crystalline matrix formulation, but a trend of the drier, the better. In another study on the same protein but in a different formulation, we found that overdrying was indeed detrimental and resulted in an increase of insoluble aggregates and a decrease of protein activity. Therefore, the range of optimal moisture content and the shape of the curve are formulation dependent.

Figure 12 shows results from a moisture optimization for a different protein product but with a similar crystalline matrix-type formulation. However, as oxidation is one of the instability mechanisms, this figure shows the percentage of oxidized protein versus storage time and moisture content. The assay of oxidation used here is reversed phase HPLC. The percentage of oxidation was calculated as the area under the peak for oxidized protein divided by the sum of the areas under the oxidized and nonoxidized peaks. The percentage of oxidized protein increased dramatically at a moisture content of 17%. However, the oxidation rate was comparable for moisture contents of 1.2% to 5.6%. Note that the percentage oxidation is lowest for the vacuum control because of the headspace vacuum. Unsealing the other vials and exposing the contents to atmospheric pressure when samples were incubated in the desiccators increased the percentage of oxidation.

MEASURE T_g OF DRY CAKES

Another way to determine the optimal moisture content for stability is to develop a relationship between moisture content and T_g of lyophilized dry cakes. T_g can be measured using either DSC or MDSC. T_g is a measure of the molecular motion of water, which is a major factor in denaturing proteins (28,29). To increase the long-term storage stability of a pharmaceutical protein, the motion of water can be limited (frozen storage) or water can be removed (lyophilization). However, even for a lyophilized biopharmaceutical product, residual moisture in the dry cake could be mobile depending on the level of the residual moisture content and storage temperature. High residual moisture content and high storage temperature may result in more mobility of water (29), and as a consequence may damage the product protein significantly as well as harm the cosmetic properties of the lyophilized cake. The glass transition temperature of a freeze-dried cake (T_g) marks an increase in the mobility of the remaining water (28). Freeze-dried products stored below their T_g will exhibit much greater stability and structural integrity than those stored above T_g. Because of the cost of storing a product at low temperatures, it is economically more desirable for the T_g of a lyophilized product to be relatively high. Each product formulation will have a different relationship between moisture content and glass transition (29). MDSC can be used to characterize the glass transition of a dry cake (30,31). By analyzing cakes of different moisture contents, the relationship between T_g and moisture content can be quantified. In the following paragraphs, the relationship between dry cake moisture content and T_g is reported for the crystalline matrix-type formulation.

Again, samples for T_g measurement are prepared similarly to those for adsorption study using desiccators. Each desiccator maintained a different relative humidity by using different saturate salts as described previously in the water adsorption study. As a result, different levels of moisture content were achieved for the samples in different desiccators. The driest samples were obtained by keeping them sealed after freeze-drying until sample preparation.