

Table 5.1 (Continued) Biopharmaceutical Tools Peculiarities and Attributes

Technique	Peculiarity	Attributes
<b>Hydrodynamics</b>		
AUC	Global structure via sedimentation, concentration-dependent aggregation, size (hydrodynamic volume), and molecular weight	Biophysical and HOS; changes in HOS
SEC	Global structure, total aggregation, size, hydrodynamic volume	Biophysical and HOS; aggregation
Field flow fractionation (asymmetrical flow FFF, hollow fiber flow FFF)	Global structure, size, aggregation	Biophysical and HOS; aggregation
Viscosity	Aggregation, biophysical	Biophysical and HOS; aggregation
LS	Static light scattering (MW), Dynamic light scattering ( $D$ , $k_b$ , $\eta$ ), small-angle x-ray scattering (SAXS)	Biophysical and HOS; aggregates
	Nano-, submicron-, micronized aggregates	Biophysical and HOS; aggregates
<b>Imaging</b>		
X-ray crystallography	Atomic level resolution (electron density)	Biophysical and HOS
Electron microscopy (cryoelectron microscopy, electron tomography)	Global surface structure, molecular shape	Biophysical and HOS
Solution scattering (SAXS, wide-angle x-ray scattering, small-angle neutron scattering)	Global surface structure, molecular shape	Biophysical and HOS
<b>Chemical Reactions (Covalent, Noncovalent)</b>		
Denaturants	Global structure	Biophysical and HOS
Extrinsic fluorescence	Global surface structure	Biophysical and HOS
Enzymatic digestion	Global structure	Biophysical and HOS
<b>Stress-Induced Spectroscopy</b>		
Intrinsic fluorescence (pH, dielectric constant, temperature, magnetic field, galvanic field, polarity, dielectric constant)	Secondary and tertiary structure; quaternary structure; fourth-dimensional interactions with formulation	Biophysical and HOS

Note: MW, molecular weight.

One of the disadvantages of the method is that it often fails to distinguish between optical and geometrical isomers and the positions of the substituent in *o*-, *m*-, and *p*- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similarly fragmented ions.

In MS, the spectra are used to determine the elemental or the isotopic signature of a sample and the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds