have a profound effect on the elution profiles. Thus, a fair amount of research has been done to use membrane materials that do not have significant interaction with the biomolecules. For protein species that are well below 1 mm in size the elution is highly dependent on the diffusion coefficient. Assuming a spherical shape for all species the molecular weight can then be estimated from the retention times, which are related to the external field strength and diffusion coefficients by a well-defined equation. The precision and accuracy of AF4 for protein and mAb characterization have been explored, and resolution compared to that obtained by SV-AUC and SEC (Liu et al., 2006; Liu, Zhu, Shire, & Demeule, 2012). The conclusion of these studies was that AF4 is a useful orthogonal method to determine the size distribution of protein aggregates and fragments. However, appropriate procedures and careful optimization are required to provide reliable quantitative information. Since this separation method is very different from that of SEC, AF4 may be useful as another orthogonal method to confirm results from SEC.

Methods to evaluate particulates in protein formulations

Large visible particulates can be assessed by visual inspection where the inspection conditions are well defined. However, human visual inspection can be problematic and often instruments designed for visual inspection are used (Borchert et al., 1986), and brief reviews have been presented (Das, 2012; Das & Nema, 2008). Subvisible particulates are analyzed using the US Pharmacopeia (USP) microscopy method. However, this method can be tedious and time-consuming. A more automated and common analysis is done using the light obscuration method with instruments such as the HIAC-Royco particle counter (Borchert et al., 1986; Demeule, Messick, Shire, & Liu, 2010, USP XXII, 1990). In this assay the sample is flowed past a small window where light is focused through for measurement with a photomultiplier tube. Particles as they traverse the light beam reduce the intensity of the light, which is proportional to the cross-sectional area of the particle. Pulses are then counted within the different amplitude ranges resulting in a particle size distribution. Both the microscopy and HIAC assays are official tests in the USP. The USP requirements for HIAC determination are that there should be at or below 6000 particles at >10 mm size and 600 particles at >25 mm size per container (USP XXII, 1990). There have been many alternative methods developed, some of which are discussed in an AAPS themed issue (Shire & Laue, 2010). Recently a device called the Archimedes device that determines particle sizes by buoyancy measurements has been compared to standard techniques for particle size determination using a mAb sample (Panchal, Kotarek, Marszal, & Topp, 2014; Patel, Lau, & Liu, 2012).

Another technology that is being used more frequently is microflow digital imaging (MDI) where photo microscopy is used to analyze distribution and overall morphology of particulates and includes instruments such as those manufactured by Occhio, Brightwell, and Flow Cam (Wuchner, Buchler, Spycher, Dalmonte, & Volkin, 2010). A comparison of four different instruments has recently been published (Zolls et al., 2013). These types of measurements may prove very useful in discerning irregularly shaped protein particulates from more spherical silicone oil droplets that are often found in prefilled syringes. This will be discussed in more detail in later chapters.