(see Equation 7.9 in Houde and Berkowitz, 2014c) (note: in conducting these measurements, information about the amount of sample injected into the SEC system is not needed, which significantly improves the accuracy of the extinction coefficient assessment). By taking the ratio of Equation 7.9 in Houde and Berkowitz (2014c) containing the experiment parameters (monomer peak areas from each detector) determined for the biosimilar and the same equation containing the experiment parameters for the RP, the same collection of proportionality constants present in each equation (which includes the instrument constant and the known unmodified amino acid sequence MW of the target biopharmaceutical that converts these values to an absolute protein extinction coefficients) will cancel, further simplifying and improving the accuracy of the comparison measurements. As a result, if the biosimilar's extinction coefficient is highly similar to the RP's extinction coefficient, this experimental ratio should be very close to a value of 1.00. Since a number of experiments where repetitive measurements on two different samples of the same biopharmaceutical, conducted by the author, have yielded mean ratio values that have shown variabilities from 1.00 of only about ±1%–2% at a 95% CL (unpublished data), experimental ratios between a biosimilar and RP that range between 0.98 and 1.02 would likely establish a high level of confidence in the biosimilarity in their extinction coefficients.

2.9 THE UNIQUE PROBLEM OF AGGREGATION IN ASSESSING BIOSIMILARITY

One of the major differences indicated in Table 2.1 between pharmaceuticals (including generics) and biopharmaceuticals (including biosimilars) concerns the propensity of biopharmaceuticals to form a unique class of high MW product-related impurities due their ability to self-associate or aggregate. This form of degradation typically refers to the physical association of monomeric units of a biopharmaceutical to form higher MW material due to their unique physicochemical surface properties and surface topology. Although some biopharmaceuticals may intrinsically display concentration-dependent self-association, which is generally reversible (and may or may not be an issue), the more common and concerning forms of stable aggregates are those generated from altered biopharmaceutical material that arises from the biopharmaceutical's exposure to a range of physical and chemical environmental conditions that stress the biopharmaceutical during its production. These stress conditions may lead to a chemical or physical PTM of the biopharmaceutical that alters its HOS and/or surface properties making it prone to aggregate. Such aggregates can span an enormous range of sizes (nanometers to microns) and display different physicochemical properties, as indicated in Figure 2.15.

Although the biopharmaceutical industry depends heavily on the use of SEC as the key analytical tool for detecting and quantitating aggregation, there is now a clear understanding of SEC's potential limitations and associated artifacts (Arakawa et al., 2010; Berkowitz, 2006; Carpenter et al., 2010). In recent years, other biophysical tools, which have existed for a fairly long time, have been greatly improved and are now also capable of providing useful quantitative physicochemical characterization information about the aggregation that is present in a biopharmaceutical