The use of albumin in formulation

By: Mikael Bjerg Caspersen
Customer solution science manager, Albumedix

Albumedix™

Published on August 25, 2016

Introduction

Development of safe and stable formulations can be a challenge, and the choice of excipients can become an important factor for success in the formulation of pharmaceuticals. For hard-to-formulate active pharmaceutical ingredients (APIs) this becomes even more profound and can be the determinate of whether such a drug candidate gets discontinued in development or becomes a successful pharmaceutical. Here, albumin has been shown to offer benefits over other excipients in the formulation of various APIs; this document aims to give the reader an overview of how albumin can help overcome certain formulation challenges.

The effects that albumin can infer on APIs in formulations will be discussed and the envisaged mechanism behind them will be outlined. At the end some suggestions of how to employ albumin in use will be given to make use of its different effects.

Albumin in short

Human albumin is the most ubiquitous protein in blood and is present at amounts around 40 g/L. It is a single chain protein of 585 amino acids of about 66.4 kDa in mass [1]. Albumin has a compact heart shaped structure with 17 internal di-sulfide bridges. Its role in blood is the shuttling of numerous smaller entities such as metals, hormones, fatty acids and toxins. However, it also makes up about 75% of the colloidal oncotic (or colloidal osmotic) pressure of blood and the single free cysteine of albumin (at position 34) makes up the majority of the reducing equivalents present in blood. All the properties are traits which are functional in employing albumin in formulation.

Albumin in formulation

Albumin has historically been used in a range of different formulations [2]. Originally, plasma sourced human serum albumin was employed. But there is seen a shift in the industry towards the use of chemically defined (recombinant) human serum albumin [3]. This stems from the fact that the recombinant product is seen to be advantageous due to factors such as: the absence of animal derived products, certainty of supply, high purity, absence of host derived proteases, high homogeneity, high free thiol content, absence of known or unknown human pathogens, batch to batch consistency and the presence of an established regulatory pathway.

Albumin in formulation has been reported to prevent:

- Surface adsorption
- Aggregation
- Fibrillation
- Oxidation

And to improve:

- Solubility
- Lyophilized cake formation
- Dissolving properties of API from lyophilized powder

The detailed mechanistic reasons for why albumin has these properties are not always unraveled when developing formulations. Often the effect will be multifactorial and not easily assignable. In the following we have bundled the mechanistic properties of albumin into four themes to enable a discussion of the functions of albumin in formulations.

Perceived mechanisms of albumin in formulation

The oxidation protection capability of albumin

This stems from the free thiol moiety of cysteine 34. This is an unpaired cysteine residue, which is present in its reduced form and it can become oxidized instead of the API during oxidative stress [4]. The thiol of cysteine 34 is positioned in a crevice in the albumin protein which shields it to a high degree from the solvent and specifically to larger molecules. The cysteine 34 of albumin thus has a relative low reactivity and is why it functions as a scavenger of predominantly highly reactive oxidation species, such as radicals, rather than as a general oxygen scavenger. Cysteine 34 of albumin therefore does not readily become oxidized and can be handled with no specific precautions during formulation process steps. It is important to appreciate that the free thiol content of commercial albumin can vary a lot depending on source and production process. Generally, recombinant sources have a higher content of free thiol compared to plasma sourced albumin. This is due to the recombinant material being based on albumin which is fully reduced at the outset of purification and that the employed purification is a gentler process than the one used for human derived albumin.

The surface covering ability of albumin

Albumin has a predisposition to cover surfaces and the protein will cover both hydrophobic and hydrophilic surfaces [5]. Historically, albumin has been used to cover the surfaces of medical devices to increase their biocompatibility. Studies have shown spontaneously

formed albumin coverage to be in the order of a single albumin molecule in height, with only 1-2 mg albumin needed to cover 1 m^2 of surface. This property is utilized for low dose/high potency drugs, where the dose could otherwise be difficult to control due to its surface adsorption during production, handling and storage. Another effect of albumin covering the surfaces is that it can hamper surface-induced unfolding and aggregation events of the API.

The direct interaction ability of albumin

Albumin has a structure which appears to have been optimized for a plethora of binding configurations to transient and non-covalently accommodate hydrophobic, charged, small and larger molecules. Albumin's promiscuous binding properties have resulted in the reporting of numerous APIs binding to albumin. They either bind in simple 1:1 ratio or other more complex ratios. This ability can convey multiple effects on the formulation. For instance albumin can, by direct binding, improve solubility of otherwise difficult to solubilize molecules or prevent otherwise detrimental interactions of molecules that could have given rise to unfolding and aggregation [6]. There are also indications of albumin having the ability to bind to the exposed hydrophobic end of growing fibrils, thereby curtailing their propagation.

The indirect interaction ability of albumin

Albumin has a positive second viral coefficient, this property describes the fact that albumin will try to avoid interacting with itself in solution [7]. This feature is effectively what brings about the oncotic pressure in blood. The effect also makes for another potential stabilizing property of albumin. As the albumin is spaced out evenly in the solution it hampers the general movement and flexibility of other entities in the solution. The decreased flexibility of the entities in the formulation can translate in to increased stability of these. The effect on this for formulation is not easily conceived or illustrated. But SAXS has showed co formulated proteins to have lower flexibility when formulated with albumin, without there being any direct interaction.

Using albumin in formulation

The four effects mentioned above will need different amounts of albumin in order to manifest themselves.

1. Surface adsorption: ≈0.1 mg albumin/mL 0.1-2 mg/mL
2. Direct interaction: Depending on concentration of API and ratio, ≈0.5-2 mol albumin/mol API

3. Indirect interaction and anti-oxidation (and in lyophilizing): ≥10-20 mg albumin/mL (in the solution prior to freeze drying)

Albumedix recombinant human serum albumin is supplied in a liquid form with albumin at 20% concentration ready to use. For a detailed description for retracting material from the container please consult our step-by-step guide which can be provided. Due to the high concentration of the albumin material a simple addition/dilution of the liquid albumin is usually workable and easily implemented in both formulation studies and at later large scale production.

Sometimes to achieve the higher concentrations of point 3, simple addition of the albumin isn't possible in an R&D setting. In this case a lyophilizing step can be used. If lyophilizing is performed it is beneficial to add the albumin to the solution prior to lyophilizing as the albumin can protect the API during the freezing, lyophilizing (cake building), and dissolving also.

If you have any further questions as to the use of Albumedix albumin products please do not hesitate to contact us. We have dedicated albumin formulation scientists who can guide and support you in the use of albumin for formulation.

Literature references

1. Fanali G., di Masi A., Trezza V., Marino M., Fasano M., Ascenzi P.; Human serum albumin: from bench to bedside. Mol Aspects Med (2012) **33** 209-290.
2. Hawe A., Friess W.; Formulation Development for Hydrophobic Therapeutic Proteins. Pharmaceutical Dev Tech (2007) **12** 223-237.
3. Tarelii E., Mire-Sluis A., Tivnann H.A., Bolgiano B., Crane D.T., Gee C., et al.; Recombinant Human Albumin as a Stabilizer for Biological Materials and the Preparation of International Reference Reagents. Biologicals (1998) **26** 331-346.
4. Roche M., Rondeau P., Singh N.R., Tarnus E., Bourdon E.; The antioxidant properties of serum albumin. FEBS Lett (2008) **582** 1783-1787.
5. Kowalczyńska H.M., Nowak-Wyrzykowska M., Szczepankiewicz A.A., Dobkowski J., Dyda, M., Kamiński, J., Kołos R.; Albumin adsorption on unmodified and sulfonated polystyrene surfaces, in relation to cell-substratum adhesion. Coll Surfaces B Biointerfaces (2011) **84** 536-544.
6. Milojevic J., Esposito V., Das R., Melacini G.; Understanding the Molecular Basis for the Inhibition of the Alzheimer's Aβ-Peptide Oligomerization by Human Serum Albumin Using Saturation Transfer Difference and Off-Resonance Relaxation NMR Spectroscopy. JACS (2007) **129** 4282-4290.
7. Ma Y., Acosta D.M., Whitney J.R., Podgornik, R., Steinmetz, N.F., French, R.H., Parsegian V.A.; Determination of the second viral coefficient of bovine serum albumin under varying pH and ionic strength by composition-gradient multi-angle static light scattering. J Biol Phys (2015) **41** 85-97.