

## ROLE OF POLYOLS AND SURFACTANTS IN LIQUID PROTEIN FORMULATIONS

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### ABSTRACT

Polyols in aqueous protein formulations, increase conformational stability of proteins by size exclusion mechanism, increasing thermodynamic stability of proteins and decreasing aqueous solubility. Polyols prevent protein aggregation; if aggregation of the protein is preceded by partial folding or denaturation while aggregation of native protein conformation, might be enhanced by polyols. Non-ionic surfactants are excluded from water structure; thus, they are expected to accumulate at the globular protein surface-water interface. Non-ionic surfactants reduce aggregation by covering hydrophobic patches on the globular protein surface result in increased interaction of their

hydrophilic components with water, thus they are expected to increase solubility of the globular protein. With respect to their effect on thermodynamic stability, this needs to be determined for each particular protein.

**KEYWORDS:** Stability, aggregation, conformation, denaturation, polyols, surfactants.

### INTRODUCTION

At present, many different recombinant proteins or peptides are approved for clinical use by the US food and drug Administration (FDA) and by the European Medicine Agency (EMA) and many, more are in development. Protein-based drugs have been formulated mainly as stable liquids or in some cases where liquid stability is limiting, as lyophilized dosage forms to be reconstituted with suitable diluent prior to injection (Walsh, 2005).<sup>[1]</sup>

Liquid formulations of protein drugs are susceptible to chemical and/or physical degradation pathways, which can negatively affect both the efficacy and safety of the therapeutic product (Wang and Pearlman, 1993).<sup>[2]</sup>

Physical instability or degradation of proteins can occur due to denaturation (loss of three-dimensional structure), aggregation and precipitation as well as adsorption of protein to container surfaces (Wang and Pearlman, 1993; Wang and Hanson, 1988).<sup>[2,3]</sup>

In order to formulate stable protein formulation, it is essential that both physical and chemical degradation pathways of the drug be minimized. Normally, the first step in most formulation procedures is to identify susceptibility of the protein to such events. This is most commonly done using “accelerated stability” protocols; examples of stress applied are temperature, pH, and shear (shaking), then to test the stability of the protein under a wide, logical formulation conditions.

Compounds commonly used as conformation stabilizing excipients and/or isotonicity agents in liquid protein formulations include; polyols and simple salts, (Nema *et al*, 1997)<sup>[4]</sup>, Polyols encompass a class of excipients that include sugars (e.g., mannitol, sucrose, trehalose and sorbitol and other polyhydric alcohols (e.g., glycerol and propylene glycol). Polyols have preference to the aqueous solvent over globular protein surface, they are called water structure breakers, since they enter the water structure and form strong bonds with water molecules. In addition to polyols and simple salts, non-ionic surfactants such as polysorbate 80 and polysorbate 20 are normally added to liquid protein formulations in order to reduce protein aggregation (Kerwin, 2008).<sup>[5]</sup>

### CONFORMATIONAL STABILITY OF PROTEINS

Although freshly isolated proteins may be folded into a distinct three-dimensional globular structure in water, this folded structure is not necessarily retained indefinitely (Timasheff, 1993).<sup>[6]</sup> Hence protein molecules must be stabilized by certain compounds and stored under certain conditions in order to maintain their conformational stability (Santoro *et al*, 1992).<sup>[7]</sup>

The marginal stability of protein conformations, mean that although hydrophobic interactions are the dominant forces in protein folding, other small interactions can contribute positively or negatively to the protein’s stability (Von Hippel, and Wong, 1965).<sup>[8]</sup>

The resistance to unfolding, also known as thermodynamic stability, varies among different proteins and depends on a combination of various forces that contribute to the stability of the protein’s globular conformation (Schellman, 1987).<sup>[9]</sup> Thermodynamic methods such as

differential scanning calorimetry (DSC) can be useful in determining the effect of temperature on protein's conformational stability (Pace, and Scholtz, 1997).<sup>[10]</sup>

The transition midpoint ( $T_m$ ) is the temperature where 50% of the protein is in its native conformation and the other 50% is denatured. The higher the  $T_m$ , the more stable is the molecule. This technique can be used to identify optimal pH, buffer species, stabilizer, etc., where protein conformation is most stable (Remmele Jr *et al.*, 1998).<sup>[11]</sup>

A protein's three-dimensional conformation may be studied in great detail by X-ray crystallography or NMR spectroscopy (Sevcik *et al.*, 2002).<sup>[12]</sup>  $\alpha$ -helical content of a protein can be estimated using spectroscopic methods, particularly far-UV (180–260nm) circular dichroism (Herman *et al.*, 1996).<sup>[13]</sup>

### AQUEOUS PROTEIN SOLUBILITY

Globular water-soluble proteins differ in the balance of charged, polar and hydrophobic amino acids that they display on their surface, and hence in their solubility under a particular set of conditions. However, the proportion of the surface that is composed of charged side chains is the most important determinant of the solubility of a protein (Shaw *et al.*, 2001).<sup>[14]</sup> Proteins usually have a net positive or negative charge that reflects the mixture of amino acids that they contain under different pH values. The *isoelectric point* (pI) of a protein is the pH at which its net charge is zero. At this pH the protein molecule is electrically neutral, least soluble and may aggregate into particles that are often too large to be soluble in water (Middaugh, 1992).<sup>[15]</sup> At a pH below the pI, the protein net charge is positive, and then negative when the pH is above the PI. The solubility of a protein is normally higher at pH values higher or lower than their iso-electric point.

### PROTEIN AGGREGATION

Aggregation of a protein can occur from the association of either the unfolded, partially folded or the native state of proteins (Chi *et al.*, 2003).<sup>[16]</sup> In order to prevent protein aggregation, it is important to understand the cause of the aggregation in a particular protein. For some proteins, aggregation can occur without or with only minor conformational changes (partial unfolding), which exposes hydrophobic domains (Chen *et al.*, 1994),<sup>[17]</sup> an example of this is the self-association of proteins such as insulin (Sluzky *et al.*, 1991).<sup>[18]</sup>

Surface adsorption is another cause of protein aggregation, some proteins tend to expose hydrophobic sites, normally present in the core of the native protein structure when an interface is present (Thurrow and Geisen, 1984).<sup>[19]</sup> These interfaces can be water/air, water/container wall or interfaces formed between the aqueous phase and devices used to administer the drug (e.g., catheter, needle). These adsorbed, partially unfolded protein molecules form aggregates, leave the surface, return to the aqueous phase, form larger aggregates and precipitate, this is the proposed mechanism for aggregation of insulin in aqueous media through contact with a hydrophobic surface or water–air interface (Twardowski *et al.*, 1983).<sup>[20]</sup>

Size-exclusion chromatography (SEC) with either UV or light scattering detection has been used for detecting and quantifying protein aggregation and is a common QC/QA method. Another method used for aggregate analysis is Native polyacrylamide gel electrophoresis (PAGE) as aggregates stay intact during PAGE (Herman *et al.*, 1996).<sup>[13]</sup>

## DISCUSSION

When using any excipient in a protein formulation, it is important to understand how the additive achieves the desired effect; in addition, it is vital to appreciate how the same excipient might have other effects on the protein formulation.

Polyols are believed to interact preferentially with water, and are excluded from protein structure thus preferential exclusion from protein surface, Preferentially excluded co-solvents increase the effective surface tension of the solvent at the protein interface, (Kendrick *et al.*, 1997)<sup>[21]</sup> whereby the most energetically favorable protein conformations are those with the smallest surface area (Levine *et al.*, 1991).<sup>[22]</sup> So they are expected to increase thermodynamic stability of the native globular protein conformation.

Structure stabilizers such as polyols can improve structural stability of proteins in water, and if aggregation of protein is preceded by partial unfolding or denaturation of the native structure, they can also prevent aggregation. If native structure is susceptible to aggregation, then structure stabilizers probably have no effect in reducing aggregation; in fact, they might enhance aggregation. The mechanism of action of commonly used structure stabilizers in reducing interaction between the globular protein with water means that structure stabilizers such as polyols are likely to reduce solubility of the native globular protein, and at high concentration they can cause protein precipitation (Timasheff, 2002).<sup>[23]</sup>

The non-ionic surfactants, polysorbate 80 (polyoxyethylene (20) sorbitan monooleate) and polysorbate 20 (polyoxyethylene (20) sorbitan monolaurate) are incorporated surfactants in marketed protein pharmaceuticals. (Kerwin, 2008).<sup>[5]</sup> The optimal amount of surfactants required, is that amount, which is needed to saturate the hydrophobic patches on the globular protein surface. The mechanism of action of non-ionic surfactants is believed to be due to preferential interaction with exposed hydrophobic patches on the surface of the globular protein (Kerwin, 2008).<sup>[5]</sup> The non-ionic surfactants have been shown to prevent aggregation and improve the water solubility of globular protein, and might stabilize protein conformation (Sluzky *et al.*, 1991).<sup>[18]</sup>

These molecules readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups to the aqueous phase, thus increasing water solubility of the protein. In addition, non-ionic surfactants compete with protein for container surface, air-water interface, ice-water interface, or any other solid surfaces, and prevent the protein from non-specific adsorption (Kerwin, 2008).<sup>[5]</sup>

## CONCLUSION

Long-term storage stability of proteins can be achieved through understanding of protein degradation pathways and how excipients interact with proteins.

As preferential exclusion, and thus unfavorable interaction, increases with polyol concentration, the native structure is stabilized largely at higher polyol concentrations. This concept can be extended to the situation in which there is self-association of the native structure. During the process of protein self-association, the surface area per protein molecule decreases, which in turn reduces the unfavorable interaction present between the solution and the protein complex or aggregates. Thus, the associated state is stable, but less soluble in the presence of stabilizing polyol; i.e., they can also enhance aggregation, and precipitation.

Optimal mechanisms for reducing aggregation of proteins depend on the cause/s of aggregation. For example, if protein aggregation were due to conformational instability then increasing thermodynamic stability of the native state as conferred by protein stabilizers,<sup>[5]</sup> would help in controlling aggregation caused by conformational instability of the tertiary structure of the protein. Aggregation of native conformations of the protein could be reduced by reducing intermolecular hydrophobic interactions between the native conformation using

aggregation suppressors such as non-ionic surfactants. The use of anti-adhesion agents such as serum albumin, would help in controlling aggregation caused by surface adsorption.

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