fairly low (< 1%, w/v) to serve as a stabilizer; otherwise at higher concentrations (>10% w/v) it can cause precipitation (53).

Poly(vinylpyrrolidone) (PVP) also is like PEG in that at low concentrations it can stabilize proteins, whereas at high concentrations it may help lead to protein aggregation and precipitation. PVP at low concentrations (≤2.0%) effectively stabilizes human IgM monoclonal antibody against heat-induced aggregation, whereas PVP concentrations ≥5.0% will cause aggregation (54).

Fibroblast growth factors, acidic and basic, are prone to acid and thermal inactivation and can be stabilized by a number of heparin and heparin-like molecules (25). Human keratinocyte growth factor, also prone to aggregation at high temperature, is stabilized by heparin, sulfated polysaccharides, anionic polymers, and citrate ion (55).

OPTIMIZING MICROBIOLOGICAL ACTIVITY

Antimicrobial Preservatives

Many products (perhaps around 25%) are commercially available as multiple-dose formulations. If a sterile product is intended for multiple dosing, then it must contain an effective antimicrobial preservative (AP) agent. AP agents are formulated with the active pharmaceutical ingredient if the product is a ready-to-use solution or is part of the diluent used to reconstitute freeze-dried products intended for subsequent multiple dosing. While rare, there are examples of AP agents formulated within the freeze-dried product and not part of the diluent.

Of 145 peptide and protein drug products listed in 2006 Physicians' Desk Reference, 36 contained preservatives (56). Most vaccine products used to contain AP agents, especially thimerosal, but by 2006, only 8 vaccine products still were formulated as multidose products.

The most common APs used in multiple-dose formulations are phenol, meta-cresol, and benzyl alcohol. Less common, especially for new formulations, but still used APs include methyl and propylparaben. Some, although very few, vaccines still contain APs with phenoxyethanol being the most common. Thimerosal used to be commonly used for vaccine products, but not today. Examples of use of these preservatives are listed in chapter 6 (Table 6-7).

Use of antimicrobial agents requires passing a preservative efficacy test (PET) (USP chap. <51> provides the directions for conducting this test). Unfortunately, the United States Pharmacopeia (USP) and the British and/or European Pharmacopeial (BP/EP) tests for PET are different in their requirements. Table 8-5 summarizes the differences between the tests. The USP basically requires a bacteriostatic preservative system while the BP/EP requires a bacteriocidal system. For example, the USP requires a 3-log reduction in the bacterial challenge by the 14th day after inoculation, while criteria A of the BP/EP test requires the same 3-log reduction within 24 hours. This great difference in compendial requirements for preservative efficacy has caused many problems in the formulation of protein dosage forms for various markets. One unpublished example involved a new protein product where the scientist developing the formulation was unaware of the different compendial requirements. The focus was minimizing instability of the new protein in the presence of the AP and used a minimal amount of AP in the formulation. The phase 1 clinical study was scheduled for a European clinic so the EP PET was performed. The formulation failed miserably and the product had to be reformulated with start of the clinical study delayed by almost a year.

Passing the BP/EP PET requires the use of relatively high amounts of phenol or cresol or other AP that may have an impact on the stability of the formulation and could result in sorption of the preservative into the rubber closure. The formulator must keep in mind that

Table 8-5 Comparison of USP and EP Preservative Efficacy Tests

Test	USP <51>	EP <Chapter 5.1.3>
Bacterial	1-log reduction within 7 days	2-log reduction within 6 hours
Challenge	3-log reduction with 14 days	3-log reduction within 24 hours
Fungal challenge	No increase after 28 days	2-log reduction with 7 days
Overall requirement	Bacteriostatic	Bacteriocidal