there was a similar trend in the viscosity–concentration profile of the fragments to that observed for the full-length mAbs, that is, viscosity increased dramatically for mAb1 $F(ab')_2$ with increased concentration and slightly for mAb2 $F(ab')_2$. Moreover the addition of NaSCN, which dramatically lowered viscosity of full-length mAb1, also reduced the viscosity of the mAb1 fragment to that of the mAb2 fragment (Figure 9.7(a)). This study showed that for mAb1 the Fab–Fab attractive interactions play an important role in determining the viscosity at high concentrations. From this result it was reasoned that if mAb1 Fab were added to full-length mAb1, there would be a reduction in viscosity due to the inhibition of network formation by binding of the monovalent Fab fragment. The surprising result was that although addition of mAb2 Fab to full-length mAb had no impact on viscosity, as expected, the addition of mAb1 Fab resulted in a twofold increase (Figure 9.7(b)). This strongly suggested that the Fab fragment itself has multiple interaction sites allowing for network formation. This was further substantiated by the difference in viscosity–concentration profile for mAb1 Fab versus mAb2 Fab (Figure 9.7(c)).

Since mAb1 and mAb2 have the same Fc it was expected that if there is significant interaction between Fab and Fc regions, and that since the Fab fragment has multiple interaction sites that the addition of mAb1 Fab to full-length mAb2 should result in a large increase in viscosity. The addition of mAb1 Fab to full-length mAb2 resulted in a small increase suggesting that mAb1 Fab alone does not form a significant interaction with the Fc region (Figure 9.7(d)).

The reversible self-association of mAb1 as a function of pH, ionic strength, and mAb concentration was further studied by Yadav et al. using the DSL-determined k_D interaction parameter, and the storage modulus, G′ determined with ultrasonic rheology (Yadav, Liu, Shire, & Kalonia, 2010). The G′ measurements as a function of pH and ionic strength showed similar trends observed for the kinematic viscosity–pH profile shown previously (Liu et al., 2005). It was also noted that the measured k_D values at the low concentration for DLS measurements at pH 5, 6, and 7 were not significantly different whereas the G′ value increased dramatically at ~ pH 6 for measurements >80 mg/mL. These results suggest that the magnitude of the existing attractive interactions at low concentration are greatly increased with increasing mAb concentration, and therefore the results of the dilute solution measurements do not necessarily reflect the extent and magnitude of these interactions at the higher concentrations. It was also observed that the pI for mAb1 determined by isoelectric focusing is about 7.8, and therefore the net charge on the mAb should be zero at pH 7.8. Thus, at pH values above 7.8 the mAb has a net negative charge and below 7.8 a positive charge, and under both conditions the mAbs should repel each other and have maximum attraction at ~ pH 7.8 since they theoretically could approach each other at close distance. This does not appear to be the case at high concentration where the pH of maximum attraction is ~6.7 as shown by the G′ and kinematic viscosity measurements. In particular, the different behavior at pH 6 versus pH 7 needed to be explained and Yadav et al. proposed a charge fluctuation model between histidine residues in close proximity to each other with pK_a at ~6 (Yadav, Liu, et al., 2010).