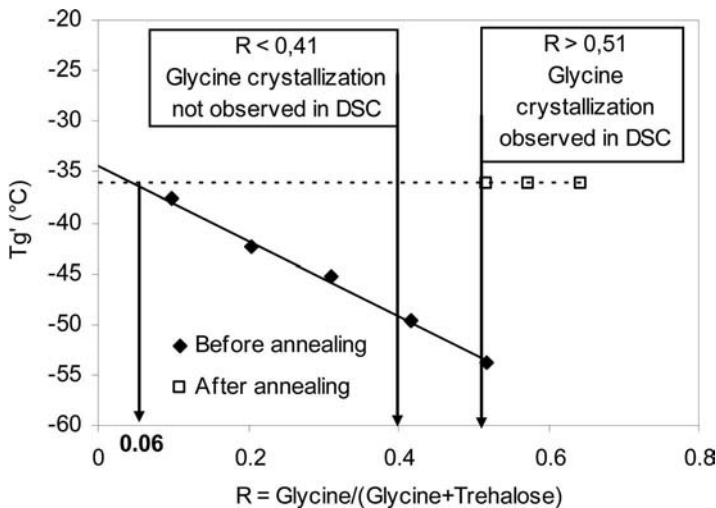


cooling rate of 1°C/min resulted in a decrease of the area of the crystallization peak of mannitol but did not allow its complete crystallization during freezing. When applying an annealing step at -20°C for 60 minutes (curve c), the DSC heating curve did not exhibit any exothermic event, suggesting the completion of mannitol crystallization during annealing. Furthermore, only a weak glass transition event at -30°C was observed (arrow 4). Freezing the MnP formulation resulted in a complex structure with a large crystalline phase composed of mannitol and a minor amorphous phase embedding the proteins and composed of PVP and low mannitol content. The resulting  $T_g'$  value is highly dependent on the thermal treatment applied to the sample.

The studies of Ma et al. (12) and Passot et al. (6) are based on a unique value of weight fraction of the crystalline agent: 40% glycine and 80% mannitol, respectively (Table 1). Kasraian et al. (26) investigated the physical behavior of solutions of various ratios of glycine/sucrose during freezing by DSC and FDM and reported the complete glycine crystallization following a cooling rate of 1.5°C/min for a glycine weight fraction higher than 71% (Table 1). The crystallization of glycine, even partial, makes it possible to perform primary drying at high product temperatures, with a value of  $T_{max}$  close to the eutectic melting temperature of glycine (-4°C). Elegant freeze-dried cakes were obtained for weight ratios of glycine higher than 57%. To develop a formulation that makes it possible to design a short freeze-drying process, it is therefore crucial to know the minimal weight fraction of crystalline bulking agent required to obtain its complete crystallization during freezing for the range of freezing rates used in the freeze-drying process.

Chatterjee et al. (28) have quantified the extent of glycine crystallization in frozen solution in a ternary model system containing water and crystallizable (glycine) and noncrystallizable (raffinose or trehalose) components by applying a systematic approach (Table 1). Aqueous solutions including different



**FIGURE 2** Glass transition temperature of freeze-concentrated phase ( $T_g'$ ) of aqueous solution including glycine and trehalose.  $T_g'$  values from the first warming DSC curve are compared with  $T_g'$  values after annealing. Source: Adapted from Ref. 28.