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Staying Current

Formulation of Biopharmaceuticals

IN THIS ISSUE

OVERVIEW

We start 2012 with this issue, but there are still a number of papers from 2011 to cover. In particular, there was a special issue of *Advanced Drug Delivery Reviews* covering topics important to protein formulation scientists. We will cover those articles, and more, as we step into 2012.

PROTEIN-EXCIPIENT INTERACTIONS

Kamerzell et al., Protein-Excipient Interactions: Mechanisms and Biophysical Characterization Applied to Protein Formulation Development. *Adv. Drug Deliv. Rev.* **2011**, 63: 1118-1159.

Abstract: This review seeks to emphasize the critical importance of protein-excipient interactions to rational development of stable formulations of proteins. In addition, the value of biophysical methods in characterizing these effects is highlighted. Different classes of excipients are covered, along with discussions of different dosage forms for protein pharmaceuticals. Finally, the value of high throughput methods for examining protein-excipient interactions is presented.

Analysis: This excellent review from the University of Kansas provides us with one of the most comprehensive summaries on this important topic that has appeared for some time. In our opinion, both formulation and analytical scientists will benefit from reading through it (and its 559 references).

OSMOLYTE EFFECTS ON PROTEIN STABILITY

Auton et al., Osmolyte Effects on Protein Stability and Solubility: A Balancing Act between Backbone and Side-Chains. *Biophys. Chem.* **2011**, 159: 90-99.

Abstract: Naturally occurring excluded solutes, called osmolytes, provide increased conformational stability of proteins under a wide variety of conditions. The transfer free energy (ΔG_{tr}) of the native protein can be predicted by summing the water-to-osmolyte free energies of exposed side chains and backbone moieties. Similarly, the ΔG_{tr} for the denatured state was estimated using a self-avoiding random coil model of the unfolded state. From this data, m values were predicted for denaturation. The correlation with experimentally measured m values for 49 proteins and nine osmolytes is quite good.

Analysis: Our examination of protein-excipient interactions continues with this fine study from Professor Bolen and his colleagues and collaborators. Using transfer free energies, they are able to reconstruct one of the key features of protein denaturation, the m-value describing the degree of cooperativity and the extent of change in solvent accessible surface area upon unfolding.

CHEMICAL AND THERMAL DENATURATION OF PROTEINS

Wang et al., Comparison of Chemical and Thermal Protein Denaturation by Combination of Computational and Experimental Approaches. II. *J. Chem. Phys.* **2011**, 135: 175102-1-12.

Abstract: A molecular understanding of the relationship between chemical and thermal denaturation of proteins is still lacking. Three different proteins (azurin, cytochrome c, apoflavodoxin) were examined using a combination of coarse-grained molecular dynamics simulations and experimental studies. It was found that there is a linear relationship between the thermal denaturation temperature and the ΔG estimated from chemical denaturation using the temperature dependence in the Gibbs-Helmholtz equation. Moreover, there is a linear relationship between the temperature of a particular structural ensemble in the absence of urea (T_b) and the temperature of the same ensemble in the presence of urea (T_u). Therefore, we conclude that the thermal and chemical denaturation processes correlate in terms of thermodynamics, although deviations were found at high concentrations of denaturants.

Analysis: This topic has been debated for some time, with some groups finding correlations between thermal and chemical denaturation and others concluding just the opposite. This work from the Universities of Houston and Umeå seem to provide us with a clearer picture of how they are coupled, along with the indication that the relationship can break down at high denaturant concentrations.

CALCIUM AND β-LACTOGLOBULIN UNFOLDING AND AGGREGATION

Petit et al., Influence of Calcium on β-Lactoglobulin Denaturation Kinetics: Implications in Unfolding and Aggregation Mechanisms. *J. Dairy Sci.* **2011**, 94: 5794-5810.

Abstract: It is known that calcium plays an important role in β-lactoglobulin fouling during milk processing. The influence of calcium on β-lactoglobulin denaturation in simulated lactoserum concentrates was examined. A change in the mechanism was observed near 80º C from unfolding to aggregation, leading to non-Arrhenius behavior. This occurred for all calcium concentrations. Calcium addition increased the kinetic rate of unfolding, especially in the higher temperature, aggregation-dominated regime, suggesting calcium catalyzes aggregation. Calcium also provides a slight protective role for native β-lactoglobulin leading to an increase in the activation energy for denaturation.

Analysis: This is a solid biophysical study from France on a protein important to the dairy industry, but also provides insights into thermal instability and the role metal ions can play in reducing or enhancing aggregation.

DRY POWDER FORMULATIONS OF ANTHRAX VACCINE

Wang et al., Stable Dry Powder Formulation for Nasal Delivery of Anthrax Vaccine. *J. Pharm. Sci.* **2012**, 101: 31-47.

Abstract: A recombinant protective antigen (rPA) vaccine with a novel adjuvant was formulated to be delivered nasally. Powders were formed by spray-freeze-drying, producing particles with a target size (D_{50}) of 25 μm. The physical properties of the powders were characterized and the structural stability of the antigen demonstrated using CD and IR spectroscopy. Powders stored for two years retained potency and the adjuvant, 48/80, was able to elicit an effective immune response in rabbits. Antibody titers after nasal delivery were comparable to intramuscular immunization.

Analysis: Professor Hickey at the University of North Carolina and his collaborators provide us with a study on preparing stable vaccine formulations using spray-freeze-drying. It also is worth noting for its focus on nasal delivery.

STABILITY OF FLU VACCINES

Patois et al., Stability of Seasonal Influenza Vaccines Investigated by Spectroscopy and Microscopy Methods. *Vaccine* **2011**, 29: 7404-7413.

Abstract: Different seasonal influenza vaccines were evaluated for stability under stress conditions (2 and 4 weeks at 25º C, 1 day at 37º C, one freeze-thaw cycle). Influvac® and Mutagrip® were both affected by all three stress conditions. Inflexal® was stable to temperature stress, but not freeze-thaw, where aggregation occurs, while Fluarix® was affected only by storage at 25º C. This study illustrates how sensitive commercial vaccines can be to reasonably modest stress conditions and demonstrates the value of using biophysical methods to monitor stability.

Analysis: This work from Switzerland shows that many vaccines exhibit some instability when stressed even by just one freeze-thaw cycle or one day at 37º C. It also illustrates that biophysical methods can and should be used to characterize the stability profiles of commercial vaccines, which have historically relied solely on activity measures.

PROTEIN STABILIZATION BY CYCLODEXTRINS

Serno et al., Protein Stabilization by Cyclodextrins in the Liquid and Dried State. *Adv. Drug Deliv. Rev.* **2011**, 63: 1086-1106.

Abstract: In the search for novel stabilizers for protein therapeutics, cyclodextrins have been investigated for more than twenty years. The research on this topic is reviewed, with a focus on their ability to reduce protein aggregation. A variety of mechanisms for cyclodextrin stabilization have been postulated and these are discussed in detail.

Analysis: This is another review from the special issue of *Advanced Drug Delivery Reviews*. This one from Novartis and Professor Winter's group in Munich is a comprehensive summary of this topic. Its focus on mechanistic aspects is particularly appreciated. This is required reading for anyone investigating cyclodextrins as excipients.

INFRARED SPECTRAL STUDIES OF A LYOPHILIZED IgG1

Murphy et al., Structure, Stability, and Mobility of a Lyophilized IgG1 Monoclonal Antibody as Determined Using Second-Derivative Infrared Spectroscopy. *J. Pharm. Sci.* **2012**, 101: 81-91.

Abstract: An IgG1 monoclonal antibody was lyophilized with increasing amounts of sucrose. In addition, some formulations contained low levels of sorbitol as a plasticizer. Maximal stability was obtained at the highest sucrose:protein ratio tested (2:1 by weight). Analysis of the high and low frequency β-sheet bands in the amide I region provides additional information on the structure of the protein, structural homogeneity and possibly mobility in the solid state.

Analysis: This study is meant to illustrate that there is greater information content in the amide I IR spectra of proteins that many may realize. An examination of band positions and bandwidths allows one to gain insight into structure as well as solid-state mobility. In addition, it shows that inclusion of even a small amount of sorbitol in an 'optimized' sucrose formulation may be beneficial for long-term storage stability.

***Note**: Some of the authors of this article are employees of Legacy BioDesign LLC*

DEVELOPABILITY INDEX

Lauer et al., Developability Index: A Rapid *In Silico* Tool for the Screening of Antibody Aggregation Propensity. *J. Pharm. Sci.* **2012**, 101: 102-115.

Abstract: A molecular computational tool is presented that could streamline the assessment of monoclonal antibody candidates by predicting the aggregation propensity via hydrophobic and electrostatic interactions. The resulting developability index is a function of the net charge, spatial aggregation propensity (SAP) and overall structure. Applications include screening of mutants and identification of drug candidates that may be difficult to stabilize.

Analysis: This is the latest work from the long-standing collaboration between Professor Trout at MIT and his group and Novartis. Using the SAP method they developed together, they now present data that it may allow one to screen antibody candidates for developability, looking to identify those that may be particularly prone to aggregation.

INHIBITING CHEMICAL DEGRADATION

Tomita and Shiraki, Why Do Solution Additives Suppress the Heat-Induced Inactivation of Proteins? Inhibition of Chemical Modifications. *Biotechnol. Prog.* **2011**, 27: 855-862.

Abstract: Thermoinactivation of proteins can be prevented by a wide range of additives. When examining the protection of lysozyme and ribonuclease A to thermal stress, it was discovered that there was a correlation between the presence of additives and the levels of deamidation and β-elimination. Remarkably, the degree of protection was quite similar between these two disparate proteins. Furthermore, the effect was seen with amino acids, salts and polyamines.

Analysis: Drs. Tomita and Shiraki in Japan have published a number of studies on stabilization of proteins over the years. Here they show that the protection at elevated temperatures is not solely due to physical stabilization, but reduction in chemical modifications.

SELF-ASSOCIATION OF INTERFERON-α_{2b} ANALOGS

Li et al., Characterization of the Self-Association of Human Interferon-α_{2b}, Albinterferon-α_{2b}, and Pegasys. *J. Pharm. Sci.* **2012**, 101: 68-80.

Abstract: The self-association of three interferon-α_{2b} analogs was determined using analytical ultracentrifugation (AUC). The self-association of interferon-α_{2b} results in formation of stable dimers and reversible oligomers. Meanwhile, self-association of albinterferon-α_{2b}, a fusion protein with human serum albumin, forms reversible dimers and trimers, while Pegasys (a PEGylated form of interferon-α_{2b}) gives only reversible dimers. The self-association seems to involve electrostatic interactions, which can be suppressed by reducing the pH to ~4.5 or adding NaCl.

Analysis: This work shows the value of AUC in characterization of the solution behavior of closely related protein therapeutics. It also illustrates how important electrostatic interactions can be to reversible oligomer formation.

BUFFER INFLUENCE ON GFP THERMAL STABILITY

de Lencastre Novaes et al., Citrate and Phosphate Influence on Green Fluorescent Protein Thermal Stability. *Biotechnol. Prog.* **2011**, 27: 269-272.

Abstract: The effect of various salts on the thermal inactivation of green fluorescent protein (GFP) was evaluated at several concentrations and temperatures (70-95° C). Thermal stability was measured in decimal reduction time (D-values). The D-values were higher for citrate and phosphate than other buffers and salts. It is hypothesized that the anions bind to GFP, thereby stabilizing it against thermal denaturation.

Analysis: In the ongoing search for protein stabilizers, case studies, like this one, illustrate how buffers can affect conformational stability and not just control changes in pH. It emphasizes how excipients may exert their influence on protein stability through a variety of mechanisms.

VISCOSITY OF CONCENTRATED PROTEIN SOLUTIONS

Jezek et al., Viscosity of Concentrated Therapeutic Protein Compositions. *Adv. Drug Deliv. Rev.* **2011**, 63: 1107-1117.

Abstract: There has been an increasing need to deliver higher concentrations of therapeutic protein over the recent years, especially for monoclonal antibodies. These highly concentrated solutions often exhibit physical properties that limit their clinical utility, such as increased viscosity. This review summarizes methods for measuring viscosity and what is known about what leads to viscosity differences, even in highly similar molecules.

Analysis: This review is another from that special issue in *Advanced Drug Delivery Reviews*. It comes from Arecor and the National Physical Lab in the UK along with collaborators at Genzyme. For anyone working on high concentration formulations, this is a valuable resource. The section on viscosity measurements is particularly useful.

ADSORPTION INDUCED ENZYME DENATURATION

Thudi et al., Adsorption Induced Enzyme Denaturation: The Role of Protein Surface in Adsorption Induced Protein Denaturation on Allyl Glycidyl Ether (AGE)-Ethylene Glycol Dimethacrylate (EGDM) Copolymers. *Coll. Surf. B* **2012**, 90: 184-190.

Abstract: The effects of protein size on denaturation that occurs upon adsorption to a particular surface (AGE-25 copolymer) were examined for different model proteins. It was determined that the extent of adsorption and denaturation is dependent on molecular weight as well as the number of glycoside groups on the surface of the protein. Therefore, surface hydrophobicity may not be predictive of adsorption behavior to chromatographic resins. Overall, the data indicate that the trend in denaturation on the copolymer is similar to that for water-miscible organic solvents.

Analysis: This work from India demonstrates that glycosylation impacts protein adsorption, so predictions based on calculations of surface hydrophobicity may not be correct. I thought the denaturation correlation between the effects of an organic solvent and those of a polymer support were intriguing and worth further study.

FIBRILLATION OF β_2-MICROGLOBULIN

Eichner and Radford, Understanding the Complex Mechanisms of β_2-Microglobulin Amyloid Assembly. *FEBS J.* **2011**, 278: 3868-3883.

Abstract: Many proteins, including β_2-microglobulin (β_2m), form fibrils in vivo, leading to disease. Yet, our understanding of the mechanisms of amyloid formation for these proteins is incomplete. This review covers what is known about the aggregation of β_2m under physiologically relevant conditions. In particular, the properties of the folding intermediate known as I_T and its role in fibrillation of β_2m are discussed.

Analysis: As important as it is to understand how β_2m forms fibrils and causes dialysis-related amyloidosis, β_2m is also a fascinating model for studying protein aggregation. The formation of fibrils seems to proceed from conformations that are quite native-like. Professor Radford's group at Leeds has worked in this area for some time and this review with Professor Eichner at Brandeis is well worth reading.

EXCIPIENT-EXCIPIENT INTERACTIONS AND PROTEIN STABILITY

Shukla et al., Molecular Level Insight into Intra-Solvent Interaction Effects on Protein Stability and Aggregation. *Adv. Drug Deliv. Rev.* **2011**, 63: 1074-1085.

Abstract: Suppression of aggregation often involves the addition of inactive ingredients, i.e., excipients. While a large number of studies have been devoted to understanding the mechanism for such stabilization, a few cases require greater detail to explain the impact on protein stability. In those cases, there are significant interactions between the excipients and that is the subject of this review. The intent is to provide new insights into additive-additive interactions and how they may contribute to protein-additive interactions. In particular, additives such as arginine, proline, urea and sugars are discussed.

Analysis: Here is another contribution from Professor Trout's group, focusing on systems where the additive-additive interactions alter their ability to affect protein stability, especially with respect to aggregation. As such, this is a very helpful overview, especially the sections on amino acids and denaturants.

ARGININE SALTS AND AGGREGATION SUPPRESSION

Schneider et al., Arginine and the Hofmeister Series: The Role of Ion-Ion Interactions in Protein Aggregation Suppression. *J. Phys. Chem. B* **2011**, 115: 7447-7458.

Abstract: L-arginine (Arg) hydrochloride has been shown to be an aggregation suppressor in a number of systems. Although the chloride salt is most often used, little is known about the impact of other counter ions on the stabilization of proteins. It was found that the degree of aggregation suppression is consistent with the Hofmeister series. It appears that attractive Arg-Arg interactions dominate the observed behavior. The ability of dihydrogen phosphate, citrate, and sulfate to have strong attractive interactions with the guanidinium head group of Arg allows the Arg molecules to cluster more easily, resulting in greater aggregation suppression.

Analysis: This study on Arg from Professor Trout's group examines the effects of counter ions of this widely used excipient. Their work has shown that the ability of Arg to self-associate is key to understanding its ability to stabilize proteins in aqueous solution.

NONCOVALENT PEGYLATION OF KGF-2

Khondee et al., Noncovalent PEGylation by Polyanion Complexation as a Means to Stabilize Keratinocyte Growth Factor-2 (KGF-2). *Biomacromolecules* **2011**, 12: 3880-3894.

Abstract: Repifermin, also known as KGF-2, unfolds at relatively low temperature (~ 37º C). Electrostatic interactions with polyanions are known to provide significant structural stabilization. In this study, Pegylated polyanions are bound to KGF-2. Highly PEGylated polyanions do not bind, but, at optimal molar rations, the T_m of KGF-2 can be increased by 9º to 17º C using PEGylated analogs of pentosan polysulfate and dextran sulfate.

Analysis: The FGF superfamily has been shown to bind polyanions, leading to an increase in thermal stability. Here, we see the ability to use these strong electrostatic interactions to allow one to PEGylate effectively without covalent attachment.

ANALYZING CHARGE HETEROGENEITY IN ANTIBODIES

Han et al., High Throughput Profiling of Charge Heterogeneity in Antibodies by Microchip Electrophoresis. *Analytical Chem.* **2011**, 83: 8184-8191.

Abstract: A high throughput capillary zone electrophoresis method was developed to evaluate the charge heterogeneity of antibodies. The method uses high-speed microfluidic devices, which compare favorably against conventional CZE for resolution. Charge variants with pI differences of 0.1 can be resolved with this method, which has an analysis time of 80 seconds.

Analysis: It is important to stay up with the latest advances in analytical technology. Here we have a method from Novartis showing that a CZE method can be converted to a microfluidic platform. In combination with sample preparation in 96-well plates, a high throughput system can be constructed.

SYNERGISTIC EFFECTS OF Arg AND Glu ON PROTEIN SOLUBILITY

Shukla and Trout, Understanding the Synergistic Effect of Arginine and Glutamic Acid Mixtures on Protein Solubility. *J. Phys. Chem. B* **2011**, 115: 11831-11839.

Abstract: The combination of Arg and Glu has been shown previously to enhance the solubility of a number of proteins. However, the mechanistic basis for this effect of oppositely charged amino acids is unknown. This study shows that the solubility enhancement is due to the relative increase in Arg and Glu molecules surrounding the protein surface. The presence of these molecules reduced protein association, leading to increases in solubility. These studies illustrate the importance of excipient-excipient interactions in modulating protein behavior.

Analysis: The theme developed in the Shulka et al. review article discussed above is the role of excipient-excipient interactions. This report is just one illustration how two oppositely charged amino acids appear to work in concert to increase protein solubility.

ARGININE AND AGGREGATION OF CONCENTRATED PROTEIN SOLUTIONS

Shah et al., Effects of Arginine on Heat-Induced Aggregation of Concentrated Protein Solutions. *Biotechnol. Prog.* **2011**, 27: 513-520.

Abstract: Arginine (Arg) was studied for its ability to affect the heat-induced aggregation of lysozyme, BSA and β-lactoglobulin. Contrary to the belief that Arg is a universal aggregation suppressor, aggregation was actually increased for BSA and β-lactoglobulin. Density function theory calculations provide molecular level descriptions of why Arg can either accentuate or diminish protein aggregation.

Analysis: This study out of Singapore provides us with increased insight into how Arg interacts with proteins, sometimes reducing aggregation, sometimes not. Moreover, the authors believe their calculations can provide an indication on what kind of additives may be helpful in reducing aggregation, based on the amino acid content of the protein.

SELF-ASSOCIATION OF ANTIBODIES

Sule et al., High-Throughput Analysis of Concentration-Dependent Antibody Self-Association. *Biophys. J.* **2011**, 101: 1749-1757.

Abstract: At high concentrations, monoclonal antibodies (MAbs) can display viscous behavior due to self-association. Using self-interaction nanoparticle spectroscopy (SINS), the concentration-dependent behavior of three MAbs was evaluated. The self-association measured by SINS correlates with static ligh scattering (SLS) methods. Overall, the antibodies display complex pH-dependent self-association behavior that is greatly affected by ionic strength.

Analysis: This work is a collaboration between Lilly and Prof. Tessier's group at RPI. It presents another method for determining the colloidal stability of proteins and shows how it can impact important properties, such as viscosity.

COULTER COUNTING OF SUBVISIBLE PARTICLES

Barnard et al., Critical Evaluation and Guidance for Using the Coulter Method for Counting Subvisible Particles in Protein Solutions. *J. Pharm. Sci.* **2012**, 101: 140-153.

Abstract: The suitability of the Coulter method for quantifying subvisible particle in protein solutions is compared to other existing methods. The effects of operational parameters and instrument maintenance on data quality are examined. Depending on the sample type, customized analysis and cleaning procedures may be necessary to use the Multisizer™ 4 for these applications. Under those conditions, it was able to provide linear and reproducible particle counts. In general, the Coulter method detects more particles than micro-flow imaging and light obscuration. This may be due to the Coulter method to detect translucent particles that may not be detected by methods that depend on the optical properties of the particulates.

Analysis: This collaboration between Beckman Coulter and Professor Carpenter's lab at the University of Colorado provides us with a detailed and careful look at the use of Coulter counting for addressing the issue of subvisible particle levels in protein therapeutics. As such, it deserves careful consideration.

STABILITY OF MAbs IN I.V. INFUSION BAGS

Sreedhara et al., Stability of IgG1 Monoclonal Antibodies in Intravenous Infusion Bags under Clinical In-Use Conditions. *J. Pharm. Sci.* **2012**, 101: 21-30.

Abstract: Compounding pharmacists often produce compounded sterile preparations (CSP). In doing so, they are responsible for setting a 'beyond use' date based on USP <797>. Recently, published studies suggest that longer use dates for CSP are acceptable. However, these studies are based in inadequate analytical testing. This work demonstrates that 'beyond use' dates must be determined very carefully, as it is shown that many monoclonal antibodies degrade when diluted and stored in i.v. bags for extended periods of time. In addition, i.v. bag agitation tests should be performed under clinical use conditions, especially when bags are shipped and transported in global clinical trials.

Analysis: This work from Genentech shows that careful evaluation of stability is required to set prudent 'beyond use' dates for CSP. The incompatibilities noted here are likely to be found for other antibodies. This work shows how important handling and packaging

can be in the overall stability of a protein therapeutic agent.

ROLE OF OLIGOMERS IN POLYGLUTAMINE PEPTIDE AGGREGATION

Vitalis and Pappu, Assessing the Contribution of Heterogeneous Distributions of Oligomers to Aggregation Mechanisms of Polyglutamine Peptides. *Biophys. Chem.* **2011**, 159: 14-23.

Abstract: Polyglutamine aggregation is associated with certain neurodegenerative diseases. Previously, it was shown that polyglutamine length and concentration affected aggregation rates. Using a homogenous nucleation model, it was found that stable, soluble oligomers are formed. In this current work, the same model was found to be robust, resulting in a free energy maximum at n = n*, where n* is the critical nucleus. This model can be used to reconcile various observations of heterogeneous oligomer size distributions and non-fibrillar aggregation pathways. The resulting modified model shows that fibril assembly is governed by the relative stability of fibril-competent and fibril-incompetent oligomers, the size of the fibril competent oligomer and the rates of interconversion between the two species.

Analysis: There is more and more being published on the role of oligomers in the formation of larger aggregates. This kinetic study from Washington University in St. Louis is helpful if you are dealing with systems that display nucleation-dependent aggregation.

PHYSICAL INSTABILITY OF PROTEIN IN A DEFINE FLUID FLOW

Simon et al., Physical Degradation of Proteins in Well-Defined Fluid Flows Studied Within a Four-roll Apparatus. *Biotechnol. Bioeng.* **2011**, 108: 2914-2922.

Abstract: The physical degradation of proteins under well-defined fluid flow conditions was investigated. The flow field was evaluated computationally and experimentally. Using lysozyme as a model protein, in situ turbidity measurements were able to monitor aggregation directly. Formation of aggregates was observed for BSA and alcohol dehydrogenase as well. Thus, the four-roll apparatus described herein proves to be a useful tool for evaluating flow-induced aggregation of proteins in a reproducible fashion. Screening experiments for therapeutic proteins using this apparatus can quickly assess the sensitivity of a given protein to fill/finish operations.

Analysis: This collaboration between the University of Erlangen and Abbott describes a device capable of establishing well-defined flow fields that can then be used to evaluate the relative sensitivity of proteins to shear damage.

SURFACTANT-MEDIATED STABILIZATION OF PROTEINS

Lee et al., Molecular Origins of Surfactant-Mediated Stabilization of Protein Drugs. *Adv. Drug Deliv. Rev.* **2011**, 63: 1160-1171.

Abstract: Surfactants not only modulate loss and aggregation at surfaces, but they can also participate in protein-surfactant interactions. The roles of surfactants in modulating interfacial protein damage are reviewed. The degree of protection depends on the physicochemical properties of the surfactant and the strength of interaction with the protein. Given these parameters, surfactants may be selected to effectively reduce loss or aggregation due to adsorption.

Analysis: As with many classes of excipients, surfactants can stabilize protein by a variety of mechanisms. This review from Amgen and Oregon State University in the special issue of *Advanced Drug Delivery Reviews*, provides us with an extensive summary on the topic.

STABILITY OF hGH IN SUPERCRITICAL CO_2

Kelly et al., Stability of Human Growth Hormone in Supercritical Carbon Dioxide. *J. Pharm. Sci.* **2012**, 101: 56-67.

Abstract: It is well known that human growth hormone (hGH) is susceptible to damage at interfaces and elevated temperatures. In order to develop a sustained release injectable delivery system, polymeric microspheres containing hGH were prepared using supercritical CO_2 ($scCO_2$). It was found that no aggregation and no fragmentation occurred during encapsulation using $scCO_2$. Moreover, no significant increase in deamidation is found and the overall conformation of hGH is unchanged. Together, the structural integrity of hGH is unaffected by $scCO_2$ processing and it can be successfully encapsulated into polymeric microparticles.

Analysis: This work done in Nottingham in the UK shows us that supercritical fluid technologies are still being evaluated for preparing controlled release delivery systems for biopharmaceuticals. In this case, even somewhat sensitive proteins, like hGH, can be handled and processed without incurring any significant damage.

SYNERGISTIC EFFECTS OF Arg AND Glu ON PROTEIN SOLUBILITY

Blobel et al., Protein Loop Compaction and the Origin of the Effect of Arginine and Glutamic Acid Mixtures on Solubility, Stability and Transient Oligomerization of Proteins. *Eur. Biophys. J.* **2011**, 40: 1327-1338.

Abstract: Addition of a mixture of 50 mM arginine (Arg) and 50 mM glutamic acid (Glu) has been shown to improve protein solubility, but the basis for this enhancement is not well understood. Data are presented that shows that the Arg/Glu mixture induced compaction by collapsing loops onto the protein core. Dressed interaction site theory is employed to demonstrate that the protein perturbs the polyelectrolyte mixture in a manner proportional to the volume of the protein. The collapsed protein should be more soluble and more stable.

Analysis: This is the same subject as discussed in the Shukla and Trout paper listed above. In this case, work done in Spain and Sweden describes a different mechanism for stabilization and solubilization of proteins in the same Arg/Glu mixture. These two papers should be read together. Together, the studies illustrate the potential of interacting electrolytes to alter protein solubility and stability.