2012). The task of detecting and quantitating the presence of such material is commonly the responsibility of analytical aggregation tools used under nonreducing and reducing conditions, for example, SDS-PAGE (den Engelsman et al., 2011) as well as MS.

2.6.2 Analytics at the Biophysical Level: HOS Including Secondary, Tertiary, and Quaternary Structure

In assessing the biophysical consistency of a biopharmaceutical, in terms of its HOS, the approach that is used is significantly different from that taken in assessing the biochemical primary structure of a biopharmaceutical, where one analytical tool, the mass spectrometer (in combination with the supporting role provided by various

TABLE 2.2
Low-Resolution Biophysical Method Being Used and High-Resolution Methods Being Investigated in Characterizing Biopharmaceuticals

Method	Key Attributes of HOS that are Assessed
Low-Resolution	
UV spectroscopy	Aromatic amino acid physicochemical environment
Fluorescence spectroscopy	Aromatic amino acid physicochemical environment
Circular dichroism	Polypeptide (secondary) structure and aromatic amino acid physicochemical environment (tertiary structure)
Fourier transform infrared spectroscopy	Polypeptide (secondary) structure via information acquired about the amide bond
Differential scanning calorimetry	Global and domain structure
Size-exclusion chromatography	Quantitative aggregation and aggregate size distribution
Analytical ultracentrifugation	Quantitative aggregation and aggregate size distribution, and shape, physicochemical properties
Asymmetric flow field-flow fractionation	Quantitative aggregation and aggregate size distribution
Light scattering (static and dynamic)	Qualitative or semi-quantitative information about aggregation and aggregate size distribution
High-Resolution	
Mass spectrometry	
Footprinting	Indirect solution structure information (approaching amino acid resolution) via chemical reporter's ability to react with the biopharmaceutical
Native	Global structure information in the gas phase via charge state distribution
Ion-mobility	Global structure information via size, shape, and charge in the gas phase
Antibody arrays	Indirect solution structure information (at the peptide level) via epitope reporter accessibility
Nuclear magnetic resonance	Structure information (approaching the atomic level) by studying the physicochemical environment of active NMR nuclei (^{1}H, ^{13}C, ^{15}N)