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White Paper

The Viscosity Reduction Platform: Viscosity-reducing excipients for improvement of filtration processes

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Introduction

Protein viscosity is a major challenge in preparing highly concentrated protein formulations suitable for subcutaneous injection. Recently, the Viscosity Reduction Platform (VRP) was introduced and its technical key features and benefits for formulations were discussed. However, highly viscous solutions do not only pose a challenge when administering a drug to a patient, they can also impose technical limitations in the manufacturing process.

The challenge arising from highly viscous proteins becomes more difficult particularly when advanced downstream processing (DSP) methods are used. As described in literature, an increasing protein concentration can promote attractive interactions between proteins that can ultimately lead to high viscosity.[1,2] The resulting decreased flux rates due to the higher flow resistance may lead to protein gelation and potentially membrane fouling.[3]

Reducing processing time, footprint and aggregate formation are critical challenges in the production of highly concentrated monoclonal antibodies.[4] Typically, tangential flow filtration (TFF) is used for the final formulation step in order to exchange the process buffer for the formulation buffer (diafiltration), clear small molecular impurities and concentrate the protein to the desired final concentration. The sizing of the unit operation and ultimately the process economics rely on the mass transfer/permeate flux during the concentration and diafiltration steps.

This white paper evaluates the effect of the excipients in the Viscosity Reduction Platform on ultrafiltration processes used to produce a highly concentrated formulation of a monoclonal antibody (mAb). Two filtration methods are demonstrated in this work.

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In a research and development environment, there is a need to generate highly concentrated protein formulations for a variety of applications like formulation optimization, stability studies, etc. Centrifugal ultrafiltration devices are widely used for this application.

In this white paper, the benefits of the Viscosity Reduction Platform in DSP are discussed with particular emphasis on tangential flow filtration.

In the first application, it is demonstrated how the Viscosity Reduction Platform can help enhance the productivity and final concentrations achieved by this approach.

In the second application, the impact of said viscosity-reducing excipients on the diafiltration step performance using a standard lab-scale TFF system is demonstrated. It is shown that a higher concentration can be reached using viscosity-reducing excipients. The concept and learnings are translatable to the design of large-scale manufacturing.

Results and Discussion

The Viscosity Reduction Platform makes it possible to achieve a higher concentration more quickly using centrifugal filters

Centrifugal filter units are typically used as a first step when preparing small amounts of highly concentrated protein solutions on a lab scale. As such, this setup was used to measure the effect of viscosity-reducing excipients on infliximab. Infliximab is a mAb with a very well-documented high viscosity at concentrations above 100 mg/mL. As previously discussed, arginine, which can be considered the industry benchmark, is not particularly efficient in reducing infliximab's viscosity, which makes the mAb an ideal test candidate to evaluate the Viscosity Reduction Platform in the downstream processing step.[6]

Table 1.

Excipients and abbreviations.

Excipient	Abbreviation
L-Arginine	Arg
L-Ornithine monohydrochloride	Orn
L-Phenylalanine	Phe
Thiamine phosphoric acid ester chloride dihydrate	TMPacid
Benzenesulfonic acid	BSAcid
Pyridoxine hydrochloride	Pyr

Table 1 summarizes the excipients within the Viscosity Reduction Platform. For reasons of clarity, the abbreviations given in Table 1 are used in the following.

The materials used to prepare the buffers, the filtration devices and the TFF cassettes used are listed in Table 2. A 0.1 M sodium hydroxide solution was used to clean the cassettes after each run. A 5 mM phosphate buffer at pH 7.2 containing 200 mM sucrose was used as the basis buffer. The excipients were used at a concentration of 75 mM in each case. pH was adjusted using hydrochloric acid and sodium hydroxide.

Table 2.

Other materials used in the presented study.

Amicon® Ultra-4 Centrifugal Filter Unit, Ultracel®-30
Pellicon® XL Cassette with Biomax® Membrane, 0.005 m², NMWCO 30 kDa
Sodium dihydrogen phosphate monohydrate EMPROVE® EXPERT BP,USP
di-Sodium hydrogen phosphate heptahydrate EMPROVE® EXPERT DAC,USP
Hydrochloric acid 1 mol/L EMPROVE® EXPERT
Sodium hydroxide solution 32%, EMPROVE® EXPERT
Sodium hydroxide solution 0.1 mol/L EMPROVE® EXPERT

The impact of the high viscosity of infliximab on concentration time can be easily determined by centrifuging a solution of 10 mg/mL starting concentration at a velocity of 2,000 × g. Without the addition of excipients, 190 minutes are required to reach a concentration of 150 mg/mL. By adding appropriate excipient combinations from the Viscosity Reduction Platform in an equimolar concentration, this time can be reduced to 120–145 minutes. This represents a time reduction of 35%. Likewise, using excipients increased the filtrate flux during buffer exchange (5 DV).

Figure 1A shows the concentration time that is required to increase the concentration of infliximab from 10 mg/mL to 150 mg/mL using an Amicon® Ultra 4 concentration unit at a centrifugal speed of 2,000 × g at ambient temperature. It is evident that adding any of the excipient combinations results in an improved concentration time. Figure 1B shows the viscosity of the respective formulations. Even for single excipients or excipient combinations that do not reduce the viscosity very well at high protein concentrations, a clear reduction in concentration time can be observed. A potential explanation is that the magnitude of the excipients' viscosity-reducing effect increases with increasing protein concentration. Thus, at a lower protein concentration, the process would be faster. The improved viscosity-reducing effect of the more efficient excipient combinations would only become relevant during the very last minutes of processing. Nevertheless, this simple experiment shows that viscosity-reducing excipients can have a measurable impact when creating a highly concentrated protein formulation.

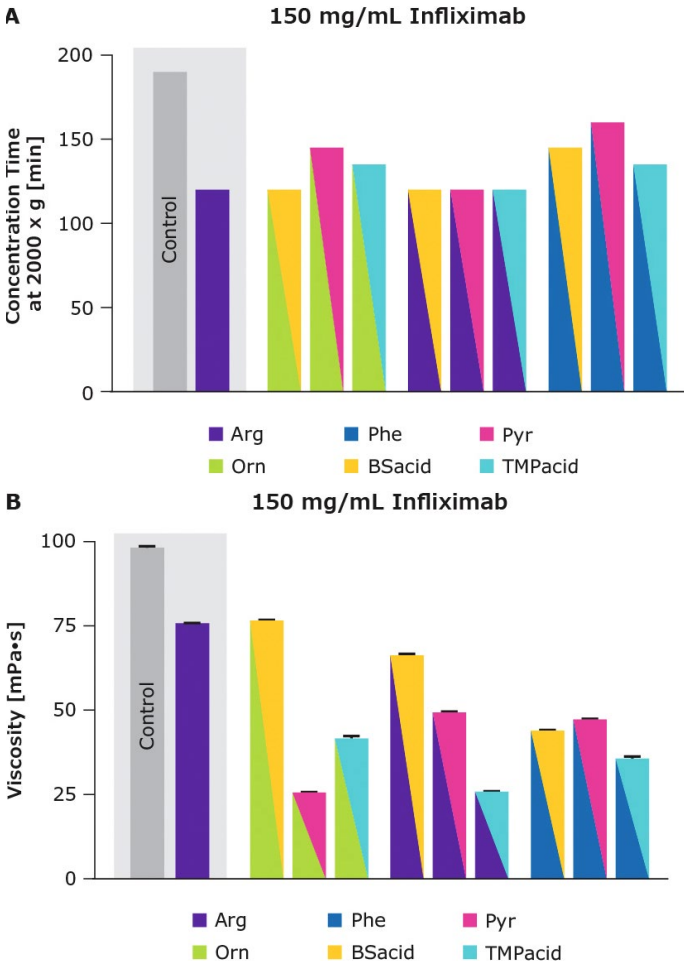

Figure 1.

Influence of VRP on A) concentration time using Amicon® Ultra-4 centrifugal filters with a 30 kDa MWCO to concentrate infliximab to 150 mg/mL and B) viscosity of a formulation comprising 150 mg/mL infliximab.

In the following study, the excipient combinations of Orn/Pyr, Arg/TMPacid and Phe/BSAcid are used, as they are the most efficient viscosity-reducing excipient combinations for infliximab.

Influence of excipients on filtration processes

While Amicon® filters offer a suitable platform to easily showcase the impact of a highly viscous solution on filtration time, the mode of action is clearly different from the tangential filtration method used at manufacturing scale. Despite their vertical membranes, Amicon® filters tend to result in strong concentration gradients within their reservoirs. Protein gelation can be observed, particularly when larger volumes are handled. In a TFF system, such gelation would not occur due to the different flow geometry.

Therefore, a lab-scale TFF system was used to investigate the impact of viscosity-reducing excipients on this process. As temperature not only affects viscosity but may also impact protein stability, experiments were conducted at ambient and cold temperatures. TFF is typically performed at ambient temperatures, which allows for more favorable hydrodynamic properties of the solution.

Nevertheless, for certain processes or for more sensitive proteins, a lower temperature could be chosen. While the protein is more stable at 2–8 °C, the viscosity of the solution will be higher.[7,8]

Experiments were performed using a Pellicon® XL cassette (Biomax® 30 kDa, 0.005 m²) with a steady feed flux of 480 L/m²/h adjusted by a peristaltic pump. For constant volume diafiltration, five diavolumes (DV) were exchanged at a transmembrane pressure (TMP) of 18 psi enforced by an automatic back pressure valve. Lastly, the protein solution was concentrated until a maximum inlet pressure of 43 psi was reached.

The batch process was started with a diafiltration step to exchange 5 DV of a 100 mL starting solution at 8 °C and 23 °C respectively (Figure 2A and B). For this buffer exchange of a 10 mg/mL infliximab stock solution, 338 minutes were needed at 8 °C, while 178 minutes were required at ambient temperature. This process step can be accelerated by adding suitable viscosity-reducing excipient combinations, reducing these times to 231 and 141 minutes respectively. In the following concentration step, using excipients also reduced the final volume reached before exceeding the pressure limit by up to 26%, indicating a higher protein concentration.

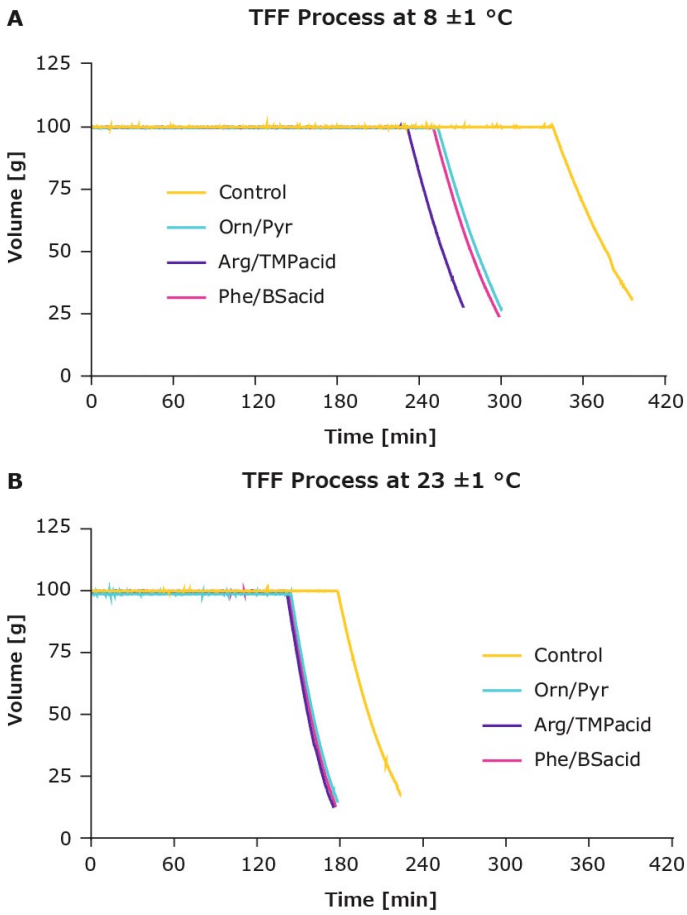

Figure 2.

Diafiltration (5 DV with basis buffer or with excipients) and concentration process of an infliximab stock solution (10 mg/mL) using a TFF system equipped with a Pellicon® XL cassette.
A) Process in cold room conditions B) Process at ambient temperature.

Critical process parameters can be improved by adding excipients

The filtration process as presented herein can be divided into two different sub-steps – the diafiltration, wherein the buffer is exchanged, and the concentration phase, where the target concentration is adjusted. The diafiltration phase in particular is relevant for the overall process time due to the high volumes used in this process step. This phase can make up 80 to 85% of the total process time and is thus critical when optimizing process economics. Figure 3 compares the different process durations for infliximab in the presence and absence of the respective excipients at 8 °C and at ambient temperature. As expected, the diafiltration time is responsible for a major part of the process time. The addition of viscosity-reducing excipients can reduce this time by up to 31%. In spite of the lower volume resulting in a higher concentration factor, the time needed can also be reduced. At ambient temperatures, a similar trend can be observed. However, as pointed out earlier, the overall process duration is reduced at higher temperatures due to the temperature dependence of the filtration process itself.

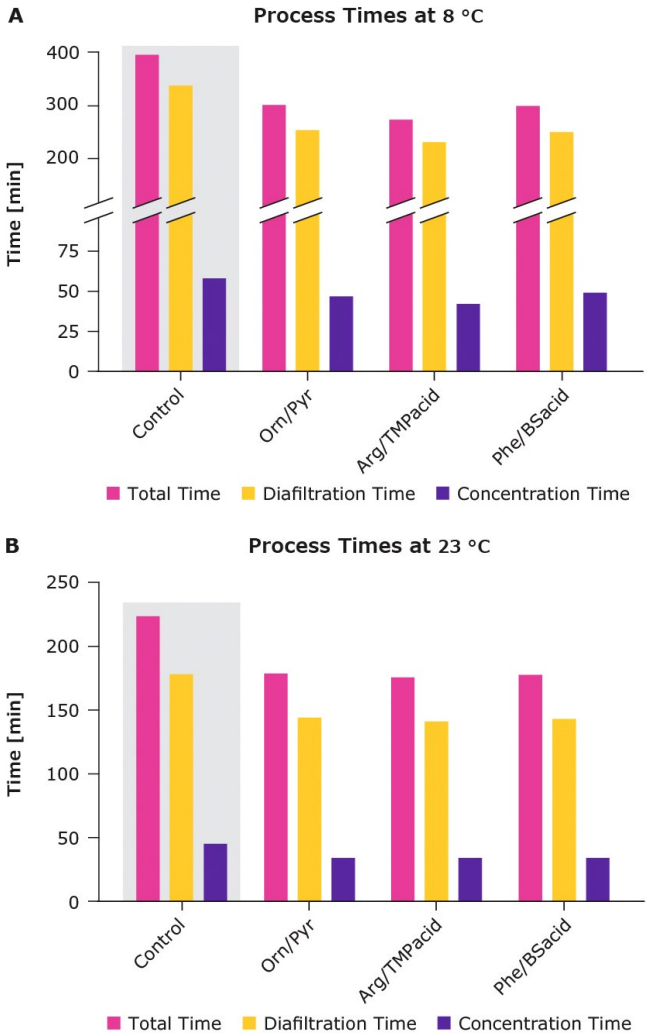

Figure 3.

Effect of excipients on process time of diafiltration and concentration step using a 10 mg/mL infliximab solution and a Pellicon® XL (Biomax®, 30 kDa) cassette. Concentration was increased to a maximum inlet pressure of 43 psi. A) Process times at 8 °C. B) Process times at 23 °C.

To identify the underlying cause of this reduction in process time, the permeability of the membrane was investigated in the presence and absence of excipients at both temperatures. It was observed that adding excipients causes the permeability of the Biomax® membrane to remain higher and also more constant throughout the diafiltration. If a more viscous solution is filtered, which is the case when no excipients are added, the membrane permeability is reduced over time, indicating a progressive fouling of the membrane itself.[3,9,10] The use of viscosity-reducing excipients prevented this membrane blocking and thus allowed for consistently high permeability. This ultimately led to the improved performance during diafiltration that was observed and discussed previously.

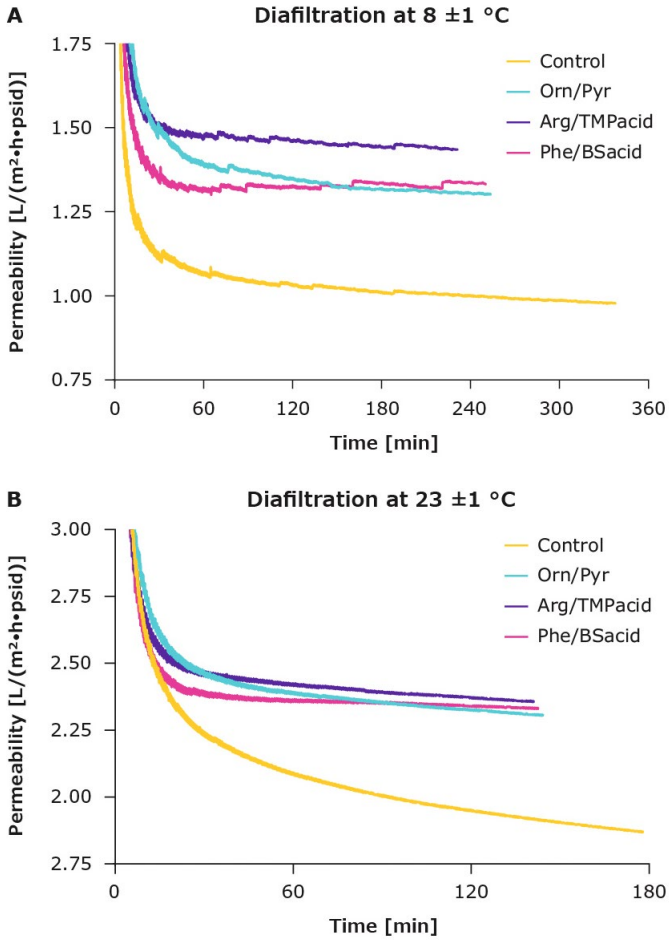

Figure 4.

Permeability during diafiltration of excipient buffer compared to a basis buffer with 5 diavolumes. A) Process in cold room conditions B) Process at ambient temperature.

To further elucidate the underlying cause of the membrane fouling connected to lower permeability, the protein-protein interactions of infliximab were analyzed by dynamic light scattering (DLS). To do so, the particle diffusion – indicated by the diffusion coefficient (Dt) – was measured at protein concentrations of 3–12 mg/mL. Diffusion at infinite dilution (D0) was obtained by linear regression and used to normalize the diffusion data. Figure 5 shows that infliximab in its basis buffer exhibits a strong negative slope, indicating strong attractive protein-protein interactions.[11] Upon addition of the excipient combinations, the slope is less negative, indicating weaker attractive interactions between the proteins. The membrane permeability is thus potentially impacted due to the intrinsic attractive interactions of infliximab in its basis buffer. This data suggests that reducing the attractive forces would be sufficient to preserve higher membrane permeability.

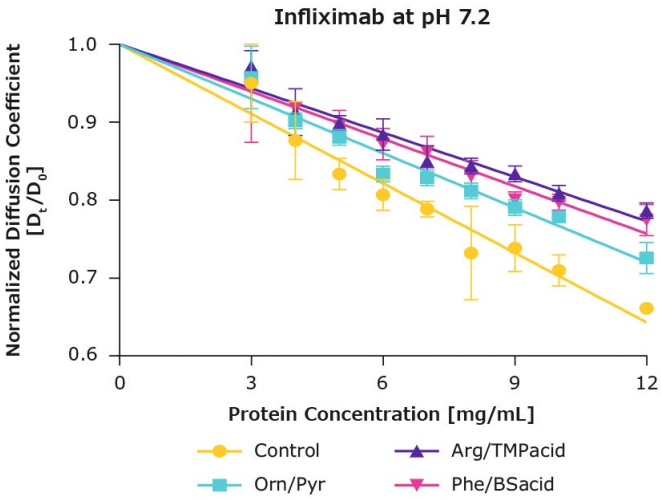

Figure 5.

Normalized diffusion coefficient of infliximab in its basis buffer and with viscosity-reducing excipient combinations.

Impact of reduced viscosity on maximum achievable protein concentration and yield

Besides the overall processing time, the yield is another critical attribute in designing an economically attractive process. Furthermore, for some modalities, the maximum achievable protein concentration can also be relevant. This is typically the case for plasma-derived proteins but can also play a relevant role in the manufacturing of antibodies.[9,12]

Figure 6A shows that when adding viscosity-reducing excipient combinations, the final yield was mostly maintained as compared to the control. The slightly lower level can be explained by the fact that no cassette depolarization step by flushing was performed in order to maintain better comparability. It is noteworthy that for the combination of arginine and thiamine phosphoric acid ester, a full recovery could be obtained even without flushing the filtration cassette.

In all other cases, a similar recovery to the control was observed. Analyzing the maximum achievable protein concentration in Figure 6B, an increase in protein concentration was observed under all conditions where viscosity-reducing excipient combinations were added. At 8 °C, the concentration was increased by 14 to 40%. At ambient temperatures, a concentration increase of 9 to 30% was observed. These two parameters show that in addition to reducing the processing time, an adequate yield can be guaranteed. The maximum achievable protein concentration can also be increased if needed.

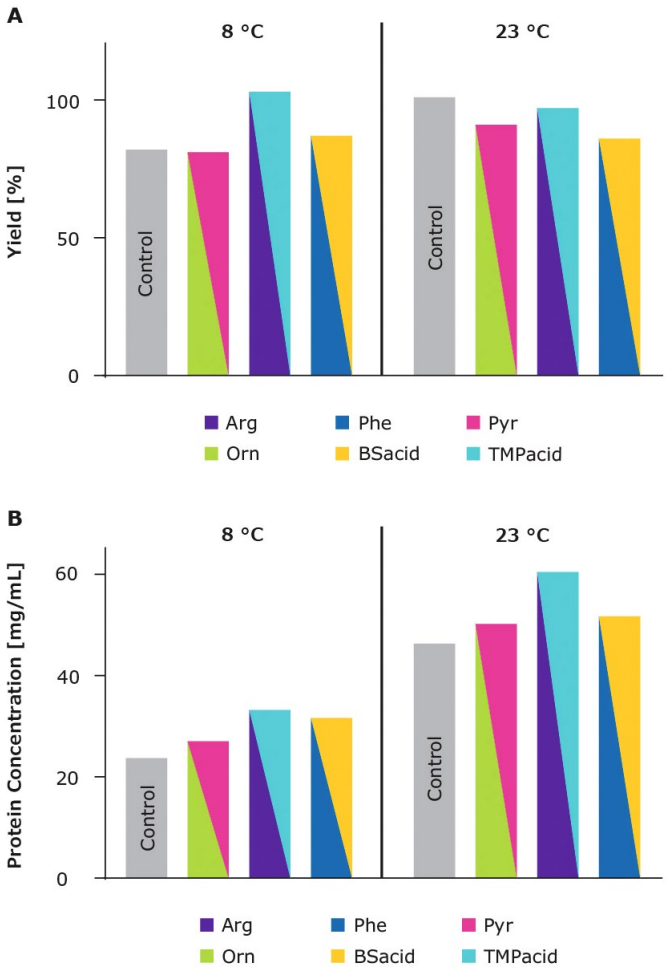

Figure 6.

Final infliximab solution after diafiltration and concentration to a system pressure of 43 psi at either 8 °C or ambient conditions. A) Yield compared to initial material input B) Protein concentration.

Just as for the diafiltration step, the underlying cause of the benefit within the concentration process was investigated further. Differences in the back pressure valve setting were already observed during this step. With excipients in the formulation, the TMP setpoint could be maintained longer before inlet pressure increased (data not shown). Looking at the system when an inlet pressure of 40 psi was reached (close to the process termination point of 43 psi), this benefit translates to a higher concentration factor (Figure 7). At 8 °C, a factor of 320% was reached, in contrast to the basis buffer with 240%. Likewise, 540% compared to 410% were found at ambient temperature. Since higher concentrations can be reached at lower

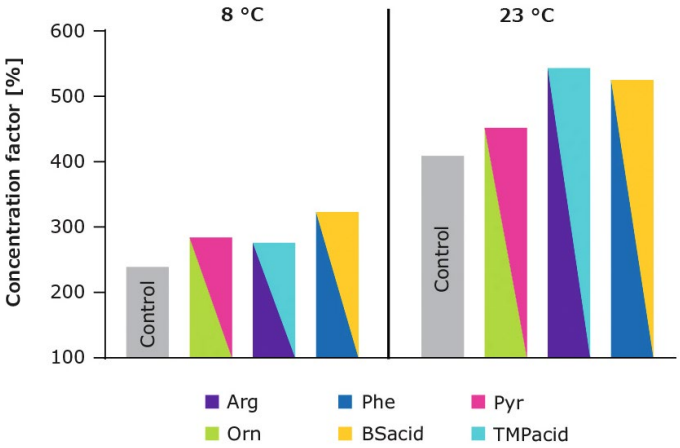

Figure 7.

Concentration factor of a 10 mg/mL infliximab formulation (100 g) after diafiltration process with or without excipients when reaching an inlet pressure of 40 psi (back pressure valve fully open).

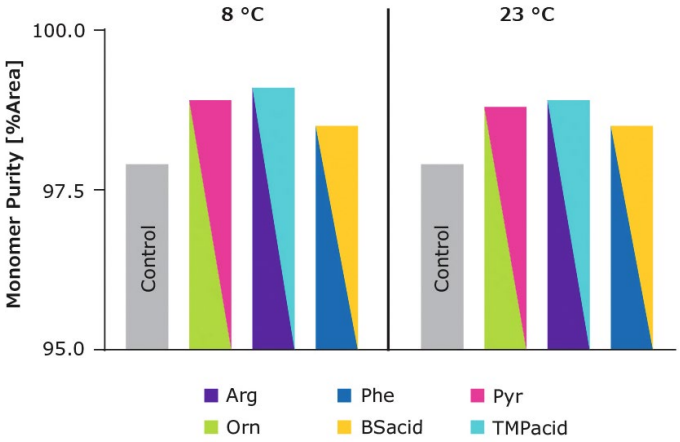

Figure 8.

Monomer purity of infliximab after finalized TFF processes diluted to 1 mg/mL for analysis.

pressure, polarization effects can be reduced.[13] The higher membrane fouling during diafiltration, as shown in Figure 4, may also have contributed to an earlier onset of the pressure increase in the concentration process.

Similarly to the diafiltration step, the concentration process was also improved. Throughout this step, permeability was higher when excipients were used. It would thus be possible to perform the diafiltration step at higher protein concentrations while maintaining the same permeability as in the control. As such, starting volume and thus the total diafiltration volume required for 5 DVs can be reduced. In the case of 100 g starting solution used here, the diafiltration buffer volume could have been reduced by 33–53%.

Impact of reduced viscosity on maximum achievable protein concentration, yield and monomer purity

As shown by the monomer purity determined by size exclusion chromatography (SEC) in Figure 8, a highly pure product was achieved under all process conditions. It is noteworthy that the addition of viscosity-reducing excipients improved monomer purity compared to a TFF run performed in the absence of said excipients. This may be due to either a slight shear-stabilizing effect of the excipients or the reduction in process time and thus in the number of pump passes.

Transferability to large-scale processes

While the process improvements achieved on a lab-scale system cannot necessarily be directly transferred to a manufacturing-scale TFF, it is possible to conceptually transfer these results. Of course, the scalability is very much affected by the specifics of the chosen setup such as the filtration membrane used, the properties of the protein, and the temperature of the process.[14,15,16]

Using viscosity-reducing excipients lowers the time required to perform a filtration, which in a manufacturing setting can be converted into a reduction in membrane area. The extent to which excipients can improve permeability depends on the much higher pressure that can be used in the commercial system and the membrane that is used. For instance, on a commercial scale, the Pellicon® D Screen with the same molecular weight cut-off could be used with a pressure of up to 80 psi.[17,18] Such differences in equipment can potentially amplify the benefit of the Viscosity Reduction Platform during bioprocessing.

Finally, the Viscosity Reduction Platform can improve the yield of pure protein by reducing shear forces. This is another very relevant parameter for a commercial-scale TFF system.

Conclusion

The Viscosity Reduction Platform contains a portfolio of excipients and is based on combining an amino acid with a second viscosity-reducing excipient.

Using excipients that reduce viscosity is a common approach to improve process economics and enable the high protein concentrations needed to meet the final formulation target. By reducing protein-protein interactions and the resulting viscosity, a higher concentration and faster diafiltration time can be achieved while maintaining an adequately high yield.

The underlying cause of the improved process economics when using excipients is the reduction in attractive protein-protein interactions. Consequently, membrane fouling and concentration polarization are

reduced. This means that membrane permeability remains more constant throughout the diafiltration step, which is thus accelerated. The achievable yield remains high. It is also possible to increase the maximum achievable protein concentration, which can be very relevant for plasma products or other monoclonal antibodies, and particularly crucial for the subcutaneous delivery of mAbs.[6] If mid-range protein concentrations are targeted, these can be reached with a lower system pressure, resulting in less polarization and thus potentially a higher yield.

As discussed previously, the Viscosity Reduction Platform enables the user to better balance protein viscosity with protein stability by using excipient combinations.[6] This white paper extends the application of the Viscosity Reduction Platform beyond the formulation of a drug product.

By reducing protein viscosity while maintaining protein stability and membrane permeability, the process economics of manufacturing mAbs can be rendered more attractive. While optimization of large-scale processes is still required, the combination of a very efficient TFF cassette such as the Pellicon® D Screen with the addition of VRP excipients can be a promising approach. Pellicon® D Screen and the Viscosity Reduction Platform are thus suitable tools for creating highly concentrated protein formulations in an economically attractive manner.[9]

The Viscosity Reduction Platform can therefore not only enable the formulation of conveniently administrable drug products but can also improve the process economics in filtration steps.

Please visit: sigmaaldrich.com/viscosity-reduction for a detailed user guide for the Viscosity Reduction Platform. For the technical sample kit as well as information on commercial licensing options, please reach out to your local sales representative.

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