particulates and immunogenicity has not been established unequivocally (Singh et al., 2010). In particular, such particulates are present in marketed products that are safe and efficacious despite a lack of monitoring. It has been emphasized that there are limitations currently in the assays available for particulate detection, and variability due to container/closure contacts, viscosity, concentration, and batch heterogeneity that make such measurements unsuitable as specifications for release and stability. However such measurements may be used to guide product development. Assays used to analyze for particulates have been discussed in Chapter 2.

If the aggregates cannot be resolubilized easily such as by temperature change or solvent conditions without denaturants such as GndHCl they are referred to as irreversible aggregates that can be separated on a gel sizing column. The aggregates may also be reversible, especially by dilution, and this type of aggregation is often referred to as self-association (Esfandiary et al., 2013). This may not be regarded as a problem since upon administration the concentration of protein is often decreased which results in loss of aggregate. However, as we will see in a later chapter on subcutaneous (SC) delivery of mAbs, self-association may have huge consequences for delivery as well as manufacture of SC formulations.

Protein aggregation is undesirable because it can lead to a reduction in activity and altered pharmacokinetics (Klotz & Thomas, 1993; Maggio, 2010; Shao, Li, Krishnamoorthy, Chermak, & Mitra, 1993). Aggregates have also been implicated in a heightened immune response (Moore & Leppert, 1980; Ratner, Phillips, & Steiner, 1990; Ring, Stephan, & Brendel, 1979; Underwood, Voina, & Van Wyk, 1974), and adverse events due to antibody aggregates have also been reported (Demeule, Gurny, & Arvinte, 2006; Ryan, Webster, & Statler, 1996). The concern over mAb aggregates especially for IV dosing has resulted in a limitation by the World Health Organization (WHO) of less than 5% aggregate in commercialized mAb therapeutics.

Protein aggregation occurs as the result of protein–protein interactions, which can increase as a function of concentration whereby the average distances between protein molecules decrease. If the aggregation is reversible it may be difficult to detect by sizing chromatography since the protein solution becomes more dilute as it separates on the column. As an example human relaxin has been shown to undergo reversible dimerization (Shire, Holladay, & Rinderknecht, 1991). When analyzed by size exclusion chromatography, essentially one peak is detected with a molecular weight at ~6 kDa which is close to the expected monomer molecular weight of 6.5 kDa as determined by amino acid composition (Figure 3.18(a); Canovadavis, Baldonado, & Teshima, 1990). The weight average molecular weight is concentration dependent as shown by analytical ultracentrifugation (Figure 3.18(b)) with an extrapolated molecular weight near zero concentration of protein to that expected of a monomer. It has been reported that increasing the loading concentration to 2 mg/mL results in relaxin eluting on sizing chromatography as a dimer (Canovadavis et al., 1990). Thus, if one is not aware that reversible self-association is occurring, sizing chromatography may not detect association due to dilution on the column.

Aggregation of proteins produced using recombinant DNA technology can occur as a result of different processing steps, usually referred to as unit operations. An