equipped with optical systems, temperature control, and interfacing with computer control and data acquisition systems. The AUC experiment can be performed in essentially two modes, sedimentation equilibrium and sedimentation velocity. In sedimentation equilibrium the centrifuge is run at sufficiently low speeds so that the sedimentation of the protein is equivalent to the diffusion, resulting in a time-invariant concentration versus radial position profile. Analysis of the concentration gradient yields molecular weight distributions. The use of specific models, such as monomer to dimer, etc., allows for the determination of association equilibrium constants for reversibly self-associating protein systems. Sedimentation velocity is conducted at higher centrifugal fields so that different sized protein molecules migrate to the bottom of the centrifuge cell. The migration of the sedimentation boundaries is dependent on both the molecular weight of the species and the shape as reflected in the frictional coefficient. Although sedimentation equilibrium is useful for obtaining actual molecular weight, sedimentation velocity has become the main use of the analytical ultracentrifuge in development of protein and mAb therapeutics. This analysis has been facilitated with the development of computer software, which fits the sedimentation data to the Lamm equation (Schuck, 2000). The data are generally displayed as a distribution function yielding peaks that are representative of the individual sedimenting species providing the species do not self-associate. Regulatory agencies are now aware of the deficiencies of SEC for determination of protein aggregates (see the discussion on SEC) and are requesting the use of alternative biophysical methods, preferably AUC, to confirm the veracity of the SEC results. The argument for using an alternative method to confirm SEC results has been presented (Carpenter et al., 2010). The quantitation of aggregates and the limits of precision and accuracy in AUC measurements have been explored using three mAbs of unspecified origin (Pekar & Sukumar, 2007). This study showed a precision of ±0.3% over a measured range of 0.6–67% aggregate. Good accuracy, as ascertained by aggregate spiking experiments, could be achieved down to aggregate levels as low as 1.5%. Examples of the use of AUC to confirm SEC results for mAbs include studies on two humanized mAbs stored at −70 °C and 40 °C (Liu, Andya, & Shire, 2006) and a study of aggregates of a humanized mAb (Andya, Liu, & Shire, 2010). In this later study, SEC and AUC were used to determine percent of aggregates in the same mAb sample. Although SEC showed a single monomer peak at 99.6% with a small amount of aggregated species the AUC analysis showed a significant aggregate peak (Figure 2.5(a)). This amount of aggregate was concentration dependent as shown by sedimentation analysis at different loading concentrations (Figure 2.5(b)) as well as a determination of weight average molecular weight by sedimentation equilibrium (Figure 2.5(c)). This again illustrates what can be missed by SEC analysis due to dilution during the chromatography.

AUC has also been used to help confirm SPR determinations. A recent publication used AUC in a competitive binding mode to determine if published SPR data comparing the binding of three inhibitors of vascular endothelial growth factor A (VEGF-A) were correct (Yang et al., 2014). Essentially, the solution-based AUC method showed that the SPR results were not correct. This was also demonstrated by using different SPR formats, which gave different results.