contact and interaction with each other, thus creating favorable spatial conditions for agglomeration and aggregation.

It was noted also that many chemical and physical processes in freeze-died formulations, both proteins and small molecules, do not follow simple kinetics law, and suggested that such observations can serve as an indirect evidence for heterogeneity of the local environments [22]. Indeed, heterogeneity would result in different populations of molecules of the active ingredient with different individual rate constants. As the common experimental methods (e.g., high-performance liquid chromatography) would measure bulk-averaged concentration of the reaction products or the extent of the conversion of a reactant, the apparent rate constant would represent a weight-averaged sum of the individual rate constants. In this case, even if the kinetics of each individual reaction corresponds to a simple reaction order, e.g., first-order, the average kinetic curve would reflect distribution of the individual rate constants, resulting in a more complex kinetic curve.

Mechanisms for the Inhomogeneity

Common mechanisms for the inhomogeneity (heterogeneity) are related to freezing (ice formation), resulting in redistribution of solutes via, e.g., inclusion inside ice crystals. In addition, we note that heterogeneity is a general property of solutions in both liquid and solid state (glasses). One specific case of heterogeneity was reviewed [33], where water clustering in solutions and amorphous solids was discussed as a probable case of heterogeneity on the sub-nanometer to nanometer-length scale. In the following two sections, we consider large-scale heterogeneities which are directly related to ice/solution interfaces.

Protein Sorption on Interfaces

Protein partitioning between bulk solution and interfaces would be an obvious case of heterogeneity, with properties of protein molecules on the interface be different from the bulk phase. Interaction of proteins with ice surface was studied for antifreeze proteins in some details. The propensity of some proteins to interact with ice surfaces is one of the defense mechanisms in nature that prevents ice growth due to increase in the curvature of the ice–water interface and thus resulting in non-colligative local freezing point depression [34–36]. The interaction of the antifreeze proteins with ice is mainly based on hydrogen-bonding mechanisms. Propensity of antifreeze proteins to ice/solution interface was used to purify antifreeze proteins, to separate them from other proteins present in *Escherichia coli* lysate [37]. In this case, non-antifreeze proteins were actually excluded from ice interface. Such exclusion of “common” proteins from ice interface is an important observation, considering that pharmaceutically relevant proteins are obviously not antifreeze proteins and