ProBio

White Paper

Challenges and Solutions in the Development of High-Concentration Protein Formulations

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High concentration formulation market

As biomedicine advances and market demands evolve, high-concentration protein formulations (HCPF) have become increasingly prominent in the biopharmaceutical industry. HCPF is particularly valued for making subcutaneous protein drug injections feasible. HCPF offers significant benefits in both clinical and commercial contexts. Clinically, it allows for patient self-administration, reducing the need for frequent medical visits, enhancing patient compliance, and lowering healthcare costs. Commercially, it supports product innovation and differentiation, easing competitive pressures and reducing the risks associated with new drug development.[1] Additionally, high-concentration protein formulations are in high demand for specialized delivery methods, such as intravitreal injections, where the unique structure of the eye necessitates extremely small injection volumes (typically around 50 μL and not exceeding 100 μL).

Market data indicates that HCPF is predominantly used in antibody products. Figures 1 and 2 show the number of high concentration antibody product (HCAP) approved by the FDA and their distribution across various administration routes from 1998 to 2023. The approval of HCAP by the FDA has shown a marked increase since 1998. In 2023 alone, 12 antibody products received FDA approval for the first time, with 6 of these being HCAPs, accounting for 50% of the total. Furthermore, nearly 70% of HCAPs are administered via subcutaneous injection.[2]

Fig 1 Trends in U.S. HCAP Product Approvals by Year (1998–2023)

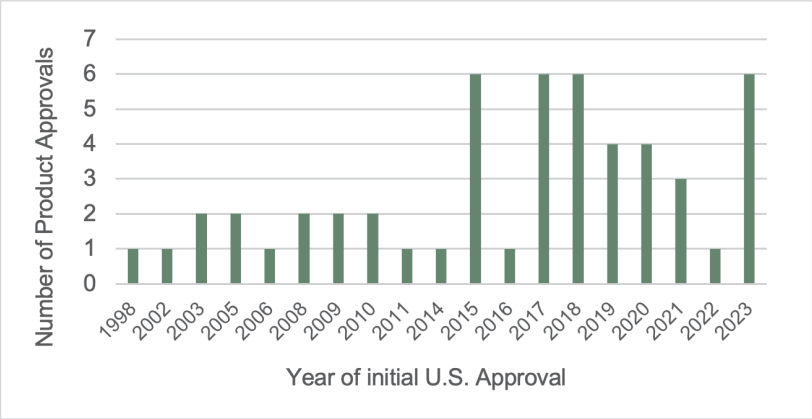
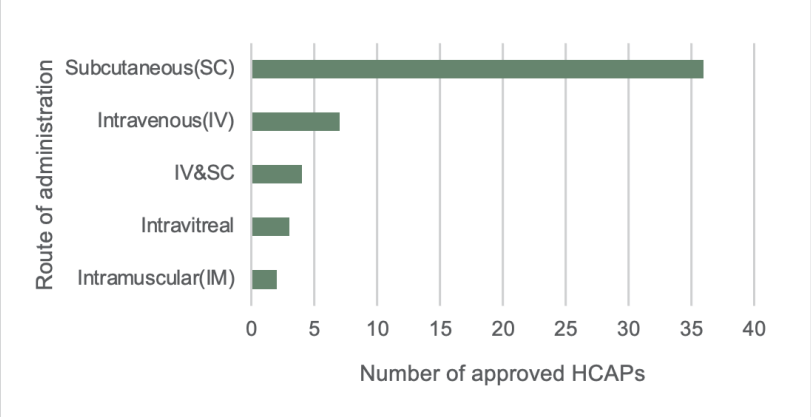


Fig 2 Approved HCAPs by Route of Administration

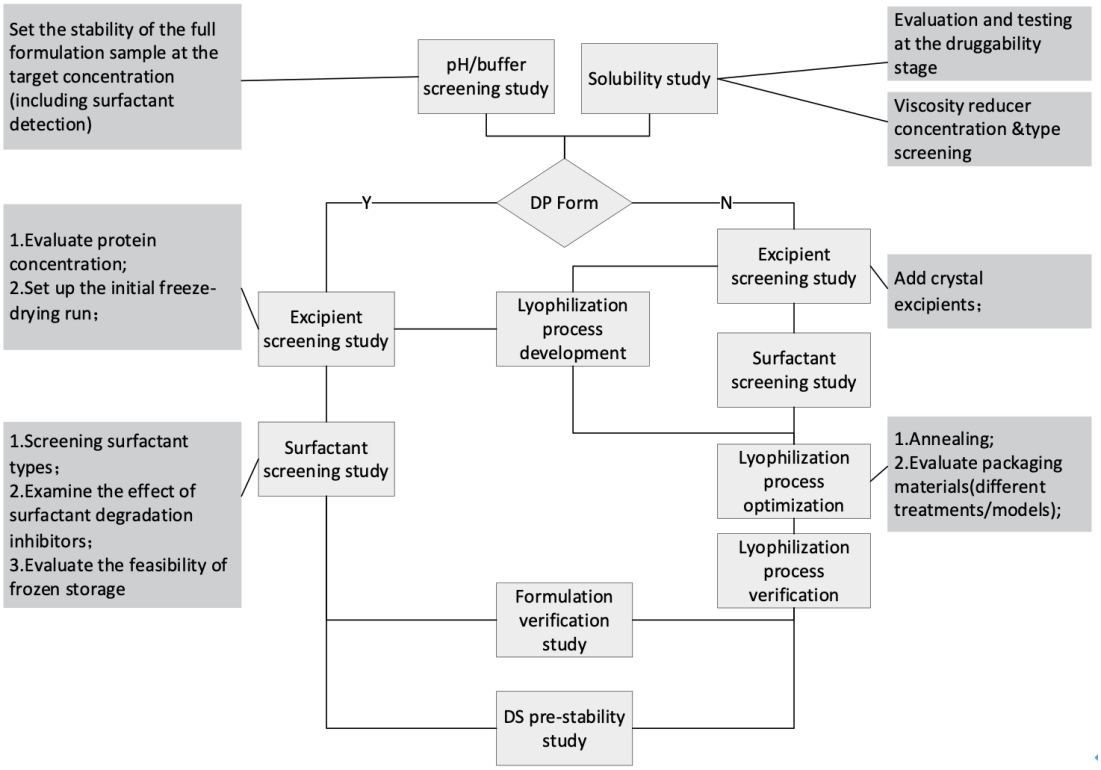


Challenges in Developing High-Concentration Protein Products

As protein concentration increases, multiple noncovalent intermolecular interactions lead to heightened viscosity and accelerated aggregation.[3,4] Additionally, higher protein concentration result in elevated levels of residual host proteins, including esterases that degrade polysorbate, a commonly used surfactant. This accelerated degradation compromises the surfactant's ability to stabilize the protein.[5-8]

Figure 3 showcases ProBio's specialized approach to tackling the challenges of high-concentration protein formulations. By leveraging extensive experience in biologics development, ProBio has crafted a formulation platform that optimizes and accelerate the development.

Fig 3 ProBio's High-Concentration Formulation Development scheme



Solutions for high concentration protein formulation challenges

Developing high-concentration formulations presents several challenges, such as high viscosity, rapid aggregation, accelerated surfactant degradation, fluctuations in protein concentration measurements, pH drift, and stringent HCP (Host Cell Protein) control. The following section delve into the primary challenges related to viscosity, aggregation, and surfactant degradation, and explore the solutions for effective formulation development.

Viscosity

Extensive research has established a clear correlation between protein concentration and solution viscosity, primarily driven by non-covalent interactions, especially electrostatic forces, between protein molecules. At low concentrations, these interactions had minimized impact to viscosity. However, as protein concentration rises, reduced molecular spacing leads to exponential increase in viscosity. Typically, the viscosity of solutions for

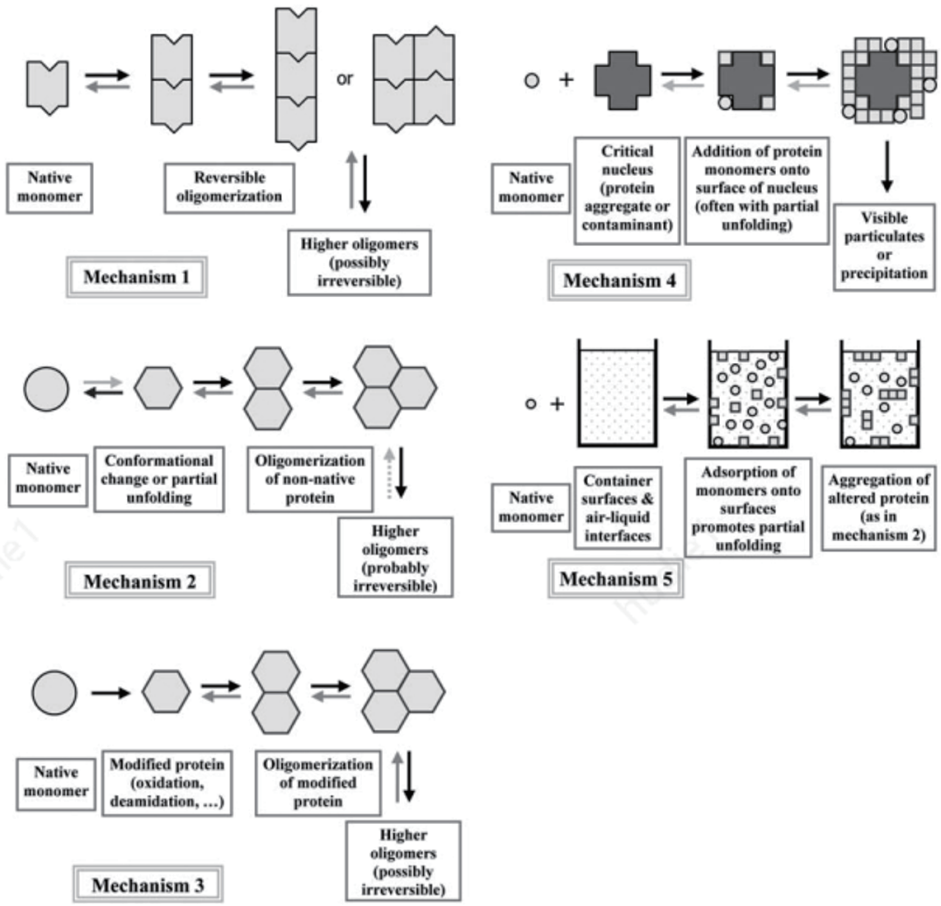
subcutaneous injection should be ≤20 cP, while intravitreal injections require even lower viscosity, usually ≤10 cP.[9]

To address viscosity issues, it is essential to reduce protein-protein interactions, typically achieved by adding viscosity-reducing excipients. Commonly used excipients include amino acids and inorganic salts such as arginine hydrochloride, sodium chloride, and proline. The effectiveness of these agents depends on the specific protein and the type and amount of the excipient used, requiring a tailored approach for each project.[10] In ProBio's experience, appropriate viscosity reducers have lowered viscosity by up to 70%. Additionally, selecting the right protein concentration is crucial, requiring a careful balance of clinical needs, viscosity, and stability.

Aggregation

Protein aggregation at the molecular level is driven by intermolecular attractive forces, with different proteins leading to diverse aggregation behaviors. As shown in Figure 4, interactions between protein monomers can produce aggregates with varying properties.[11] At high concentrations, reduced molecular spacing intensifies these interactions, further elevating the risk of aggregation through mechanisms like electrostatic interactions, van der Waals forces, and hydrophobic effects.

Fig 4 Diagram of Common Aggregation Mechanisms



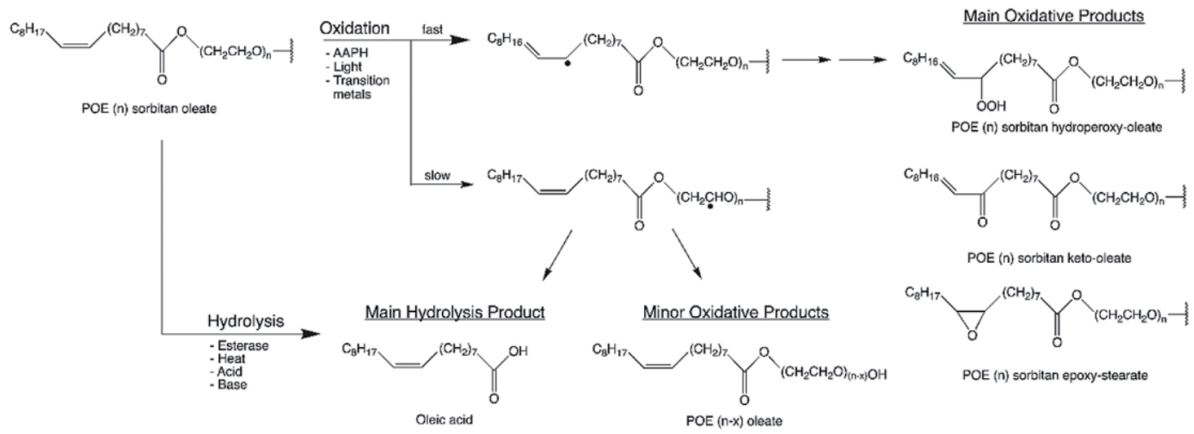
Single-factor (OFAT) or DoE (Design of Experiments) methods are used to optimize the formulation by evaluating the effect from additives and their combinations on stability. HCPF requires extra attention to stability which may compromised by viscosity reducers and high protein concentration.

Leveraging its extensive experience with protein aggregation, ProBio has developed a method to predict the aggregation tendencies of candidate formulations by evaluating protein conformational stability with nanoDSF or DSC and colloidal stability using kD measurements. These predictive evaluations, combined with accelerated stability studies, help to significantly shorten development timelines while minimizing risks.

Surfactant degradation

Polysorbate 20 and 80 are the most common surfactants in protein formulations. In high-concentration formulations, they can degrade through oxidation, hydrolysis, and enzymatic breakdown.[10,12] Identifying the causes of degradation is essential for developing targeted solutions.

Fig 5 Polysorbate Degradation Pathways



ProBio conducts comparative studies early in the formulation development of high-concentration formulations. These studies(such as adding methionine, EDTA, using non-histidine buffer systems, or incorporating enzyme inhibitors)help identify the causes and risks of surfactant degradation and provide a foundation for developing effective solutions.

Different from typical oxidation and hydrolysis, enzymatic degradation usually occurs only in HCPF. This degradation is caused by esterases from Host Cell Proteins (HCPs) that specifically catalyze the cleavage of ester bonds in polysorbate molecules, compromising their ability to protect proteins. Esterases is concentrated in ultra filtration step among the target protein. And high esterases concentration could accelerate the degradation of polysorbate molecules. This issue is typically addressed by controlling esterase levels through upstream and downstream processes, using surfactants without ester bonds, or applying lyophilization.

Other challenges

Long reconstitution time

High-concentration protein formulations have high solid content, dense cake layers, and high viscosity after reconstitution. During reconstitution, the solution exhibits poor wettbility, low tendencies for rehydration and disintegration, and low porosity, all of which contribute to a prolonged reconstitution time, sometimes even extending to several hours[13-17]. Improving the crystallinity during the freezing process of the solution helps address the issue of long reconstitution times[14]. Based on ProBio's project experience, the reconstitution time can be reduced by more than half. However, depending on the product's application scenarios and market

strategies, the shortened reconstitution time may still be unacceptable, in which case, reducing the protein concentration appropriately should be considered.

pH drift

pH drift is relatively common in HCPF due to the Donnan effect, where protein molecules specifically adsorb certain ions in the solution, causing pH changes. This can be mitigated by pH correction during buffer preparation, adjusting the solution's pH to offset the drift cased by Donnan effect.

UF/DF risks

Over concentration is always applied during UF/DF step to increase the recovery rate. For HCPF, over concentration might lead to membrane clogging which caused by high viscosity. Therefore, close coordination with downstream processes is essential during formulation development to ensure scalability under target conditions. Additionally, downstream purification should optimize UF/DF membrane pore size, material, and process parameters to reduce production risks.

Big variation in concentration measurement

Conventional UV spectrophotometry requires diluting protein solutions for accurate concentration measurements. For high-concentration protein formulations (HCPF), high viscosity and large dilution factors can lead to significant measurement variability. Recent advances allow for direct measurement of HCPF without dilution, greatly reducing this variability. Alternatively, gravimetric dilution can help control measurement variability if differences in solution density before and after dilution are accounted for.

Summary

HCPF poses challenges such as high viscosity, rapid aggregation, and surfactant degradation. Comprehensive effort from upstream, downstream, formulation, and analytical department is needed to address the challenges.

ProBio leverages its extensive experience and industry-leading expertise to successfully support clients in delivering high concentration formulation projects.

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