Figure 9.5 Sedimentation equilibrium measurements using preparative centrifuges. Figure created by Sandeep Yadav.

equilibrium, providing sufficient time to carefully remove the centrifuge tubes and place them into the automated microfractionator. Obviously once the individual fractions are diluted the gradient is destroyed, but a total concentration at the radial position of sampling is obtained resulting in the creation of an absorbance versus radial position plot (Figure 9.5). Generally the analysis of analytical ultracentrifugation data is done under sufficiently dilute conditions so that for an ideal sedimenting species the following equation is used (Yphantis, 1964):

$$c(r) = c_0 \exp\left[M_w (1 - \nu\rho) \omega^2 \left(r^2 - r_0^2\right) / 2RT\right] \qquad (9.19)$$

where c(r) is the mass concentration at radial position r, c_0 is the initial loading mass concentration, r_0 is the radial reference position, ν is the partial specific volume for the protein, ρ is the solution density, ω is the angular velocity, R is the gas constant, and T is the absolute temperature during centrifugation. However for high-concentration protein/mAb solutions the conditions are no longer ideal due to the presence of protein–protein interactions and the determination of the actual weight average molecular weight, M_w, from the experimentally determined apparent molecular weight, M_{app}, requires the use of an activity coefficient as described by the following equation (Chatelier & Minton, 1987):

$$M_{app} = M_w \left[1 + c\, (d \ln \gamma/dc)\right] \qquad (9.20)$$

where γ is the activity coefficient for the protein or mAb. Since mAb2 and mAb3 viscosity data could be adequately described by the excluded volume equation of Mooney it was assumed that mAb2 and mAb3 were essentially monomeric in solution. This assumption results in a multiplicative correction factor as a function of mass concentration, c, when M_w is set equal to 150 kDa. After applying this correction to the apparent molecular weight data for mAb1, a corrected weight average molecular weight was obtained. These corrections are estimates that may not account for complete correction,