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Intermolecular interactions in highly concentrated formulations of recombinant therapeutic proteins

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The subcutaneous administration of recombinant therapeutic proteins requires the use of highly concentrated protein formulations to provide the desired dosage in a single injection. These highly concentrated formulations can have very high viscosities, creating challenges in processing (e.g. by ultrafiltration), storage (e.g. enhanced aggregation), and delivery (e.g. injection through small bore needles). Recent work has begun to identify the key intermolecular interactions governing the behavior of these highly concentrated formulations, including the effects of different excipients that have been shown to reduce viscosity and enhance the stability of these formulations. These intermolecular interactions also have a significant effect on the filtrate flux and maximum achievable protein concentration that can be obtained during ultrafiltration used for final concentration and formulation of these therapeutic proteins.

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Introduction

Much of the growth in the biotechnology industry during the past 20 years has been driven by the successful clinical development of monoclonal antibodies (mAb) and more recently antibody-derived therapeutics like Fc-fusion proteins [1–3]. Antibody products for cancer treatment are often delivered by intravenous injection, but the use of subcutaneous injection is much more convenient for the patient and can even accommodate self-administration at home [4,5]. Many of these recombinant therapeutic proteins require high dosing levels (on the order of milligrams of drug product per kilogram of body weight). Thus, very highly concentrated formulations (mAb concentration >100 mg/mL) are needed to provide the required quantity of drug within the 1–2 mL that can be accommodated in a single injection.

These highly concentrated protein formulations often have very high viscosities, creating challenges in processing (e.g. by ultrafiltration) and delivery (e.g. injection through small bore needles). In addition, protein aggregation is often enhanced at high concentrations, which can significantly reduce the storage stability. This article provides an overview of the intermolecular protein–protein interactions that govern the behavior of these highly concentrated solutions, including a discussion of the role of excipients in reducing the extent of protein aggregation and lowering the solution viscosity. Particular emphasis is placed on the impact of these intermolecular interactions on the filtrate flux and maximum achievable protein concentration during ultrafiltration, which is the dominant technology used for the development of these highly concentrated formulations.

Protein–protein interactions

Intermolecular interactions between proteins are governed by the detailed molecular properties of the protein (determined by the amino acid sequence and three-dimensional folded structure) and the solution conditions (including the pH, temperature, buffer, and presence of specific excipients and salts). Many studies have used classical colloid theory to describe the interactions between proteins, with the intermolecular forces described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory in combination with appropriate solvation, hydrodynamic (e.g. lubrication), and specific short-range (lock-and-key) interactions [6,7]. More recent efforts have used coarse-grained (CG) models to evaluate local electrostatic and Lennard-Jones (dispersion) interactions involved in the aggregation of mAbs, explicitly accounting for the Y-shaped structure and induced conformational changes in the protein that can occur upon aggregation [8]. These CG models have also been used to predict the viscosity of different mAbs at high protein concentrations based on the specific charged and hydrophobic groups within the variable region of the mAb [9]. These models have provided important insights into the network structure [10] and liquid–liquid phase separation of concentrated protein solutions [11].

The strength of protein–protein interactions in solution are commonly characterized by the magnitude of the second virial coefficient (B_2), which for a simple spherical

solute is related to the integral of the potential of mean force [12•]:

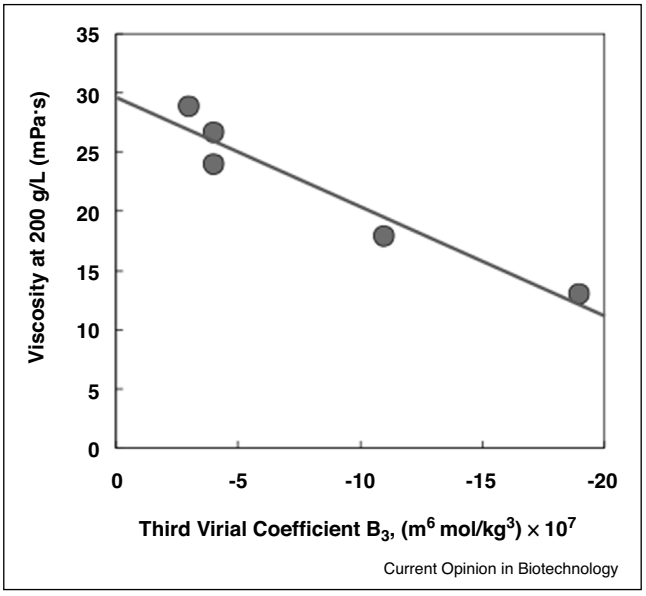
$$B_2 = B_{HS} + 2\pi \int_{2r_s}^{\infty} \left\{ 1 - \exp\left[\frac{-W_{ij}(r)}{k_b T} \right] \right\} r^2 dr \qquad (1)$$

where B_{HS} is the contribution from the protein excluded volume (often referred to as the hard-sphere contribution), k_b is the Boltzmann's constant, T is the absolute temperature, r is the radial distance, and W_{ij} is the potential of mean force. Although the value of B_2 can provide important insights into the overall interactions between proteins in highly concentrated solutions, this approach is typically unable to identify specific geometric configurations or charge distributions that can lead to strong local interactions that govern protein aggregation [13,14] and can strongly influence solution viscosity [15•,16,17].

B_2 can be evaluated experimentally using a variety of techniques, including light, neutron, and small-angle X-ray scattering; both size exclusion and self-interaction chromatography; sedimentation equilibrium; and membrane osmometry. Positive values of B_2 correspond to net repulsive interactions and tend to dominate under conditions where the protein is highly charged (i.e. at pH well above or below the isoelectric point) and at low ionic strength. Slightly negative values of B_2 are well correlated with crystallization [18,19], while large positive values of B_2 have been used as a predictor of protein stability. Osmotic pressure measurements can also be used to evaluate the third virial coefficient (B_3) based on data at very high protein concentrations. Negative values of B_3 correspond to local attractive interactions, likely related to geometric complementarity. Binabaji *et al.* [20] found a strong relationship between B_3 and the viscosity of a concentrated mAb solution (Figure 1), suggesting that localized interactions between proteins at very short intermolecular distances are critical in determining the rheological properties of these protein solutions, consistent with results for the viscosity of concentrated solutions of mAb variants [17].

There is extensive experimental evidence that the magnitude of intermolecular interactions and the conformational stability of the protein can be strongly influenced by the choice of buffer and the addition of specific excipients [21,22••,23]. Histidine is commonly used as a buffer in mAb formulations in both the liquid and solid (lyophilized and frozen) states [24]. Histidine can enhance protein stability during storage and freeze-drying, significantly reducing the extent of conformational changes [25,26]. Histidine has also been shown to reduce the viscosity of some mAbs, although recent data with an IgG4 antibody showed a maximum in viscosity at an intermediate histidine concentration of around 20 mM

Figure 1



Viscosity at a mAb concentration of 200 g/L as a function of the third virial coefficient (B_3).
Data replotted from [20].

[18]. The addition of histidine also increases the hydrodynamic radius of a mAb (measured using dynamic light scattering), and this appears to be correlated with an increase in both B_2 and the viscosity as shown in Figure 2 [27].

Downstream processing

Ultrafiltration (UF) and diafiltration (DF) are currently used for the final concentration and formulation of nearly all therapeutic proteins [28]. It is well-established that the filtrate flux during UF decreases with increasing protein concentration due to the increase in osmotic pressure and viscosity, both of which increase dramatically at high protein concentrations due to intermolecular interactions. The increase in viscosity can also lead to back-filtration near the module exit due to the large pressure drop associated with flow through the feed channel [29]. The net result is that the maximum achievable protein concentration, that is, the protein concentration at which the filtrate flux decreases to essentially zero, varies inversely with the solution viscosity as shown in Figure 3 [30••].

A number of studies have demonstrated that the filtrate flux during UF/DF can be increased by reducing the viscosity of the protein solution [31–33]. This includes the use of specific excipients such as arginine, histidine, or imidazole, with the addition of these excipients increasing the filtrate flux by as much as a factor of two with a corresponding increase in the maximum achievable protein concentration [34]. The filtrate flux can also be increased by performing the UF at elevated temperature (e.g. 37°C), although this could lead to a loss in product stability.