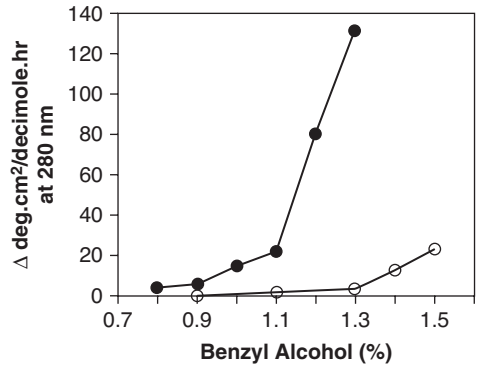

Loss in ellipticity at 280 nm 280 of rhIFN-γ as function of benzyl alcohol concentration in 16 mM acetate buffer at pH 5.0 (○) and 16 mM succinate buffer at pH 5.0 (●).

Figure 8-8 Effect of benzyl alcohol on recombinant human interferon gamma aggregation. *Note*: Tobler, et al. J Pharm Sci, June, 2004 used hydrogen-deuterium isotope exchange detected by MS to detect tertiary structure changes that involve only a limited part of this protein still causing irreversible loss of activity. Benzyl alcohol causes protein to unfold forming very large aggregates. *Source*: From Refs. 62 & 64.

increasing the concentration of APs may have a negative impact on protein physical stability (precipitation, aggregation, etc). Increasing AP levels will increase the hydrophobicity of the formulation and could affect the aqueous solubility of the protein. Increasing AP concentrations also increases the potential for toxicological hazards.

It is well known that APs not only protect insulin formulations against inadvertent contamination, but also may have a significant effect on protein stability. For example, phenolic preservatives have a profound effect on the conformation of insulin in solution (57) and the assembly of the specific type of LysPro insulin hexamer (58). Furthermore, phenol and/or *m*-cresol in insulin solutions will have a tendency to be adsorbed by and permeate rubber closures (59). Therefore, rubber formulations must be designed to minimize these potential problems.

APs are known to interact with proteins and can cause stability problems such as aggregation. For example, phenolic compounds will cause aggregation of hGH (60). Phenol will produce a significant decrease in the α-helix content of insulinotropin resulting in aggregation of β-sheet structures (61). Benzyl alcohol, above certain concentrations and depending on other formulation factors, will interact with recombinant human interferon-γ causing aggregation of the protein (Fig. 8-8) (62). Other examples are granulocyte-stimulating factor and recombinant interleukin-1R (56). These examples point out the need for the formulation scientist to understand the importance of potential effects of preservative type, concentration, and other formulation additives on the interaction with proteins in solution while balancing the needs for antimicrobial efficacy.

In determining the appropriate AP agent or agents, insulin was studied as the protein to be preserved and combining insulin with different types of AP agents either alone or in combination (63). These formulations were challenged with the five USP PET organisms and D values[2] determined. The D-value determination allows a single-quantitative estimate of the AP effectiveness of a certain agent or combination of agents in a specific formulation against a specific microorganism. The preservative combination of 0.2% phenol and 0.3% *m*-cresol gave the lowest D-value (fastest time required for a 1-log reduction in the initial inoculum of *S. aureus* and, thus, was the most effective AP system in this particular insulin formulation.

There are instances where a manufacturer, because of concerns regarding aseptic processing and sterility assurance of the product throughout its shelf-life, will add an AP agent in

[2] D value = Time required for a 1-log reduction in the microbial population due to the effect of the antimicrobial preservative system. The smaller the D value, the greater the effect of the preservative on the microorganism in question. Covered in chapter 18.