separation technologies), is the key analysis tool. In the case of biophysical characterization, the approach that has evolved has resulted in the use of a number of different low-resolution biophysical analytical tools (see Table 2.2). However, the poor resolution of these tools coupled with the driving need to obtain more detailed information about a biopharmaceutical's HOS has led to the search for more informative, yet still practical, biophysical tools. In the last decade, this situation is beginning to show significant signs of being realized as a growing number of more advanced high-resolution biophysical tools (again see Table 2.2) are being investigated and developed in an attempt to achieve a more detailed characterization of a biopharmaceutical's overall HOS (Berkowitz et al., 2012; Marino et al., 2015).

2.6.2.1 Commonly Used Low-Resolution Biophysical Tools to Assess HOS

The most common and readily available, and therefore frequently employed, biophysical tools used in the biopharmaceutical industry assess the HOS of biopharmaceuticals from a global perspective. Such biophysical tools probe and collect the signal output from a number of physicochemical structural elements on a biopharmaceutical that may be different or identical (but located in different physicochemical environments) that nevertheless emit a highly similar signal output that changes little from their unaltered state even when the physicochemical environment of these structural elements is changed. In so doing, they frequently have little capability to detect small subtle changes in the HOS of a biopharmaceutical, especially when one includes their inherent low signal-to-noise ratio.

This difficulty is illustrated by a hypothetical case where the CD measurements are conducted on an RP and its biosimilar that consist of a number of separate α-helix segments that are spread across different locations of the entire biopharmaceutical molecule. Since CD measurements from virtually all α-helices produce effectively the same CD spectrum (signal output), if only a small part of one α-helix segment in the biosimilar is altered (e.g., converted to a random coil) relative to the RP, that altered region of α-helix segment will yield a different CD output spectrum that still overlaps extensively with the normal CD outputs from the rest of the unaltered α-helix material still present in the biosimilar sample. As a result, the small altered CD signal output between the RP and biosimilar will need to be extracted from nearly the same large normal background CD spectrum generated from the intact (unaltered) α-helix material (along with the measurement's associated noise) still present in both samples. This large background of normal α-helix CD signals will significantly reduce one's ability to see the actual small difference that exists between the RP and the biosimilar relative to an ideal hypothetical case where each individual α-helix segment generated its own unique CD spectrum that didn't overlap. In the latter case increasing the concentration of both samples would improve one's ability to assess the difference in the altered α-helix CD spectrum between the RP and the biosimilar over the noise that is present in the physicochemical measurements.

However, in the real case where the CD spectrum from the normal and altered α-helix material present overlap significantly, the approach of increasing concentration would not be very effective since the large normal CD background spectrum from the unaltered α-helix material would also increase. In this situation, one is faced with the typically difficult problem of trying to extract a small signal