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Key Considerations and Strategies for Optimizing High-Concentration Protein Formulations

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ABSTRACT

Protein therapeutics, particularly monoclonal antibodies (mAbs), are increasingly formulated as high-concentration antibody products (HCAPs) for subcutaneous (SC) delivery to enhance patient convenience and compliance. This route allows self-administration, reduces hospital visits, and lowers treatment costs. However, developing HCAPs presents challenges, including increased viscosity, protein aggregation, and solubility issues, which can compromise stability, manufacturability, and patient adherence. High viscosity exacerbates formulation, storage, and delivery complexities, influencing injection force, patient comfort, and device functionality. Approaches to mitigate these issues include strategic protein engineering, optimizing formulation pH, incorporating excipients to reduce viscosity, and employing advanced downstream bioprocessing techniques like tangential flow filtration (TFF). Excipients such as sodium chloride, arginine, and caffeine effectively lower protein-protein interactions, though their concentration must be carefully balanced to maintain protein stability. Additionally, combining excipients and leveraging advanced filtration methods enhance the efficiency of biopharmaceutical production while preserving protein integrity. Understanding the biophysical principles, such as the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, and leveraging modern analytical methods to assess protein behavior in solution, provides critical insights for developing stable, high-concentration formulations. Strategies encompassing candidate selection, antibody optimization, and tailored formulation development are essential to overcoming these challenges, enabling efficient SC delivery of HCAPs with improved patient outcomes.

Keywords: High-Concentration Protein Formulations (HCPFs), Monoclonal Antibodies (mAbs), Protein Aggregation, Viscosity and Solubility Challenges.

1. INTRODUCTION:

The standard route for protein drug delivery is intravenous (IV) administration due to its low bioavailability for all alternative routes of administration, improved oversight during clinical application, and streamlined pharmaceutical innovation. Most of the monoclonal antibody drug approvals fall into a similar class that are elite among low protein concentrations, usually less than 30 mg/ml, and administered intravenously as infusion, particularly in neoplastic diseases. Nevertheless, in some therapeutic applications (especially chronic conditions like asthma, psoriasis or arthritis), the use of an alternative route of administration (subcutaneous delivery) become more significant.[1] This route, particularly when used in conjunction with prefilled syringe and autoinjector technologies, facilitates home administration and improves patient adherence to treatment protocols.[4]

Reasons driving the development of protein formulations for subcutaneously administration are: [1]

The normal volume of injection for a subcutaneous administration can be limited to 1 to 1.5 mL. This volume restriction is a result of back pressure exerted by the subcutaneous tissue that can actually push the administered back out and contribute to the discomfort and pain observed with injections.[1]

HCAPs allows the administration of greater of protein within a single dose volume of less than 1.5 mL compared with intravenous (IV) products, which often require a hospitalization or healthcare provider supervised trip to an infusion center. Subcutaneous (SC) administration can be carried out at even at home without visiting a doctor.[2]

However, the volume restriction can be mitoigated by co-formulating with hyaluronidase, which is administered alongside with the drug product. This approach allows a subcutaneous administration of up to 10 mL.[1]

High concentration antibody products are defined as monoclonal or polyclonal antibody injectable products that have an inclusive product concentration greater than100 mg/mL. These high concentration protein formulations bring extra problems of maintaining physical stability. Thus, for the therapeutic usage of HCAPs, formulation, stabilization, development and manufacturing processes must be sufficient and reproducible.[2]

In this setting, a high-concentration antibody product (HCAP) is a solution or lyophilized injectable that attains a concentration of greater than 100 mg/mL after reconstitution. According to other laboratory writings, concentrations in the "high concentration protein formulation" range should be aimed at concentrations in which protein-protein interactions, that depend on crowding, the characteristics of the solution including high viscosity, become crucial, and thereby materially influence the stability and administration of these formulation.[6]

1.1 EXAMPLES OF HIGH-CONCENTRATION PROTEIN FORMULATIONS:

Monoclonal antibodies (mAbs) High-concentration mAb formulations (>100 mg/mL) are more commonly utilized for subcutaneous delivery, decreasing mAb injection volumes.

Enzyme-based human therapeutic substances: Some types of enzyme-based therapeutic substances employ high-concentration formulations to enhance efficacy and minimize the frequency of administration.

1.2 ADVANTAGES:

i.The higher the concentration of active pharmaceutical ingredient (for example a protein or peptide) that can be applied to the skin the less volume needs to be applied to achieve the same dose.

ii.Very few doses delivered can be a big plus for patients, since it enables at-home self-administration of the drug via subcutaneous injection instead of needing to go to a hospital to undergo a long, intravenous infusion.

iii.Most conventional subcutaneous injections are less than 2ml.

iv.For those who suffer from chronic illness like asthma, dermatitis, colitis, or arthritis that requires regular dosing of their drug, the convenience of auto-injection devices is especially significant.

v.It also allows less frequent dosing compared to intravenous application with a slower systematic availability improving pharmacokinetic and pharmacodynamic (PK/PD) profiles.

vi.Other routes of administration may also be associated to high protein concentrations, depending on the molecule chosen and dose requirements.

2. KEY CONSIDERATIONS IN HIGH-CONCENTRATION PROTEIN FORMULATIONS:

2.1 Stability of proteins: Higher concentrations of proteins are more likely to aggregate or denature. Appropriate excipients like stabilizers (e.g., sucrose, trehalose) or surfactants (e.g., polysorbates) are used to minimize aggregation.

2.2 Viscosity: Increasing protein concentration leads to increased viscosity of the solution which can create challenges for injectability, manufacturability and comfort for the patient. Common approaches include changing the buffer composition due to solubility issues in such cases, as well as using low-viscosity proteins.

2.3 Buffer Selection: A proper buffer system (e.g., phosphate, acetate) retains the stability of the protein and maintains that the pH remains in an optimal range for the activity of the protein. The pH and ionic strength are tightly controlled.

2.4 Excipients: To prevent protein aggregation, excipients are used (NaCl, $MgCl_2$) to maintain proper osmolality in addition to stabilizers and surfactants.

2.5 Filtration and Sterilization: Due to their use in sterile applications, high-concentration protein solutions need to be sterilized, usually necessitating aseptic processing or filtration steps to eliminate potential contaminants while preserving the structural integrity of the protein itself.

3. CHALLENGES:

To facilitate patient convenience, monoclonal antibodies (mAbs), are currently formulated above 100 mg/mL concentrations. However, these high concentrations pose some real challenges for product stability and performance.[3]

Some of the challenges with pharmaceutical development of HCAF (high concentration antibody formulations) include increased viscosity and physical instability including irreversible aggregation and low solubility. In particular, these hurdles can make it difficult for drugs to be manufactured on a large scale, stored for long periods of time, and then delivered/aliquoted to patients.[5]

However, high viscosity, protein self-association, and aggregation are especially worrisome.[3]

A major issue is the increase in viscosity, which appears to result from concentration-dependent interactions between proteins that lead to self-association. The increased viscosity makes it difficult to formulate as well as manufacture the device and the drug to be injected.

Gelation, phase separation and other forms of self-association, were encouraged by negative molecular properties or solution conditions.

Moreover, factors like temperature, agitation, light exposure and high concentration play an important role regarding protein aggregation. . Aggregation compromises patient safety and product shelf-life, hence presents manufacturing difficulties. Thus, the prevention and control of protein aggregation in high-concentration formulations are essential for the successful development and production of stable therapeutic proteins and mAbs.[3]

3.1 Solubility:

Obtaining high protein concentrations can be highly dependent on solubility and it can be hard on some enzymes or cytokines since they can have very limited solubility. [1] The complexity of factors influencing protein solubility is higher than that of small synthetic molecules, therefore, specific approaches must be adapted to tackle solubility problems in proteins.[4]

The solubility of a protein is the maximum amount of protein that can be present in solution with co-solutes, in which the solution remains optically clear (without visible protein precipitates, crystals or gels) and does not sediment upon application of centrifugal force at 30,000g for 30 minutes. [9]

This means that the solubility of proteins depends on numerous factors, including ionic strength, salt type, pH, temperature, and specific additives. This is accounted for by changes in the surface tension of the surrounding water, as well as by the tendency of proteins to interact with water molecules and ions, versus their tendency to self-associate. How binding of the proteins to certain additives, salts, etc., guides their solubility, either by altering protein structure or by shielding specific amino acids important for self-interaction. Additionally, certain sugars, amino acids, sugars and salts can enhance protein solubility by hydrating and stabilizing them into more compact conformations.[10]

Kinetic and Thermodynamic solubility is one such definition to define solubility, a critical parameter. The way to tell them apart is through the mode of solubility measurement.

Kinetic solubility refers to the ease with which a protein forms an amorphous or crystalline precipitate upon introduction to a new solvent. This means resuspending it in a concentrated stock solution in a particular solvent before diluting it in a specific aqueous solution to see how it performs at that concentration. Determination of protein solubility is typically performed after the removal of insoluble protein by filtration or centrifugation.

Solubility is an equilibrium parameter from a thermodynamic point of view. That is, solubility, being a thermodynamic property of proteins, is related to the chemical potential of the corresponding molecules. More specifically, protein precipitates appear when the chemical potential of a protein molecule in solution becomes equal to or greater than that of the protein in a certain solid phase (which can be either crystalline or amorphous). Thus, it can be said that protein solubility is the concentration at which the chemical potential of the dissolved protein has already been equal to that of solid protein phase, under defined environment including the pH, ionic strength and temperature.

Hence, the thermodynamic solubility governs the amount of a compound that can dissolve (or go from solid to liquid state).[1]

3.2 Viscosity:

One of the main features of how high protein concentrations influence viscosity is that their dynamic viscosity increases not linearly with concentration but exponentially. Even a small difference in protein concentration can have a large effect in terms of the potential difference in viscosity for a drug product and the variability in that viscosity. Similarly, dynamic viscosity is closely dependent on the product temperature and the applied shear stress.

High viscosity not only create processing and storage difficulties but also creates formulation and delivery challenges.[11]

For highly viscous protein solutions, the force one has to apply on the syringe during injection becomes huge, which will likely cause massive pain to the patient.[17] It might lengthen injection times. This may cause discomfort in patients and potentially have a negative impact on medication compliance and adherence. In drug development, the highly viscous nature of such solutions leads to product loss by its adherence to primary packaging surface, hence should be accounted.

For autoinjectors, keeping the device well-functioning for the duration of its shelf-life is very difficult, requiring intensive modelling and accelerated aging to replicate the loads that it will face throughout its lifetime.

A viscosity range appropriate for subcutaneous injection depends on the patient population. For these patients with limited dexterity, we usually constrain to a viscosity less than 10 cps for the 1mL prefilled syringe. In individuals with normal dexterity, this limit might go as high as ~20 cps. In instances wherein patients with higher viscosity solutions, autoinjectors may be selected to allow for self-administration and decrease the time for administering an injection to under 10 seconds.[11]

Concentration dependent self-association of mAbs leads to increased viscosity which can create challenges in the ultrafiltration diafiltration (UFDF) unit operation of manufacturing. This can slow operations or impose unacceptable tensions on the system. With moderate viscosity, other measures can be implemented such as single pass tangential flow filtration (TFF) or increasing the recirculation temperature to address these situations.

A higher viscosity can also affect the filling process with an effect on both accuracy and fill rate.

Interruptions or extreme decrease in the fill rate can contribute to a phenomenon known as drying at the filling needles, where aggregates or particulates are developed in the final end of the drug product or force flushing of fill lines, culminating in yielding loss. Moreover, due to its high viscosity, it can interfere with the use of pre-filled syringes during administration, particularly for subcutaneous administration with a syringe and needle.[8]

Differences in the way antibodies associate with each other likely explain the differences in their solution viscosity behaviors. Intermolecular interactions between each antibody molecule cause differences in their imbedding hydrophobic/electrostatic/Hydrodynamic/HC/residual interaction. (Figure 1)

This interaction results in the formation of networks of antibodies and other complex structures in their solutions. The surrounding environment, as well as the relative proximity of these molecules, affects this interaction, with the interaction being mostly pairwise at low concentrations. This is manifested in the diffusion interaction parameter (kD). However, at higher concentrations, the effects of higher order interactions become increasingly relevant and can have a strong influence on the viscosity of the solution.[20]

The Derjaguin-Landau-Vervey-Overbeek DLVO theory, originally developed for colloidal systems, can also explain some protein behaviors in solution. This approach is mediated on a pairwise basis between the two interactions and aims to rationalize overall properties in these Van der Waals systems including overall stability and aggregation behavior. DLVO theory, when applied to proteins in solution, takes into account how such pairwise attractions and repulsions between protein molecules influence their behavior, such as their propensity to aggregate, stability under different environmental conditions, and their viscosity in solution at particular concentrations. Hence, DLVO theory serves as a foundation to scrutinize and predict solution behaviours of proteins based on their intermolecular interactions.[21]

Electrostatic characteristics include, but are not limited to, net charge, distribution of partial atomic charges, isoelectric point (pI), and dipole moment and determine the type, frequency, and lifetime of intermolecular interactions occurring in solution at a certain antibody concentration. Hydrophobic interactions arise from the repulsion of biological macromolecules by water and the tendency toward self-association. Such interactions take place when molecules are spatially nearby and show complementary forms at their interfaces. Unlike hydrophobic interactions, that are distance and shape complementary, electrostatic interactions are effective over a large number of intermolecular distances.[22]

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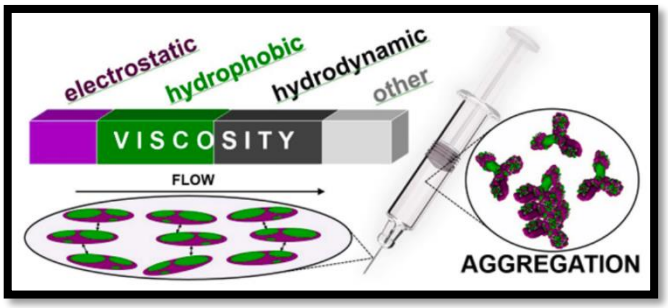

S I T

Figure 1: Effect of intermolecular interaction on aggregation and viscosity

3.3 Aggregation:

Antibody aggregation is a major concern because aggregates are generally expected to be immunogenic, possibly hyper-potent, and in contrast they can reduce overall efficacy.[12]

While crystalline aggregation found during the stability testing could decrease product shelf-life, posing risks to supply chains. Both high molecular weight species can arise from weak, nonspecific interaction among proteins (self-association) and through covalent bonds (covalent aggregation).[13] In either case, the protein can aggregate to form soluble entities (dimers, trimers, etc.) or insoluble aggregates that often take the form of particulates.

Protein aggregation is affected by multiple factors, such as molecular attributes, formulation composition (including the concentration of actives), agitation, temperature, light exposure, and interactions with particular materials. In addition to controlling processing conditions, storage, shipment and handling should also help mitigate aggregation, though it cannot be avoided altogether. In such instances, gaining knowledge and optimizing molecular properties and formulation composition are important to minimize stability risks.[14]

Raising the recirculation temperature can also lead to increased level of Protein aggregation and chemical degradation.[8]

4 APPROACHES

Understanding pH behavior of the protein is the first step in planning a suitable dosage form and formulation. Choosing the formulation pH is key to avoiding the many potential degradation mechanisms, such as aggregation, insufficient solubility (which is related in part to the protein's isoelectric point) and the chemical degradation that can occur via oxidation, deamidation and hydrolysis, etc. [15] The majority of the pathways of chemical degradation can be managed effectively by lyophilization.

4.1 Approach to overcome viscosity:

Proteins normally have low viscosity below 75 mg/mL. For some proteins, concentrations in the range of 100 to 200 mg/mL may produce higher viscosities that exceed the typical injectability limit of approximately 20–25 mPa·s. Over this range, proteins can self-associate to form transient clusters that increase viscosity.

Above 200 mg/mL proteins are in closer proximity and protein–protein interactions can occur unless the proteins have specific self-interactions. Viscosity reducing excipients can also affect protein-protein interactions in both scenarios but negative impacts typically occur at concentrations less than 200 mg/mL.[17]

Viscosity is due to noncovalent intermolecular interactions that trap a network of molecules. In this way, any molecule or substance able to interfere with this network can be used to lower the viscosity of this sample. Because charge-based interactions can influence molecules over longer ranges than hydrophobic interactions, they usually dominate.

For instance, sodium chloride reduces viscosity by shielding charges on proteins, resulting in less electrostatically driven protein-protein interactions (PPI). Alternatively, it has been proposed that arginine operates by direct contact with the protein surface. Such binding action could protect charges and also reduce hydrophobic forces by associating with aromatic residues.[8]

Caffeine showed the ability to decrease the viscosity and also outperformed sodium chloride and arginine in some cases.[16]

The molecular basis of intermolecular interactions between proteins is the same as that for intramolecular interactions that structurally stabilize proteins. Thus, excipients which reduce viscosity by targeting protein-protein interactions may also destabilize proteins.[17]

Protein stability is very much concentration dependent, and this is well known. Excipient act primarily as stabilizer in lower concentration but their increased concentration adversely affects the protein stability. Thus, keeping the excipient at the right concentration is an important part of the strategy in preserving protein stability.[18]

Excipient combinations present an effective way to reduce protein viscosity and retain protein stability – and as a result provide a more convenient route of drug administration through the subcutaneous route. A combination of an amino acid and an anionic excipient is more effective at lowering viscosity than either used alone, even at higher concentrations.

Combining excipients to decrease viscosity can thus be added as a valuable tool for TFF in the downstream workflow.

4.1.1 Downstream Bioprocessing

Downstream bioprocessing refers to the steps involved in purification and concentration of molecules from natural sources (e.g., including but not limited to clarification, chromatography, and filtration).

One of these methods, tangential flow filtration (TFF), is commonly used in downstream bioprocessing for two main reasons:

A. Ultrafiltration: For increasing the solute concentration in solution by size.

B. Diafiltration: Buffer exchange.

These approaches can have particular uses such as:

1. Removing organic solvents in the Manufacturing of antibody-drug conjugates.

2. Concentrating plasmid DNA and exchanging chromatographic buffers in plasmid DNA production.

3. High-viscosity drugs concentration has been increased.

4. Pre-concentrating mAb before anion exchange chromatography.

This is where TFF comes in, it enhances the efficiency and effectiveness of these processes in the production of biopharmaceuticals.

In contrast to classical normal flow filtration (NFF), tangential flow filtration (TFF) pushes the feed parallel to the membrane, not through the membrane directly. (Figure 2) This design has some advantages compared to others: the possibility to reduce the risk of filter clogging and the gentler treatment of shear-sensitive products.[19]

Strategy I: Selection of antibody candidates with low viscosity.

Strategy II: Optimizing the lead antibody candidate(s) to get reduced viscosity.

Strategy III: Optimizing the formulations of antibodies.

Strategy I: Selection of antibody candidates with low viscosity:

The primary benefit of this mode is that it is the most proactive and cost-efficient, ideally applied within early stages of the drug discovery process when typically, 10 – 100 lead candidates are being pursued. At this point, the focus should shift to therapeutic antibody candidates likely to demonstrate low viscosities at high concentrations, with all other attributes—such as potency, safety, physicochemical stability, and pharmacodynamics/pharmacokinetics.[20]

Strategy II: Optimizing the lead antibody candidate(s) to get reduced viscosity

This decision can be taken at a stage in the drug discovery process when only 1–5 or at most 10 molecules are selected. At this point, only, the amino acid sequences of the antibody can be optimized to reduce viscosity without negatively impacting biological

activity and physical-chemical stability. This strategy requires a detailed knowledge of the sequence–structure properties of the drug candidates and the use of protein engineering and molecular biology facilities. [23]

Strategy III: Optimizing the formulations of antibodies.

Understanding how formulation components e.g. buffer, pH, salt, surfactant, and other excipients impact intermolecular interactions is essential for designing a well-founded antibody drug candidate formulation optimised for reduced viscosity. However, formulation components have been studied more in depth on their effect on aggregation, stability, and solubility than on the viscosity of antibody solutions. Nevertheless, some recent work has elucidated the contributions of certain formulation components to viscosity of antibody solutions. [20]

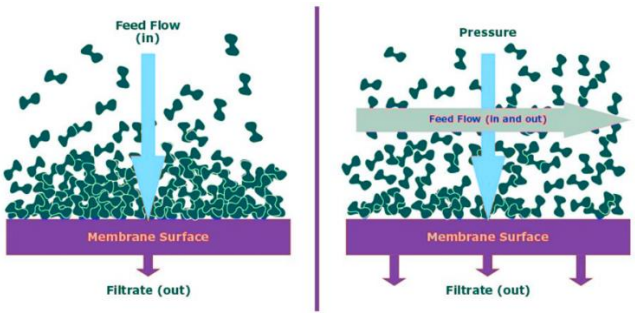

Figure 2: Normal flow filtration (NFF) vs Tangential flow filtration (TFF)

4.2 Approach to overcome self association and aggregation:

High-Concentration Protein Formulations: Challenges and Solutions for Aggregation and Self-Association Here are some concrete ways to help prevent that:

4.2.1 Protein Engineering

4.2.1.1 Surface Charge Optimization:

Rearrange amino acids to gain or eliminate charged residues, minimizing electrostatic attractions.

Change the pI of the protein (isoelectric point) to prevent neutral charge scenarios where aggregation is more probable. [30,32]

4.2.1.2 Hydrophobicity Reduction:

Decrease hydrophobicity of the surface by mutating hydrophobic residues facing the solvent.

Hydrophobic patches on fusion tags or through chemical modifications such as PEGylation protect each other from the other neighboring proteins. [30,33]

4.2.1.3 Improving Inherent Stability:

Incorporate disulfide bonds or other structural features to stabilize the protein's native state.

To increase thermal and colloidal stability by directed evolution or rational design.[33]

4.2.2 Optimizing Formulation Conditions

4.2.2.1 Buffer Selection:

Keep the pH at the one that is favorable for the most stable form of the protein, well away from the protein pI.

Use buffers such as histidine, citrate, or phosphate that can provide stability across a wide range of pH.[31,35]

4.2.2.2 Ionic Strength Adjustment:

Supplement with salts (e.g., NaCl, KCl) in moderate concentrations to screen electrostatic interactions and diminish aggregation propensities.

Do not use a very high concentration of ionic strength, which could induce aggregation through salting-out. [30,34]

4.2.2.3 Temperature Control:

Store and handle formulations under conditions that avoid aggregation-inducing stress.

Add cryoprotectants (e.g., sucrose) for freeze-thaw stability.[35]

4.2.3 Use of Stabilizing Excipients

4.2.3.1 Sugars and Polyols:

Some sugars, like sucrose, trehalose or sorbitol stabilize proteins by preferential hydration [34,35].

4.2.3.2 Surfactants:

Supplement non-ionic surfactants, eg polysorbate 20 or 80 to reduce aggregation caused by contact with surfaces.[31,34]

4.2.3.3 Amino Acids:

Reduce self-association by disrupting protein-protein interactions by adding arginine, lysine, or glycine. [32,36]

4.2.3.4 Cyclodextrins:

Coat exposed hydrophobic regions with cyclodextrins to reduce aggregation.

4.2.4 Regulating the Concentration of Proteins

In case aggregation continues, change the protein concentration to achieve a compromise between therapeutic efficacy and physical stability.

Use co-formulations with other stabilizing proteins or inert ingredients to reduce crowding. [32,35]

4.2.5 Processing Techniques and More

4.2.5.1 Low-Shear Mixing:

Minimize shear and use gentle mixing practices to prevent stress-induced aggregation during manufacturing.

4.2.5.2 Microfiltration:

Microfilters or tangential flow filtration to remove pre-formed aggregates.

4.2.5.3 Freeze-Drying:

Optimize the lyophilization formulations with reconstitution buffers to minimize aggregation. [31,35]

4.6 Predictive & Analytical Tools

Assessment of excipients and buffer condition for aggregation reduction by high-throughput screening.

Use molecular dynamics simulations to pinpoint aggregation-prone segments and direct strategies for protein or formulation alterations.

Use analytical ultracentrifugation (AUC) and dynamic light scattering (DLS) to follow self-association.[32]

4.7 Novel Formulation Approaches

4.7.1 Liquid-Liquid Phase Separation (LLPS) Studies:

Identify mechanisms by which proteins can separate into stable concentrated phases without aggregation.[35]

4.7.2 Nanoparticle Encapsulation:

Incorporate proteins in biocompatible nanoparticles to encapsulate proteins from aggregation conditions.[32]

5. CONCLUSION:

HA molecules are widely used in the formulation of parenteral products, especially in the formulation of high-concentration antibody products (HCAPs) for subcutaneous formulations, which improve the convenience of use to patients and reduce the burden on healthcare. Nonetheless, problems like high viscosity, aggregation of proteins, and solubility constraints require novel approaches. Optimized antibody design, excipient engineering, and cutting-edge bioprocessing techniques, such as TFF, are among the key strategies to surmount these challenges. A keen knowledge of protein-protein interactions and factors that affect stability creates formulations that are easy to administer and helpful to the patient. Solving these challenges is what HCAPs do all day, every day, revolutionising therapeutic delivery (particularly in chronic conditions) by reducing technical and economic barriers leading to improved access, adherence, and outcomes.[35]

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