>100 μm has left a large gap in our ability to assess the level and distribution of very low micron and submicron-size aggregate particles that are felt to be an important size range of aggregates responsible for generating an immunogenic response (Carpenter et al., 2009). Hence, a significant effort has been under way for almost a decade to develop new and improved analytical tools capable of detecting and quantifying aggregates in this size range to fill this gap. In so doing, significant progress is being made in this area. This progress is supported by the development of such techniques as flow imaging microscopy, nanoparticle tracking analysis using light scattering, and other more exotic physical techniques (e.g., resonant mass measurements, Archimedes) (Hawe et al., 2014).

Clearly, in assessing biosimilarity in terms of aggregation, the level of aggregates present is critically important. Here the goal is not to match aggregate levels determined in the RP, but rather not to exceed an upper aggregation limit based on experimental aggregation information obtained on the RP. In addition, given the importance of the physicochemical properties of the aggregates, biosimilar manufacturers should also consider conducting some appropriate level of comparability work focused on characterizing the structural nature of the aggregates found in the RP to those found in its biosimilar using various physicochemical approaches that have been reported recently in the scientific literature (Iacob et al., 2013; Iwura et al., 2014; Remmele et al., 2006). Such work would be focused on detecting the presence of significantly new forms of aggregates in the biosimilar relative to the RP that might be associated with different properties and therefore different immunogenicity and toxicological effects.

2.10 THE ROLE OF PHYSICOCHEMICAL MEASUREMENTS IN ASSESSING THE IMMUNOGENICITY OF BIOPHARMACEUTICALS

In generating physicochemical characterization data to adequately support the biosimilarity of a biosimilar to its RP, there is one area where present physicochemical analytical technology still has significant shortcomings in adequately assuring that biosimilarity exists. This gap in assessing biosimilarity concerns the area of immunogenicity. At present, much of the physicochemical investigation effort in this area has been focused on trying to improve the detection and quantification of aggregation, as discussed in Section 2.9. Unfortunately, little in terms of definitive useful information has been gathered about what specific attributes of a biopharmaceutical aggregate's structure one should be concerned with in terms of immunogenicity [other than some ideas concerning size and the presence of native-like repetitive structural elements (Rosenberg, 2006)].

Although great strides have been made in establishing analytical physicochemical biosimilarity (as a way to avoid the need to conduct costly and lengthy clinical trials), at present the complexity of factors that can play a role in immunogenicity (Mukovozov et al., 2008) makes it unavoidable to realize that physicochemical or biological data cannot adequately override the need for some clinical work to establish the absence of any immunogenicity concerns with a given biosimilar. Although