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Wisniewski, R. 1988. Design objectives for aseptic seals. *Proceedings of the Bioprocess Engineering Symposium of the American Society of Mechanical Engineers*, 27 November–2 December, in Chicago, IL.

Wisniewski, R. 1992. Principles of the design and operational considerations of large scale high performance liquid chromatography (HPLC) systems for proteins and peptides purification. *Bioseparation* 3:77–143.

Wu, S. L., A. Figuero, and B. L. Karger. 1986. Protein conformational effects in hydrophobic interaction chromatography. *J. Chromatog.* 371:3–27.

Wu, D., and R. R. Walters. 1992. Effects of stationary phase ligand density on high-performance ion-exchange chromatography of proteins. *J. Chromatog.* 598:7–13.

Yamada, M., S. Adachi, and Y. Shirai. 1989. A simulated moving-bed adsorber with three zones for continuous separation of L-phenylalanine and NaCl. *J. Chem. Eng. Japan* 22:432–434.

Yamamoto, S., K. Nakanishi, and R. Matsuno. 1988. *Ion-exchange chromatography of proteins.* New York: Marcel Dekker.

Yamamoto, S., N. Nomura, and Y. Sano. 1990. Predicting the performance of gel-filtration chromatography of proteins. *J. Chromatog.* 512:77–87.

Yamamoto, S., K. Nakanishi, R. Matsuno, and T. Kamikubo. 1983. Ion exchange chromatography of proteins: Prediction of elution curves and operating conditions. *Biotechnol. Bioeng.* 25:1465–1483.

Yang, V. C., and R. Langer. 1985. pH-dependent binding analysis: A new and rapid method for isoelectric point estimation. *Anal. Biochem.* 147:148–155.

Yang, B. L., and S. Goto. 1993. Affinity purification by tapered bed. *J. Chem. Eng. Japan* 26:752–754.

Yeung, E. 1986. *Detectors for liquid chromatography.* New York: John Wiley and Sons.

Yun, T., and G. Guiochon. 1994. Modeling of radial heterogeneity in chromatographic columns: Columns with cylindrical symmetry and ideal model. *J. Chromatog.* 672:1–10.

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LYOPHILIZATION OF PROTEIN PHARMACEUTICALS

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There are numerous unique, critical applications for proteins in human healthcare. Many protein drugs are already on the market, and over 200 candidate proteins and peptides are currently in clinical trials (Geisow 1992; Wang and Pearlman 1993; Talmadge 1993). However, even the most promising and effective protein therapeutic will not be of benefit to human health, if its stability cannot be maintained during packaging, shipping, long-term storage, and administration. For ease of preparation and cost containment by the manufacturer, and ease of handling by the end user, an aqueous protein solution is often the preferred formulation. Unfortunately, water fosters protein degradation by providing a medium for molecular movement and conformational perturbations, as well as serving as a reactant in chemical degradation pathways (e.g., hydrolysis).

Because the free energy of stabilization for a native protein is only about 50 ± 15 kJ/mol (Jaenicke 1991), proteins are readily denatured (often irreversibly) by the many stresses arising in solution, such as heating, agitation, freezing, pH changes, and exposure to denaturants (Arakawa et al. 1993). In addition, various chemical

modifications of amino acid side chains can occur relatively rapidly in aqueous solution (Manning et al. 1989, In press). These physicochemical modifications often result in undesirable by-products, including soluble and insoluble aggregates, and inactive and/or antigenic species. Such alterations may not only compromise the clinical efficacy of the protein drug, but may also increase the risk of adverse side effects (e.g., Thornton and Ballow 1993). Thus, the inherent instability of the protein, and/or the logistics of product shipping, storage, and use, often preclude preparation of the protein as an aqueous solution (Pikal 1990a,b). Also, simply preparing stable frozen protein formulations, which is a relatively straightforward process, is not a desirable alternative. Shipping and storing products at subzero temperatures are not technically and/or economically feasible in many markets.

The practical solution to the protein stability dilemma is to remove the damaging component—water. Lyophilization (freeze-drying) is the method most commonly used to prepare dehydrated proteins. In this process the product is first frozen at atmospheric pressure. Then water is removed by reducing the chamber pressure of the lyophilizer and collecting the water as ice onto a condenser. Theoretically, lyophilized proteins should have the desired long-term stability at ambient temperatures. Such stability would allow the product to be handled conveniently and distributed to a wider market, including those markets in Europe that do not guarantee refrigerated delivery.

However, as will be described in this review, without the proper insight into the lyophilization process and how it affects proteins, it is not a simple task to remove water by freeze-drying, without damaging the protein. Recent infrared spectroscopic studies have documented that the acute freezing and dehydration stresses of lyophilization can induce protein unfolding (Prestrelski, Tedeschi, et al. 1993; Prestrelski, Arakawa, et al. 1993, 1994 [148–169], Dong et al. 1995). Unfolding can not only lead to irreversible protein denaturation, if the sample is rehydrated immediately, but can also reduce storage stability in the dried solid (Chang, Beauvais, et al. in preparation; Prestrelski et al. 1995). Thus, when one is trying to prepare a lyophilized protein product, one must develop a formulation that stabilizes the protein during freezing, the lyophilization cycle itself, and subsequent storage of the dried solid. Simply obtaining a native protein in samples rehydrated immediately after lyophilization is not necessarily indicative of adequate acute stabilization, nor is it predictive of storage stability. Many proteins unfold during lyophilization but readily refold if rehydrated immediately (cf. Prestrelski, Tedeschi, et al. 1993; Dong et al. 1995). Without directly examining the structure of the dried solid (using infrared spectroscopy, as described below), it is not possible to know whether an unfolded protein with poor storage stability is present or not. Finally, even if the protein is native in the dried solid, this may not be adequate if other crucial physical factors—the glass transition temperature and the residual moisture of the dried solid—are not optimized.

To develop a protein formulation that has both acute and long-term storage stability, it is crucial that the specific conditions (i.e., pH, specific stabilizing ligands) for optimum protein stability be established *and* the appropriate nonspecific stabilizing additives (i.e., those excipients that generally stabilize any protein) be incorporated into the formulation. For acute stabilization the appropriate excipients must be chosen to protect the protein during *both* the freezing and drying steps (Carpenter et al. 1993; Prestrelski, Arakawa, et al. 1993). For storage stability, in addition to providing acute protection, the excipients must also form an amorphous solid (i.e., a glass) with the protein, and, hence, provide an environment that is restrictive to physical and chemical degradation (Franks 1990; Franks et al. 1991; Roy et al. 1990). Furthermore, the glass transition temperature of this amorphous solid varies inversely with the sample residual moisture, which is then greatly influenced by the lyophilization cycle itself (e.g., final product temperature). Therefore, the lyophilization cycle parameters can also greatly affect the storage stability conferred by a given formulation.

Finally, in addition to protein stability, a lyophilized pharmaceutical product must also have acceptable cake morphology and dissolution properties. Often the most desired cake has a strong, porous structure, formed from a crystalline bulking agent, into which is incorporated the protein/stabilizer amorphous phase. Therefore, additives must also be selected, and the lyophilization cycle designed, such that freeze-drying takes place without the collapse of the amorphous phase of the cake. Finally, the formulation must maintain protein stability and cake integrity during a rapid and efficient lyophilization cycle (i.e., without the need for excessively rigid control of cycle parameters such as chamber pressure and shelf temperature [Pikal 1990a; Chang and Fischer 1995; Nail and Gatlin 1993, 163–233]).

Optimizing the protein and cake stability during the lyophilization cycle appears and can be a daunting, complicated process. However, as will be documented, achieving these goals can be relatively straightforward if the underlying physical principles are understood and a rational approach is taken. In general, for long-term

storage stability of dried proteins, it appears that only four criteria must be met:

1. Acute lyophilization-induced unfolding must be minimized and, ideally, the protein should be native in the dried solid.
2. The dried powder must have a glass transition temperature that is higher than the desired storage temperature.
3. The residual moisture must be relatively low (i.e., $\approx < 0.01$ g H_2O per g dried solid).
4. Specific formulation conditions (e.g., pH) must be developed to inhibit chemical degradation pathways, which might arise even in native proteins.

The purpose of this review is to provide an overview of the principles that must be followed, and how essential physical data are obtained and used to meet these criteria. In addition, in order to provide a rational basis for choosing excipients, a description will be given of the mechanisms for protein stabilization by additives.

At this point it is important to note that the principles and mechanisms to be discussed should be generally applicable to any protein. However, obtaining a stable lyophilized formulation of a given protein may not necessarily be as straightforward as it appears based on the generalizations provided in this review. Each protein has unique physicochemical characteristics, which produces its unique "personality." Sometimes this personality manifests itself as a protein that "cooperates fully" during formulation development. At other times the protein seems to be a "spoiled child who follows or breaks the rules in an apparently illogical pattern of frustratingly inconsistent behavior." Interestingly, with such a "recalcitrant" protein, once the problems are analyzed carefully, often one learns that the protein did follow the rules, but the patterns of consistent behavior were too complicated to discern during the initial phases of the research. Currently, it is not possible to predict if a protein will fall into one of these extremes or somewhere in between, which in a way is beneficial to the careers of formulation scientists. If all that was needed to obtain stable proteins was the purchasing of a "kit of magic excipients" or simply following a single, simple recipe, then there would not be much need for highly skilled protein stabilization experts in the industry or for further advances in the field from basic researchers. Since this is not the case, there is a great need to increase the fundamental understanding of protein formulation and

to document by case studies the applicability of general rules to individual proteins.

Since the purpose of this chapter is to outline the main principles that are important, at least as a starting point for guiding the development of stable lyophilized protein products, there will *not* be an exhaustive review of all of the published studies, many of which are excellent, on acute and storage stability of lyophilized proteins. There will not be an in-depth exploration of some of the rigorous physical mechanisms and theories that govern the design of lyophilization cycles (e.g., the connection between sample collapse temperature and glass transition temperature [$T_{g'}$] of the frozen sample). Excellent reviews of these issues are already available (see MacKenzie 1975 [277–307], 1976; Pikal 1990a; Pikal and Shah 1990; Nail and Gatlin 1993). Instead, selected examples from the literature will be used to illustrate the theoretical and practical elements of developing an optimally stable, lyophilized protein product.

This chapter will consider, in order, the following individual topics, with special consideration of how the individual areas are interrelated and impact on each other:

- The first topic will be how to design an econonical lyophilization cycle that results in the desired cake properties and residual moisture. As described, an important part of this process is the use of differential scanning calorimetry to obtain essential physical data (i.e., $T_{g'}$ and eutectic crystallization temperatures of formulations) that dictate the cycle parameters.
- Consideration will then be given to how to design formulations that stabilize proteins during both freezing and drying, and the mechanisms for stabilization by additives. An introduction to the use of infrared spectroscopy to monitor protein conformation directly in frozen and dried samples will also be provided. Although this method is just beginning to be used in commercial formulation development, it should soon become an invaluable part of this process, because structural information about the protein during lyophilization and storage is crucial.
- In the final section the optimization of formulations for long-term storage stability will be discussed. A major part of this discussion will focus on the impact of the physical properties of the dried solid (i.e., T_g and protein conformation) on

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