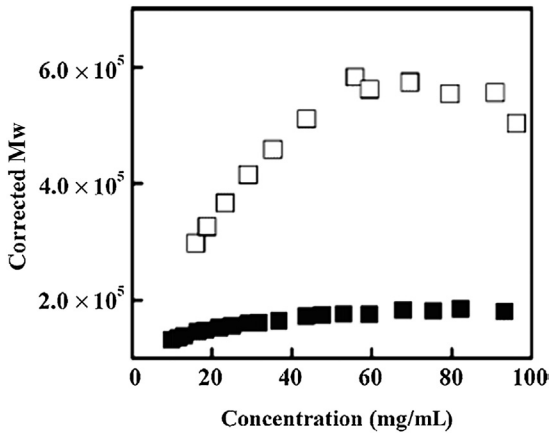

Figure 9.6 The corrected weight average molecular weight as a function of concentration for mAb1 at a loading concentration of 100 mg/mL in 240 mM trehalose, 40 mM histidine, 0.04% polysorbate 20 at pH 6 with no added salt (open squares) and with 150 mM NaCl (solid squares).
From Liu et al. (2005).

but the results clearly show that mAb1 self-associates as a function of concentration, that is, reversible, and after adjustment of ionic strength to ~150 mM with NaCl these interactions as reflected by the determined M_w are greatly reduced (Figure 9.6). Moreover, this nicely correlated with the reduction in mAb1 viscosity strongly suggesting that the attractive interactions are linked to the increased viscosity. Additional verification of these results was obtained by using SLS to determine molecular weight as a function of concentration for protein or mAb solutions at high concentrations (Scherer, Liu, Shire, & Minton, 2010). Analysis of the data using adhesive hard sphere models showed that at lower ionic strength (40 mM) the mAb1 data could best be described using a monomer–n-mer equilibrium association where the best-fit parameter for n was 6. Interestingly this is similar to the sedimentation equilibrium analysis that shows the Mw at higher concentration leveling off at ~4x the monomer molecular weight of 150 kDa (Figure 9.6). It was also shown that mAb2 does weakly self-associate to a dimer so that the initial assumption that there were no interactions beyond those of an excluded volume model was not correct, and this may account for the difference in highest molecular weight attained by self-associating mAb1 molecules as determined by AUC and SEC analysis.

Specific interactions in mAb1 that result in increase of viscosity

The reversible self-association of mAb1 and mAb2 was further studied by Kanai et al. (Kanai, Liu, Patapoff, & Shire, 2008) using full-length mAbs as well as Fab and $F(ab')_2$ fragments. It was proposed that a "network" or clustering of mAbs at high concentration was a primary determinant for the observed high viscosities. The concept of protein network formation as a function of concentration has been proposed previously (Porcar et al., 2010; Stradner et al., 2004) and is not without controversy (Shukla et al., 2008a, 2008b). Recently, additional studies using small angle neutron scattering of hemoglobin solutions have provided additional support for the