Have you ever wondered why it is that when you are cooling your vials of solution in the freeze drier, they never freeze at the freezing point? Or why it is that your vials always freeze at different times, and temperatures?

There are simple factors to be taken into consideration, for example there is freezing point depression and temperature variation across the drier shelves. If we consider a 10 % w/v solution of sucrose the freezing point depression is about 0.63 °C. Taking that with the largest typical variation across shelves of 5 °C, it is difficult to explain why solutions of sucrose will typically freeze at -8 to -12 °C. Put a small volume of the same solution in a DSC pan and cool it and it will typically freeze between about -13 and -20 °C. Those of you who are familiar with DSC have probably asked yourselves why the freezing temperature (cooling trace) is always much lower than the ice melting temperature (warming trace), and why do your vials in the freeze drier always freeze at higher temperature than the sample in the DSC?

This apparently odd behaviour of water is in fact something we see almost every day. Certain types of clouds, called Middle or Family B clouds, exist at a height of about 6,500 to 13,000 feet, approximately 2,000 to 4,000 metres. Altocumulus and altostratus clouds for example belong to this family. Although temperatures at this altitude are typically in the range +2 °C to -11°C, these clouds are composed of droplets of liquid water. Why don't they freeze?

All of these observations are explained by the physics of freezing. Freezing is a crystallisation event and has the same properties as any other crystallisation, although it is a little unusual in that it is the solvent and not the solute that crystallises. Crystallisation events have two distinct phase nucleation and growth.

Freezing is a liquid to solid transition and is initiated by the nucleation of the solid phase (ice) in the undercooled liquid (water). Through the process of random Brownian diffusion, a number of molecules must come together to produce an transient embryo which resembles the crystal in spatial orientation, and has a sufficiently long life time for further condensation of water molecules to occur. Because Brownian diffusion is random, the greater the number of molecules in the samples the greater the chance that sufficient molecules will come together in the right orientation. Nucleation therefore is a probability event, the more molecules there are, the more probable is embryo formation.

Diffusion also has a temperature component. The speed of diffusion increases with temperature as higher temperatures impart greater kinetic energy. This affects the rate at which embryos might be formed and the lifetime of the molecules in the embryonic configuration. Thus the probability of nucleation is a function of temperature and volume (or mass, the number of molecules available to form a nucleus).

The nucleation rate J(T), at a temperature T, is described by the equation:
\[ J(T) = A^{(K\tau)} \]

sometimes expressed as \( J(T) = A \exp((K\tau)) \), where \( \tau = \left[ (\Delta T)^2 T^3 \right]^{-1} \), \( \Delta T \) is the degree of undercooling \( (T_m - T) \) below the melting point \( T_m \), and \( A \) and \( K \) are constants.

Thus nucleation is a first order kinetic rate process but it is not of the Arrhenius type. The nucleation rate \( J(T) \) *increases* with *decreasing* temperature.

The second phase of freezing is ice crystal growth. Although the ice morphology and crystal size distribution (product texture) depend in a complex manner upon the viscosity, cooling rate, temperature, nucleation density and the heterogeneity of the substrate, crystal growth itself shows Arrhenius temperature dependence. The higher the temperature the higher the growth rate.

It is, however, the first step of freezing, nucleation which leads to variability of freezing. The freezing point of water is not 0 °C, simply because freezing is a probability event, and therefore there is no unique and reproducible freezing point. At 0 °C, the probability of nucleation occurring is vanishingly low and thus water, or an aqueous solution will always cool below the equilibrium melting point. How far it cools below the melting point is a matter of probability.

The reason that the sample in the DSC pan freezes at a lower temperature than the product in a vial is simply the volume. The larger volume in the vial has a larger the number of molecules, a higher probability of nucleation, and hence it is more likely to nucleate at a higher temperature, or lower degree of undercooling. A small volume of water in a DSC pan, or indeed a droplet within a cloud, can undercool significantly. In practice the probability of nucleation becomes so high in the region of -40 °C, that this imposes a practical limit to the degree of undercooling, but it is quite feasible to store small droplets of water, or aqueous solutions, at -20 °C, unfrozen, for a period of years.

The random nature of nucleation introduces an unpredictable and uncontrollable variability into the process, and the onset of freezing cannot be synchronised between two samples which are simply cooled. The probable freezing temperatures are given by a Gaussian distribution about a mean, with a spread of approximately ±5 degrees. Thus an array of vials on a shelf will be seen to freeze at different times, and therefore at different temperatures.

In theory it is possible to influence ice crystal dimensions, by manipulating the cooling rate. Fast cooling leads a higher degree of undercooling; nucleation is favoured over growth resulting in a higher number of smaller, often spherulitic, ice crystals. Slower cooling favours ice crystal growth over nucleation, resulting in a smaller number of larger dendritic ice crystals. The latter are to be preferred as they lead to better connectivity and higher sublimation rates. Unfortunately the rate of heat transfer out of the sample is determined by the latent heat of crystallisation, the specific heat and the thermal conductivity. Water and ice have large specific heats and low thermal conductivities, both of which mitigate against rapid heat transfer. In practice, on a freeze dryer shelf, the vial contents will cool at a rate of no more than 0.3 to 0.5 °C/minute, even if the shelf is cooled more rapidly. This is quite slow on a physical scale. Substantial degrees of undercooling require the water to be cooled at rates exceeding 1,000 °C / second, unattainable under freeze-drier operating conditions.