of salt may not significantly impact the overall thermodynamics of mixing. However, a large amount of buffer salt, which is not generally recommended for a variety of reasons (44), could have a significant impact on phase behavior and is discussed later in this chapter. Additionally, there are other factors to consider during the process, such as the freeze concentration effect that occurs as a result of removal of water from the solute phase via crystallization. This concentration effect can be very dramatic, resulting in concentrations as high as 50 times the original solution concentration (45). Several models exist for the understanding of phase separation in dilute polymer solutions. The most widely used and accepted model is the Flory-Huggins theory [eq. (5)], which uses a lattice model to predict the compatibility of polymers. However, the strong influence of orientation-dependent interaction forces (i.e., hydrogen bonding) makes the Flory-Huggins theory inadequate for predicting phase separation in systems of direct interest to freeze-drying (17,19). Many studies have focused on modifying the Flory-Huggins theory to incorporate the effects of such interactions (19,46). The results of such work may be useful for the prediction of phase separation during freezing, but only one such thermodynamic model has been developed thus far.

In the late 1960s, Edmond and Ogston developed a model to study phase separation in dilute polymer solutions, which uses the osmotic virial equation truncated after the second term (47). Although this model was developed for dilute systems and does not directly apply to concentrated, frozen systems, it has been extrapolated to such systems to assess the likelihood of phase separation during the freezing step. In the late 1990s, Heller et al. directly applied the model to freeze-dried systems (17). Although there were several approximations of dubious validity involved, they were able to demonstrate the potential for phase separation to occur during the freezing process (17). Additionally, they addressed the presence of what they refer to as a "phase separation envelope" (Fig. 3), which introduces the kinetic factor that arises because of an increase in viscosity and eventual glass transition at subzero temperatures. Essentially, phase separation may be thermodynamically favored because of concentration and temperature effects, but kinetically hindered because of immobilization of molecules in the glassy state. Thus, there is a period of time in the early freezing process, prior to the onset of the glass transition, which is critical for the occurrence of phase separation.

The most widely studied systems that show phase separation are polymer systems, which can include protein-polysaccharide (23,48), sugar-protein-polysaccharide (49), protein-protein, protein-surfactant (50), polymer-polymer (18), and polymer-polyelectrolyte systems (24,33). Phase separation in such systems is generally the rule, rather than the exception, and is dependent on temperature, concentration, molecular weight, and the presence of other components such as salts. In a freeze-dried system this would directly translate to low temperatures and high concentration, the most unstable condition for many of the systems listed above. As stated previously, the main contribution to phase separation in polymer-containing systems is the large molecular weights of the polymers that limits the entropic gain upon mixing and large orientation-dependent positive enthalpies of interaction upon mixing, resulting in phase separation (19). It is widely speculated that such phase separation occurs during the freezing process with proteins and their intended stabilizers, as a protein can be viewed as a polymer. However, proteins are structurally different than synthetic polymers, and little proof exists for such occurrences in pharmaceutically relevant formulations.