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High purity BioXPro™ Arginine as a viscosity reduction agent

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Abstract

To administer the desired efficacy of many biologic modalities, like monoclonal antibodies (mAbs), a high concentration may be required in the formulation. Thus, the concentration may be above 100 mg/mL, which can be categorised as a high protein concentration formulation. Although this ensures that the desired effect is achieved, increasing the concentration can lead to some adversities during manufacturing and administrating.[1]

At high concentrations, the viscosity may be so high that it leads to administration challenges, especially for therapies that require self-administration. This arises as more force is required to overcome the higher viscosity, which in turn, can apply more pressure at the administration site or cause more syringe movement during administration. Ultimately, if the viscosity is not combated, there could be low patient compliance due to pain at the site of injection.[2]

One solution to lower viscosity within the formulation is to add a viscosity reducing agent. In order to utilise such agents effectively, a careful comprehension of the mechanism and causes of the viscosity increase needs to be considered. One rationale for the higher viscosity is the crowding effect, whereby the protein is forced into a closer proximity to itself. This amplifies any attractive or repulsive forces, like electrostatic, hydrophobic or steric.[3] Ultimately, these forces cause the whole formulation to have a higher resistance to movement.

Arginine has been repeatedly shown to have this effect, and it is used in formulations to reduce the viscosity of high protein formulations.[4]

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Arginine in monoclonal antibody formulations

Arginine has been widely used in solution-based protein formulations as an excipient. The effects of arginine as an excipient can be categorised into (i) enhancing protein refolding and solubility, (ii) stabilising protein and suppressing aggregation, (iii) reducing viscosity of high concentration protein formulations and (iv) reducing non-specific surface adsorption. For this reason, arginine is included in a substantial number of marketed formulations (**Figure 1**).[4] In the last few years, arginine is included in 20 % of the subcutaneous-injectable marketed antibody formulations. This highlights the importance arginine has in many antibody formulations.

Arginine as a viscosity reducing agent

There are many proposed mechanisms of how arginine acts as a viscosity reducing agent.[5] For example, arginine hydrochloride has been found to inhibit the protein-protein interactions between constant domains of the monoclonal antibodies (mAbs) in the fragment crystallisable (Fc) region but not between fragment antigen-binding (Fab) domains.[6]

In marketed antibody formulations containing arginine, the arginine concentration ranges from 25 to 200 mM. For example, Ultomiris, Benlysta, Dupixent, Alhemo, and Spevigio all contain 25 mM arginine for each of their drug delivery systems, whether that is solution for injection, prefilled syringe, pen, autoinjector, or intravenous. At the other end of the range, arginine is present at 200 mM, in marketed formulations such as Xolair, Leqembi and Omlyclo. The concentration of arginine in the formulation is often determined by the maximum solubility or its impact on osmolality.

A limiting factor of the arginine concentration is its impact on osmolality. Normal serum osmolality is between 275 and 295 mOsm / kg, which means the formulation ideally would have an osmolality in this range to be isotonic. Each administration route has a different upper limit depending on the sensation of pain they cause; for subcutaneous the upper limit is 600 mOsm / kg.[7] Any salts or sugars already in the formulation contributes to the osmolality, so the maximum concentration of arginine is limited by the residual allowed osmolality.

Arginine reducing the viscosity of gamma globulins

To have a universal evaluation of **BioXPro™ Arginine** as a viscosity reducing agent, the full range of gamma globulins are assessed from both bovine and human. In this comparison, it would highlight how arginine may impact a wide range of antibodies that are within the gamma globulins. The antibodies are formulated in a range of buffer systems and at different pH, such as citrate, acetate, histidine, and phosphate buffer systems. For formulated solutions, the pH is normally between 5 and 7.5, to decrease the irritation it causes when administered.

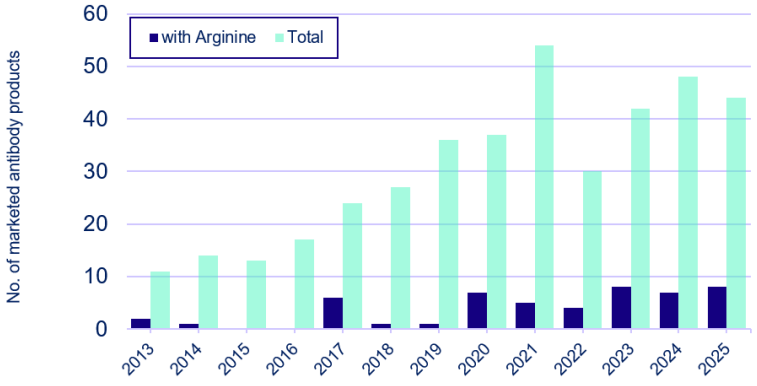

Figure 1: Frequency of arginine in marketed antibody formulations.

Arginine may interact differently in different pH and buffer systems, thus it is pertinent to know how it may affect the physicochemical properties of the solution, like pH. When adding 100 mM of **BioXPro™ Arginine** or **BioXPro™ Arginine Hydrochloride**, there is a clear difference between the impact they have on the pH. **BioXPro™ Arginine** shifts the pH up by around 2, whereas **BioXPro™ Arginine Hydrochloride** only drops the pH around 0.1. The minimal impact is observed when adding arginine-HCl to any histidine buffer at each pH.

Each of the gamma globulins were formulated in two pHs in each of the buffer systems at 250 mg/mL with varying concentrations of **BioXPro™ Arginine Hydrochloride**.

The dynamic viscosity was measured by the Honeybun (Unchained, U.S.). In comparison of non-arginine formulations, there is a difference in dynamic viscosity not only between the buffer systems and pH, but the gamma globulins as well. The difference between the gamma globulin may be in relation to their isoelectric points. Human gamma globulins isoelectric point is around 6.8.[8] The initial value highlights the dynamic viscosity that is required to be reduced by a viscosity reducing agent.[9]

In histidine, the different pH has a more significant difference with the human than bovine gamma globulins. Albeit all histidine formulations have a dynamic viscosity decrease of around 20 % from the addition from 0 to 250 mM **BioXPro™ Arginine**. A similar effect is seen with the acetate buffer in both gamma globulins. Conversely, **BioXPro™ Arginine** does not influence the dynamic viscosity in the citrate and phosphate as clearly for both gamma globulins. **BioXPro™ Arginine** reduces the dynamic viscosity of the human gamma globulins more than bovine, and phosphate is inconclusive on its positive impact.

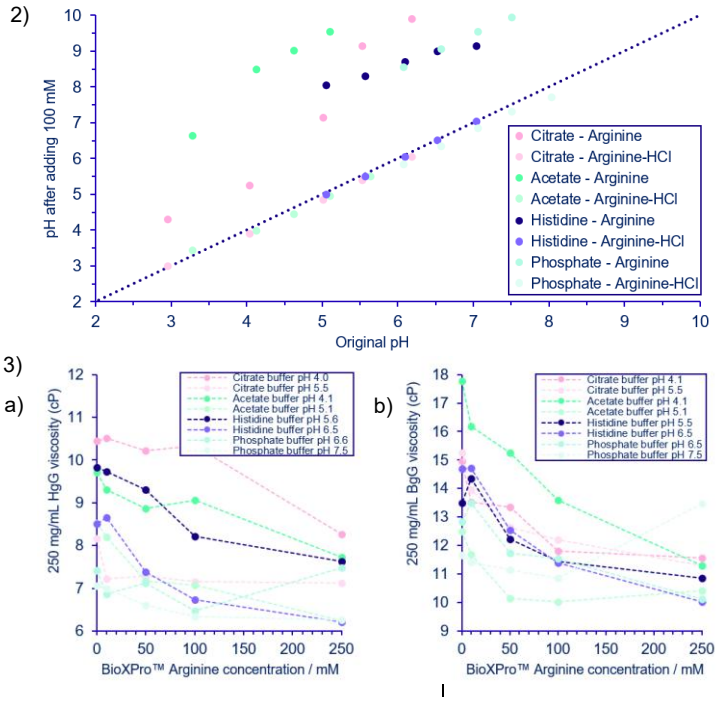

Figure 2: The comparison between the pH of different buffer systems (citrate, acetate, histidine, and phosphate) before and after adding arginine or arginine-HCl.

Figure 3: The reduction of the dynamic viscosity of both a) HgG and b) BgG with increasing concentration of BioXPro Arginine.

This may be the rationale that out of the 48 marketed antibody formulations that contain arginine, 32 are in a histidine buffer, 11 are in an acetate buffer, 4 are in a phosphate buffer, and 1 is in a Tris buffer.

To conclude, **BioXPro™ Arginine Hydrochloride** has proven to be a pertinent excipient in antibody formulations, especially with the high concentration required for its desired efficacy. **BioXPro™ Arginine Hydrochloride** has many useful properties that it imparts to the formulation, more so, acting as a viscosity reducing agent.

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