nonlinear regression analysis where the parameter k/v was used as a fitting parameter. This semiempirical modified Mooney equation (Equation 9.18), based on Mooney's original equation for spherical particles, involves only repulsive interactions between molecules due to molecular crowding at high concentrations (Mooney, 1951). This effect commonly termed "excluded volume" is simply the decrease in free volume to accommodate a particular large macromolecule due to its overall size (Zhou, Rivas, & Minton, 2008). Thus, viscosity increases with concentration can be predicted with this model provided there are no other interactions. Most importantly, the size and shape of the protein dictates the excluded volume that results theoretically in the lowest viscosity at a particular concentration that the protein can attain. As seen in Figure 9.3, the viscosity–concentration profile for mAb2 and mAb3 are adequately described using the modified Mooney equation, suggesting that there are no additional interactions beyond the repulsive interactions due to excluded volume for these mAbs. However, the viscosity–concentration profile for mAb1 could not be fitted using the modified Mooney equation, that is, the viscosity increases with concentration to a much greater extent than what would be expected for purely excluded volume effects. Since these three mAbs have similar size and shape, this strongly suggests that there are additional interactions between mAb1 molecules that cannot be accounted for with a simple excluded volume model. Adjustment of the ionic strength to ~150 mM NaCl results in little change to mAb2 and mAb3 viscosity profile resulting in a good fit to the modified Mooney equation, whereas the viscosity decreases dramatically for mAb1 with a viscosity–concentration profile that can be roughly fitted with the modified Mooney equation (Figure 9.4) (Liu et al., 2005; Liu, Nguyen, Andya, & Shire, 2006). These results suggest that the interactions are electrostatic in nature and that they are reversible.

Determination of the DLS interaction parameter at low concentration has proven useful as a predictor of high viscosity at high concentration (Connolly et al., 2012) as well as static light scattering (SLS) (Saluja et al., 2007; Yadav, Laue, Kalonia, Singh, & Shire, 2012) and analytical ultracentrifugation (Saito et al., 2012) for determination of the second virial coefficient, B22, again at low concentration. Although in general these determinations at low concentration are able to predict viscosity and assess protein–protein interactions at high concentration there have been discrepancies. It would be highly desirable to assess molecular weight as a function of concentration, especially at the higher concentration. This has been a formidable challenge since the most common assay to evaluate molecular size has been SEC that because of dilution during the chromatography results in reversible aggregates that dissociate to monomers (see Chapter 3 and Figure 3.18). Analytical ultracentrifugation can be a useful technology to assess these interactions at high concentrations, but because of the large refractive gradient formed during centrifugation at high concentrations the refraction of the light beam from the photoelectric scanner may result in it not being in alignment with the detecting phototube (Svensson, 1954a, 1954b). Minton and Lewis have shown that it is possible to acquire data at high protein concentration, using myoglobin at a loading concentration of about 180 mg/mL, and thin window gasket materials for the centrifuge cell centerpiece, which results in very small light paths (Minton & Lewis, 1981). Although this technique can be used, it is difficult due to wrinkling of the gasket, leaks, etc. An alternative technology using preparative