

observed splitting of XRD peaks. It was also estimated using molecular modeling that the observed shifts in the peak positions are equivalent to applying a hydrostatic pressure of 2–3 kbar.

Furthermore, in a separate and a carefully designed study of water–sucrose system it was shown that, when the trapping (and therefore a probability of creating regions with elevated pressure) was minimized, a single-glass transition event was observed, followed by the onset of ice melting [21]. In that investigation, solution enclosure by ice crystals was prevented by using either scraped-surface freezing process or slow growing of ice crystals from solution containing ice nucleus.

To summarize the discussion on heterogeneity of protein environments due to solution inclusion by ice crystals, we note that the absolute majority of pharmaceutical formulations contain more than one solute. The solutes have different diffusion coefficients and/or interaction with ice surface, and therefore can be expected to develop variable extent of spatial heterogeneity under nonequilibrium freezing conditions. Protein environment in such materials will obviously be different in different parts of the sample, which could lead to distribution of degradation rates. An additional mechanism for the heterogeneity, i.e., due to local pressures as a result of volume expansion during water-to-ice transformation, should also be taken into consideration.

Inhomogeneity as a General Property of Solutions and Glasses

Inhomogeneity on the length scale of sub-nanometer to nanometer is a fundamental property of amorphous liquids (solutions), as summarized earlier [33]. Moreover, even a single-component system such as liquid water is nonhomogeneous on the molecular level, as illustrated by the Frank and Wen's model [53] (Fig. 2).

Many important events in solutions, such as crystal nucleation are expected to be dependent on local heterogeneity, with small clusters of molecules serving as nucleation centers. In addition, larger-scale heterogeneities (on the scale of hundreds of nanometer) were observed in solutions under certain conditions, [55] although the origin of the driving force for such large-scale heterogeneity is obscure.

For amorphous solids (glasses), their intrinsic heterogeneity and its pharmaceutical significance were discussed in [22], and briefly outlined below. The heterogeneous nature of glasses is reflected in non-exponential behavior of the structural relaxation, as expressed in the well-known Kohlrausch–Williams–Watts (KWW) equation:

$$X(t) = \exp\left(-\frac{t}{\tau_{\text{KWW}}}\right)^{\beta_{\text{KWW}}}$$

where $X(t)$ is a property of the material, τ_{KWW} and β_{KWW} measure the average relaxation rate and the extent of non-exponentiality and the distribution of relaxation times, respectively. Values of β_{KWW} vary from 0 to 1, with 1 representing a single-exponential relaxation process. Typically, many organic amorphous materials have