipitation, but formation micron-scale "droplet" structures dispersed in an isotropic phase. These droplets formed a variety of plate and rod structures depending on the concentration of the two components. These "droplet" structures began forming at a higher concentration (above 2% TMV, 0.5% albumin or PEG) than the polymer concentrations used in the present study (0.15% HA, 0.5% additive). However, the molecular weight of the HA used in this study was at least an order of magnitude larger than either the PEG or albumin used in the TMV study, which should cause phase separation at a lower concentration.

Although the cellulose derivatives and alginate derivatives used in this study do not have the extreme rigidity of TMV, the increased scattering observed in our systems is consistent with increased concentration fluctuations in the solution. The scattering observed qualitatively matched those samples where viscosity enhancement occurred. Solutions with high scattering exhibited enhanced viscosity. Only one system, the HA and CMC solution showed an increase in viscosity without high scattering. The discrepancy between observation and MALS data might be eliminated if scattering at smaller angles were measured, such as the small angle scattering reported in Chapter 4.

Since the fluctuations observed visually must be of the same length scale as the wavelengths of visible light, scattering at small angles should reflect these fluctuations. Future work could examine depolarized small angle light scattering, which could expose birefringence in regions with elevated concentrations of the stiff polymer. Studies of xanthan gum (another rigid polysaccharide) in dilute solution (0.5%) that were believed to be a weak network were shown instead to be a dispersed nematic phase.[23] Similarly, the enhanced viscosity observed in solutions of cellulose and alginate derivatives may be due to such a dispersed phase. The presence of high molecular weight HA would increase the tendency of these more rigid polymers to collect together.

Addition of cellulose and alginate derivatives to HA solutions enhances the viscosity of the solution and makes it less sensitive to HA molecular weight. The origin of the enhanced viscosity in these systems is hypothesized to be due to an increased tendency to form a dispersed phase rich in the more rigid polymer driven by HA. When the HA concentration is kept constant, the viscosity enhancement measured as the ratio of the binary solution to the sum of the viscosities of the two single component solutions shows a maximum as the concentration of the viscosity enhancer is increased, implying that the viscosity enhancement induced by the dispersed phase may be overwhelmed at higher concentrations by the high viscosity of the concentrated polymer solution. Further research may reveal greater viscosity enhancements. In particular, the magnitude of the maximum enhancement and the concentration of the viscosity modifier at which it occurs may both increase if the concentration of HA is increased. In addition, the effect of increasing the molecular weight of the more rigid polymer has yet to be examined. The phase behavior of tobacco mosaic virus and flexible polymer is more sensitive to the concentration of the flexible polymer than the rigid polymer, indicating that this parameter may be particularly important in tuning the system. Also, the second virial coefficient for a mixture of rods and coils is predicted to scale with length of the rod to the first power;[24] thus increasing the length of this component should greatly increase the interaction driving phase separation.

6.5 Figures and Tables

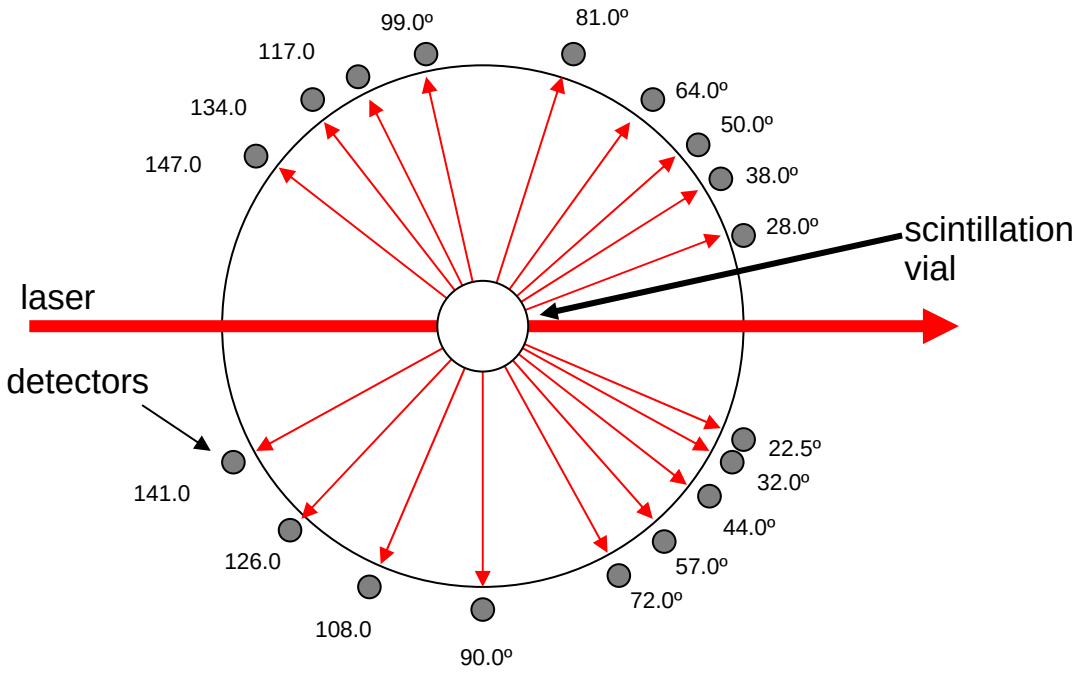

Figure 6.1 Schematic of apparatus for measuring light scattering from polymer solutions. Top view of the apparatus is shown. Angles of all detectors (shown) dictate the discrete value for which measurements are made (Figure 6.10).

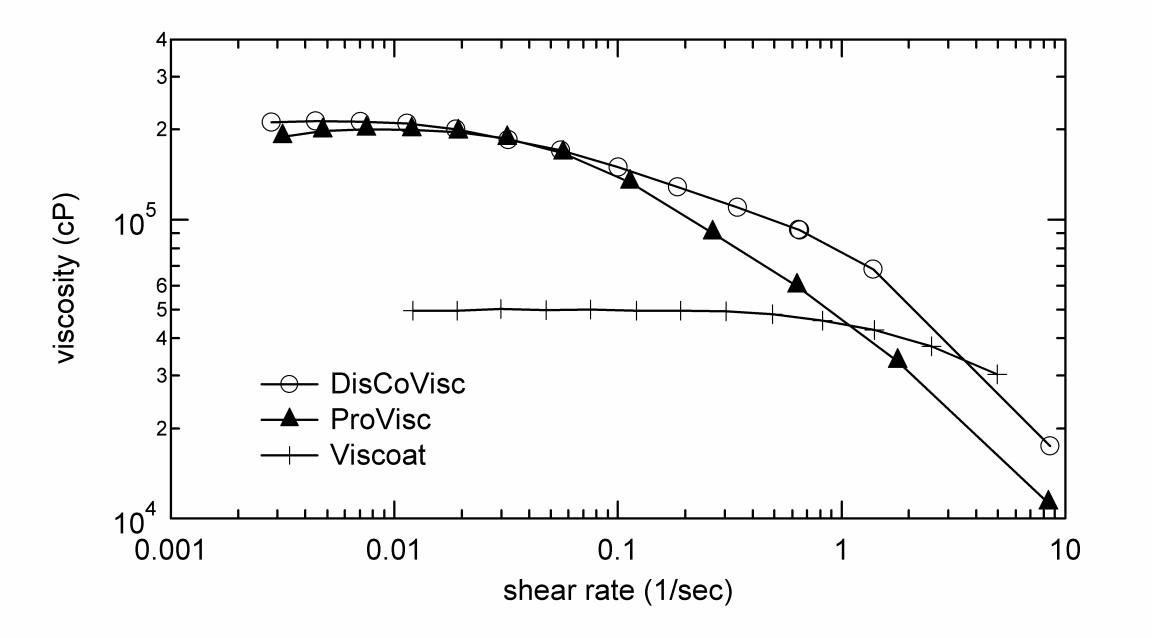

Figure 6.2 Viscosity of viscoelastic solutions for ophthalmic surgery.

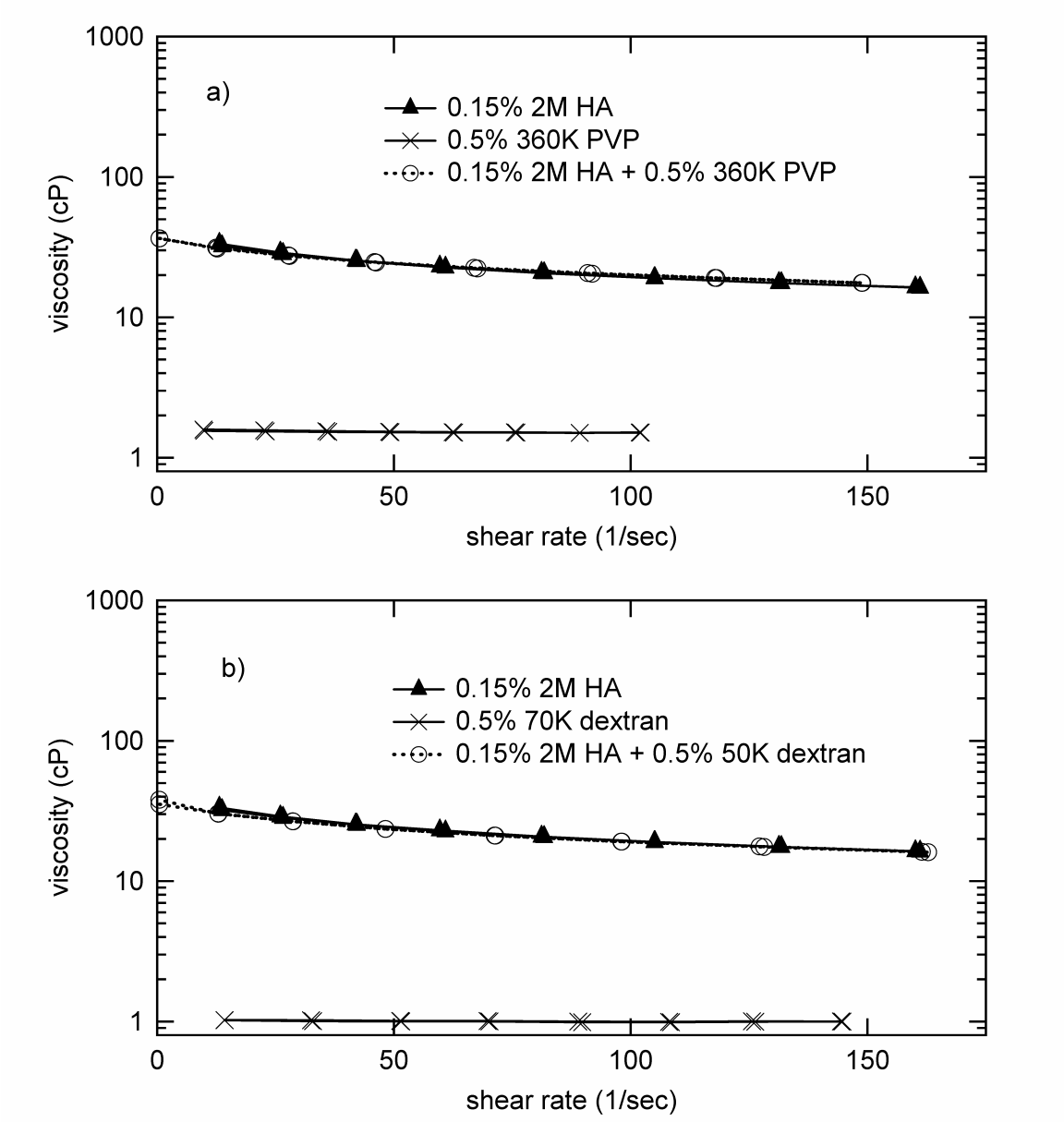

Figure 6.3a-b Hydrophilic polymers which show no viscosity enhancement when mixed with hyaluronic acid: a) polyvinylpyrrolidone b) dextan.

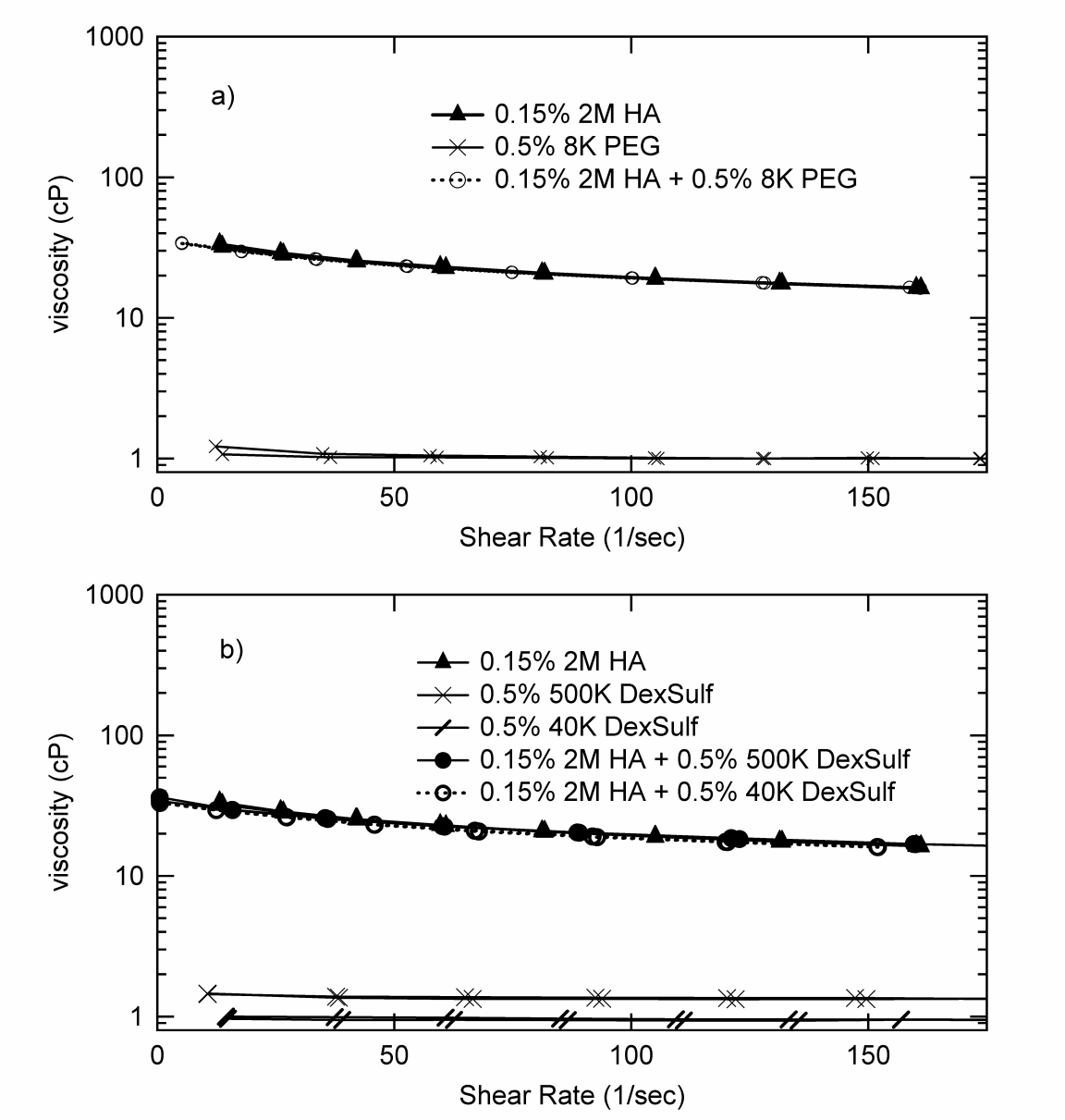

Figure 6.3c-d Hydrophilic polymers which show no viscosity enhancement when mixed with hyaluronic acid: c) polyethyleneglycol d) dextran sulfate.

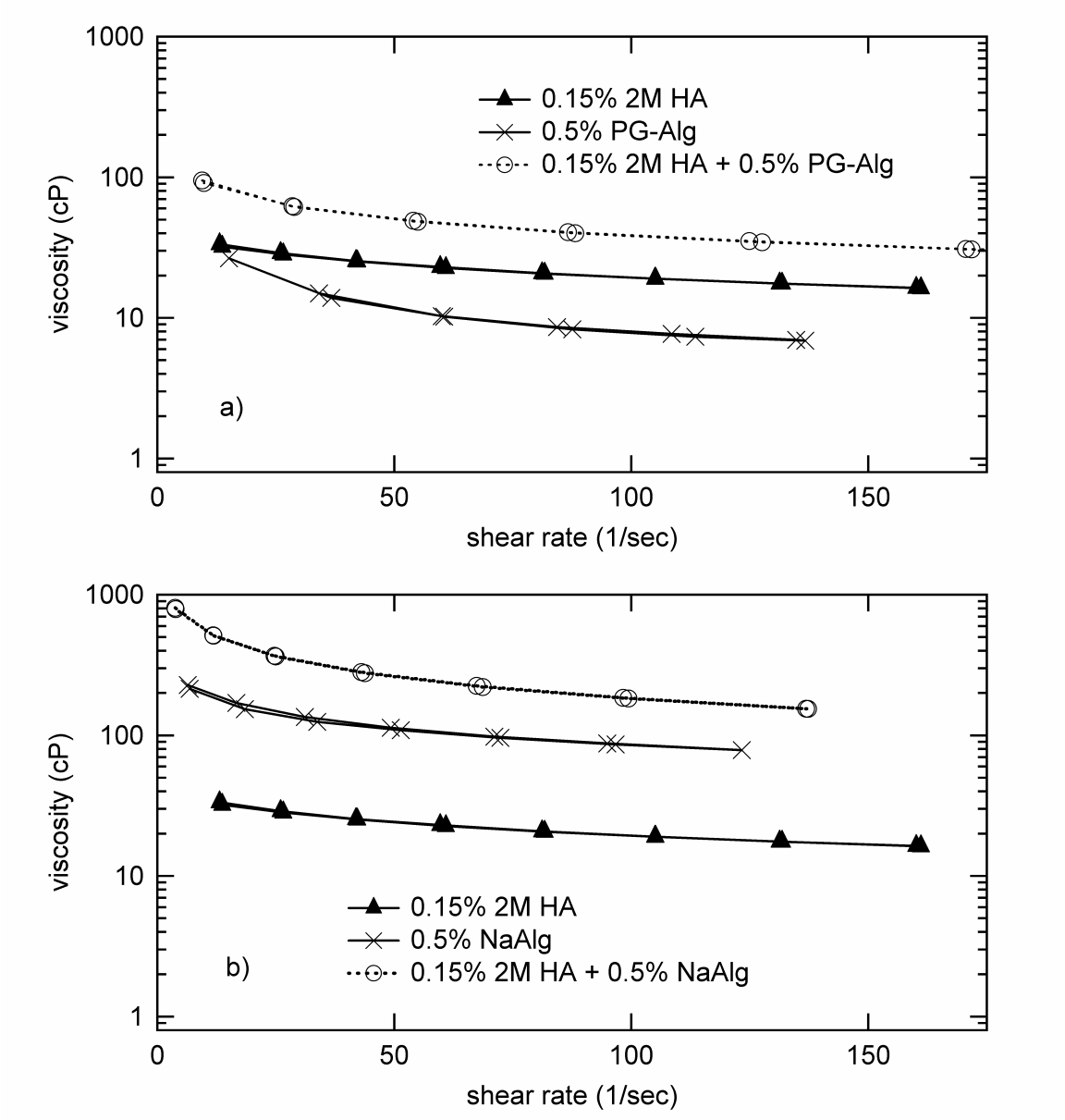

Figure 6.4a-b Hydrophilic polymers which show viscosity enhancement when mixed with hyaluronic acid: a) propyleneglycol alginate. b) sodium alginate.

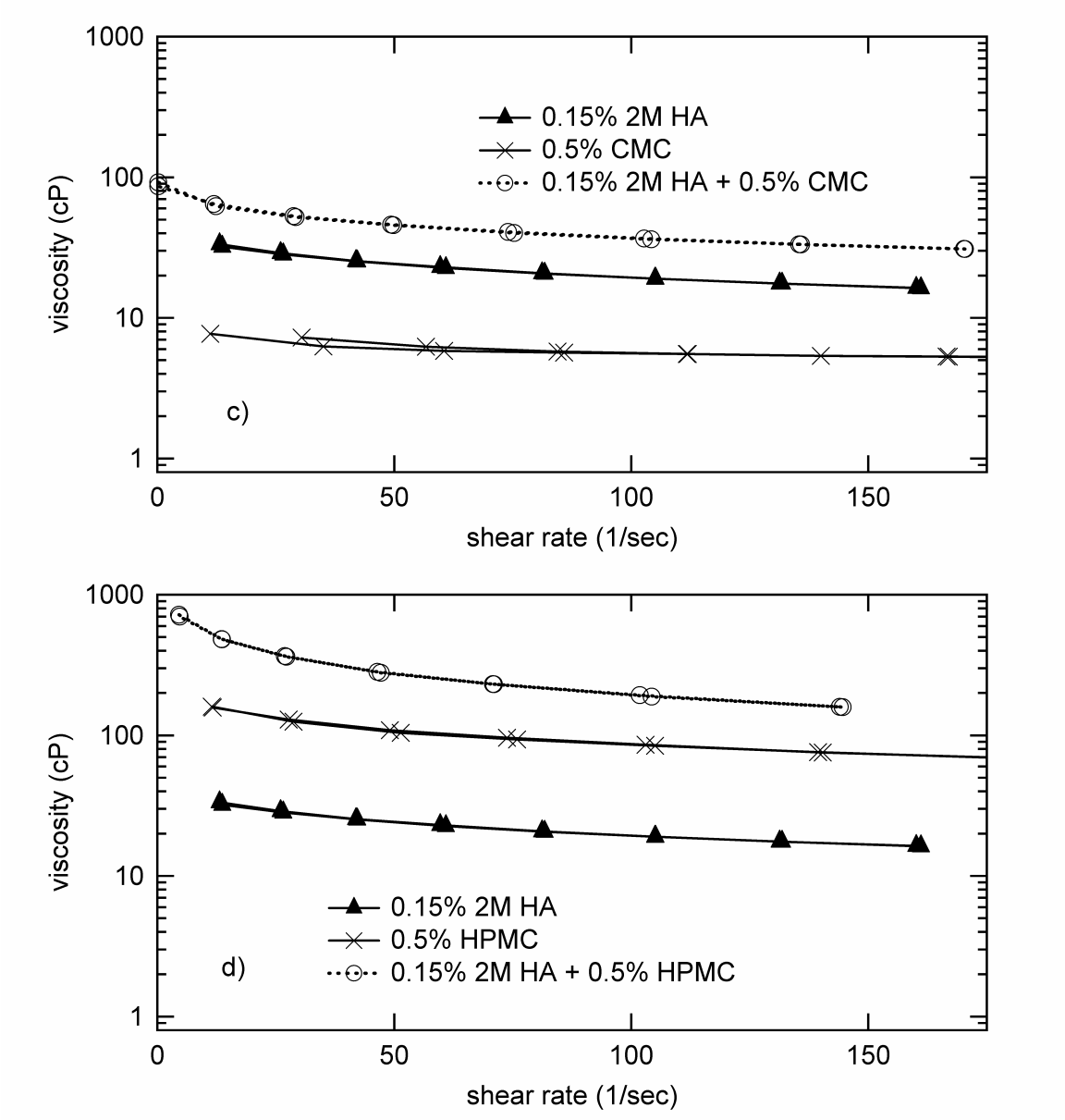

Figure 6.4c-d Hydrophilic polymers which show viscosity enhancement when mixed with hyaluronic acid: c) carboxymethylcellulose. d) hydroxypropyl methylcellulose.

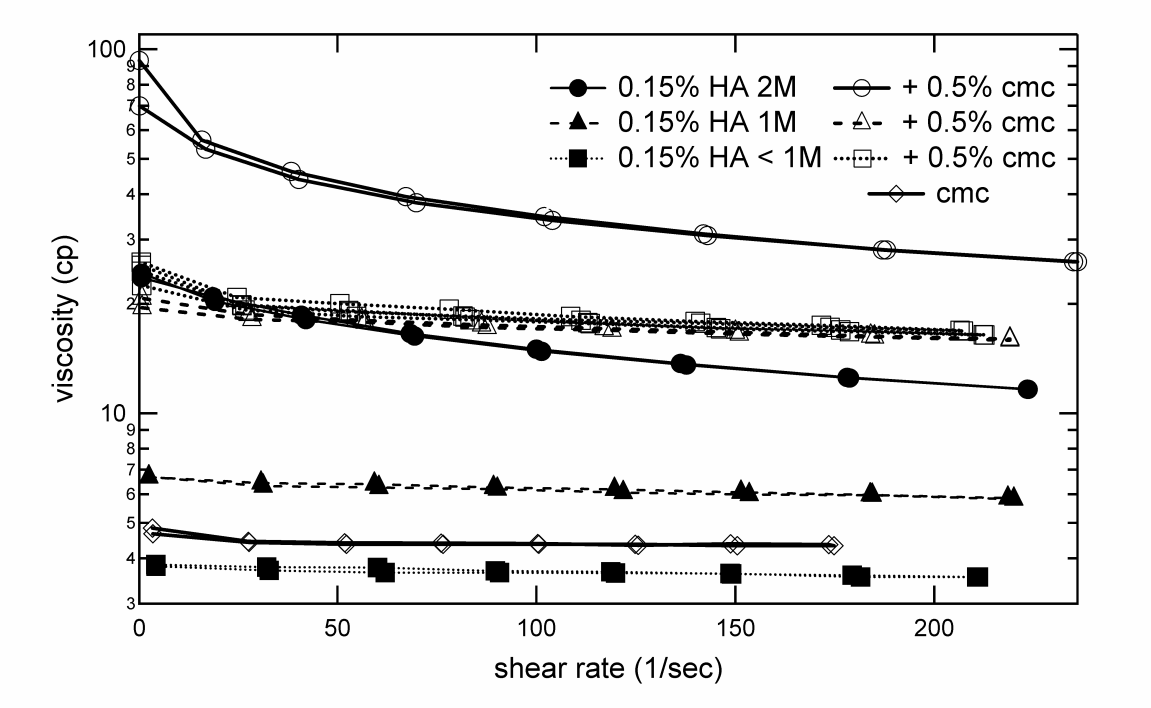

Figure 6.5 Viscosity enhancement by CMC on various molecular weights of hyaluronic acid.

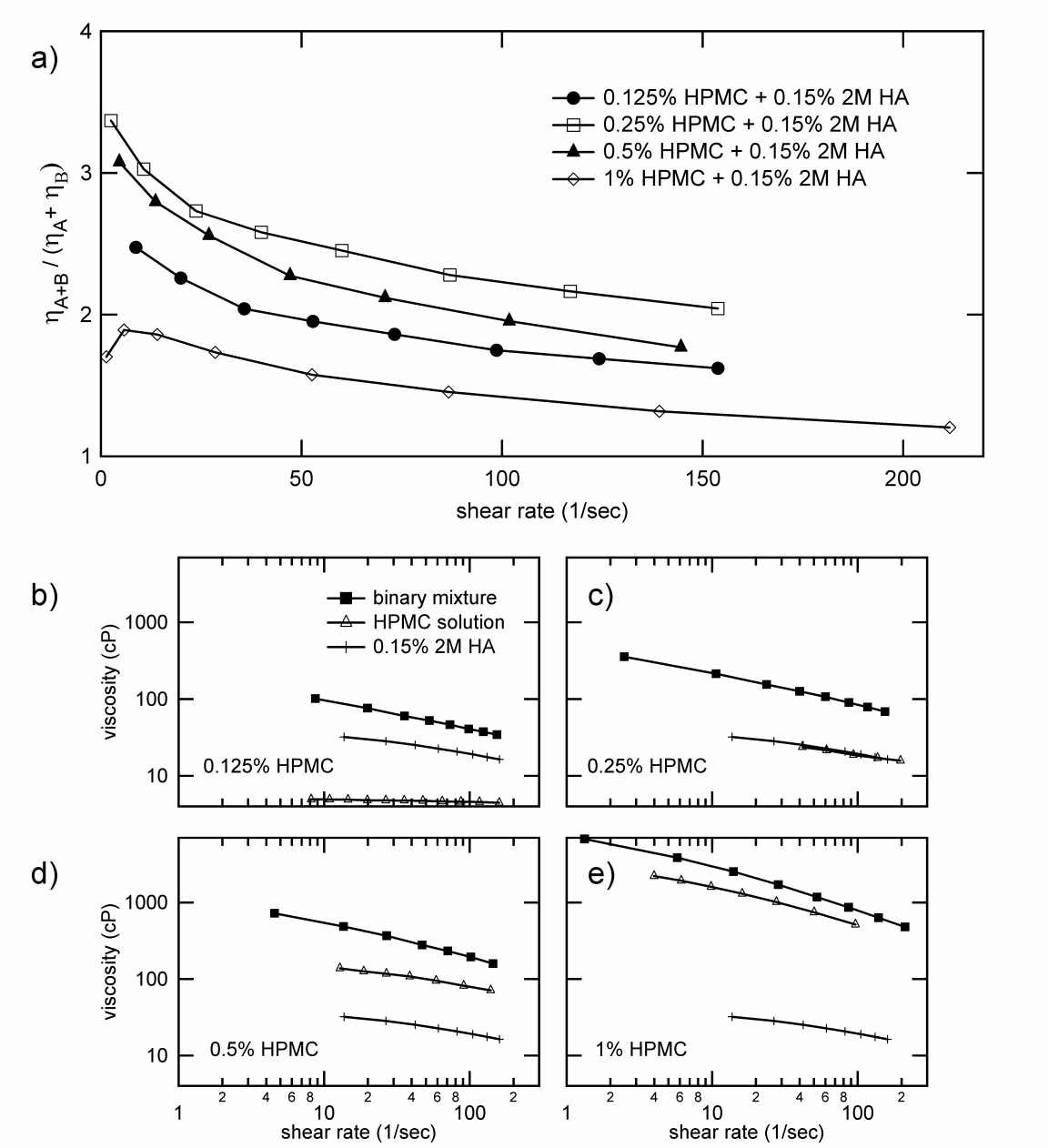

Figure 6.6 a-e Viscosity of binary solutions of 0.15% HA and HPMC with increasing concentrations of HPMC. a) Ratio of the viscosity of the binary solution to the sum of the viscosities of the single component solutions, shown in parts b-e. See Figure 6.6 f-g for the rest of the caption.

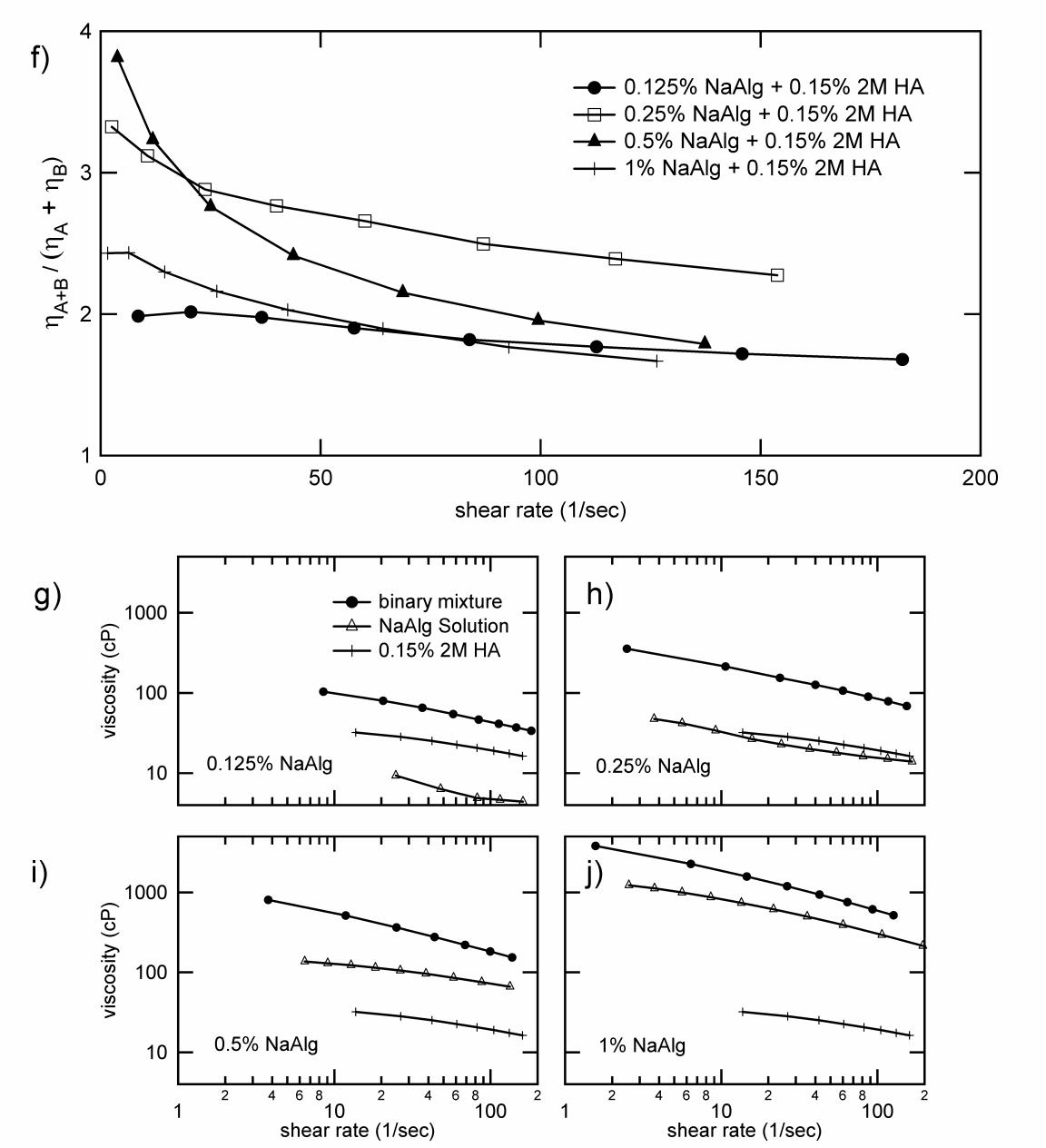

Figure 6.6 f-j Viscosity of binary solutions of 0.15% HA and NaAlg with increasing concentrations of NaAlg. f) Ratio of the viscosity of the binary solution to the sum of the viscosities of the single component solutions, shown in parts g-j. Note that the HA viscosity curve is the same in all parts b-e and g-j because HA concentration is always 0.15%. Normalization by the sum of the viscosities of the individual solutions provides a

measure of the nonlinear enhancement of the viscosity due to the interaction between the species (see text).

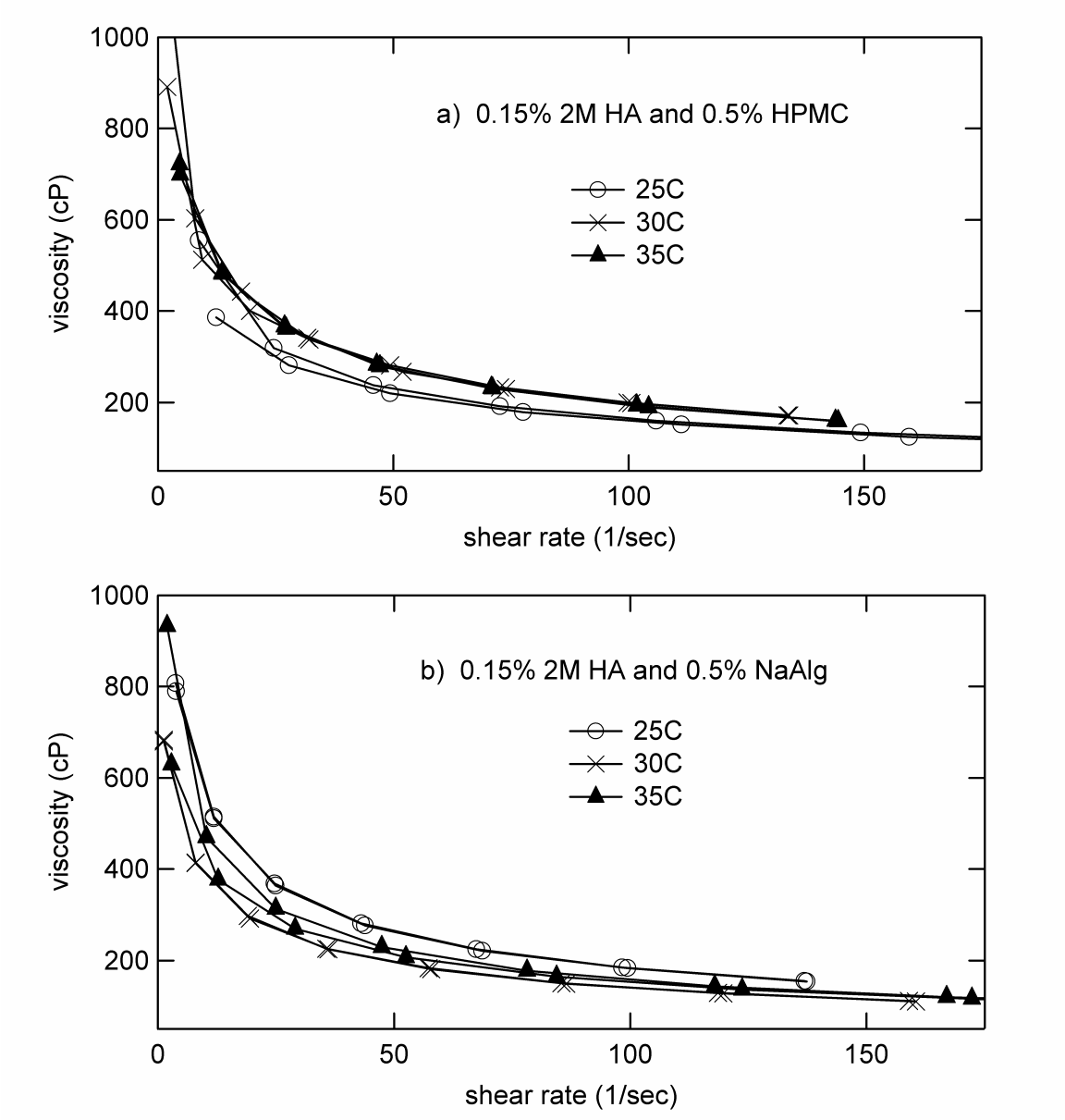

Figure 6.7 Temperature effects on the viscosity of solutions of 0.15% HA and a) 0.5% hydroxypropyl methylcellulose b) 0.5% sodium alginate

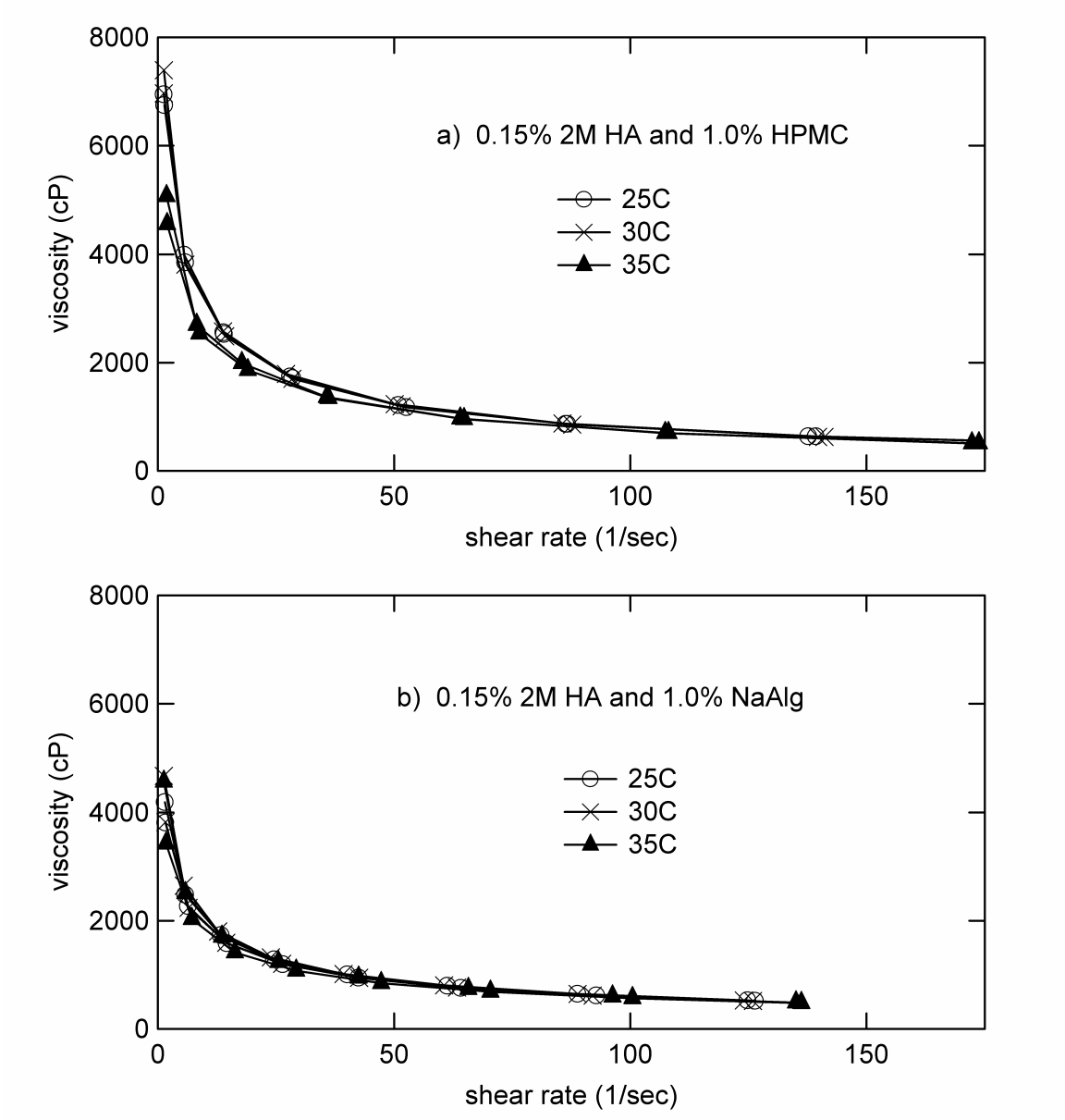

Figure 6.8 Temperature effects on the viscosity of solutions of 0.15% HA and a) 1.0% hydroxypropyl methylcellulose b) 1.0% sodium alginate

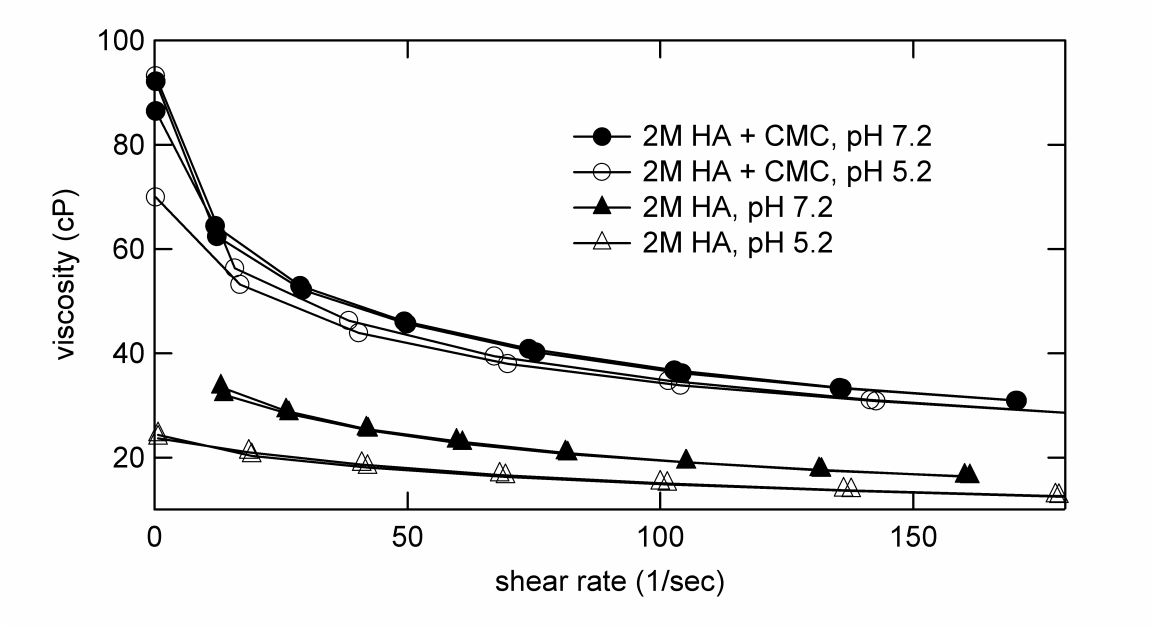

Figure 6.9 Effect of pH on viscosity of 0.15% HA and 0.15% HA with 0.5% CMC.

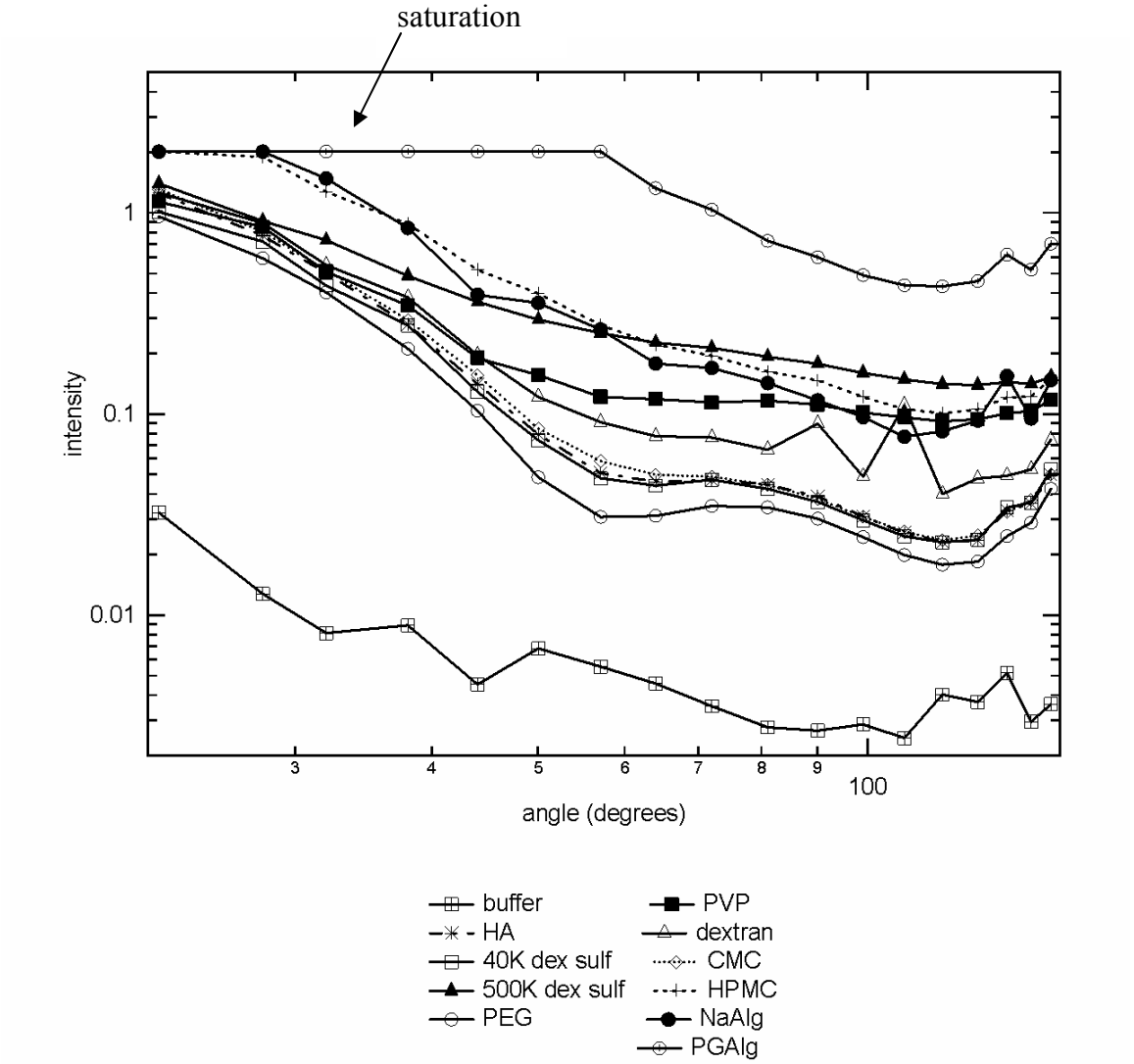

Figure 6.10 Light scattering from solutions of 0.15% hyaluronic acid and 0.5% additional hydrophilic polymer. Saturation of detectors occurs at an intensity of 2.

polymer	persistence length (mm)
NaAlg	15-9[25-27]
CMC	12-16[15, 16]
HA	4[18, 19]
dextran	1.8-1.5[17]
PVP	1.5-1.1[28, 29]
PEG	0.9[30]

Table 6.1 Persistence length of hydrophilic polymers. The values of the persistence length of PG-Alg, HPMC, and dextran sulfate were not available. Since these polymers share a backbone with one of the polymers in the table—HPMC with CMC, PG-Alg with NaAlg, and dextran sulfate with dextran—the persistence lengths of the polymer pairs can be assumed to be similar.

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