Impact of net charge versus localized surface charge distribution on protein–protein interactions and viscosity as a function of mAb concentration

Proteins and mAbs with a net charge are expected to contribute to repulsive interactions and although many solution properties such as solubility and viscosity at low concentrations can be interpreted with this colloidal view, the work of Yadav et al. (Yadav, Shire, et al., 2010; Yadav, Shire, & Kalonia, 2011; Yadav, Shire, & Kalonia, 2012) and recently Sarangapani et al. (Sarangapani, Hudson, Migler, & Pathak, 2013) have discussed the limitations of the colloidal model when applied to high concentration mAb solutions. At low concentrations at a given pH the mAb molecules behave as particles with a net charge and essentially can be treated as point charges. It has been shown that dilute protein solutions exhibit a minimum viscosity at the pI and increasing viscosity at pH values away from the pI (Buzzell & Tanford, 1956; Cofrades, Careche, Carballo, & Colmenero, 1993; Komatsubara, Suzuki, Nakajima, & Wada, 1973; Tanford & Buzzell, 1956). This has been attributed to electroviscous effects (Rubio-Hernandez, Carrique, & Ruiz-Reina, 2004). The primary effect is due to the diffuse double layer of counterions surrounding the molecule which increases the "drag" of the molecule due to the additional counterion layer whereby near the pI where the net charge is zero there would be significant reduction of this double layer leading to a minimum in the viscosity. Viscosity measurements of bovine serum albumin at higher concentrations show an opposite effect where the viscosity is a maximum at the pI. This phenomenon can be ascribed to the fact that at low concentrations the molecules sense a net charge repulsion, whereas at high concentration the surface distribution of charges can lead to large attractions via charge–dipole interactions. This was further demonstrated by determining the effective charge at 5 mg/mL mAb and viscosity at different concentrations for four different mAbs (Yadav, Shire, et al., 2010). The viscosity at concentrations >50 mg/mL did not correlate with the net effective charge of the mAbs. Whereas the viscosity differences at pH 6 and 15 mM ionic strength were mAb4>mAb1>mAb 5>mAb3, the effective net charge differences were mAb5> mAb3>mAb4>mAb1 (Figure 9.8). Thus, the net charge does not correctly predict the rank order of viscosity for the monoclonal antibodies, whereas the surface charge distribution does account for the different viscoelastic behaviors of the different mAbs (Yadav, Laue, et al., 2012).

Aggregation and viscosity of mAbs

As discussed, reversible attractive interactions that result in clustering of mAbs appears to be a driving force for the high viscosities observed at high concentration. A general schematic view of the role of aggregates in the generation of highly viscous solutions has been presented (Figure 9.9) (Yadav, Laue, et al., 2012). It was suggested that irreversible close contact mAbs aggregates would result in decreased viscosity where in the extreme these aggregates are suspensions. This simplistic model has been criticized since it has been shown that closely associated aggregates can indeed generate higher