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White Paper

The Viscosity Reduction Platform: Viscosity-reducing excipients for protein formulation

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Introduction

Protein viscosity is one of the major obstacles in preparing highly concentrated protein formulations suitable for subcutaneous (subQ) injection. SubQ application of highly concentrated protein formulations enables a higher patient convenience and might thus lead to reduction in health costs. This can be achieved by reducing the time requirement of the patient to administer therapy, e.g. by enabling self-injection at home and reducing time required to do so. However, highly viscous protein solutions would require a significant force to be applied to the syringe for injection. As a result, the patient could experience a considerable amount of pain. In many cases, injectability would not be possible.[1,2]

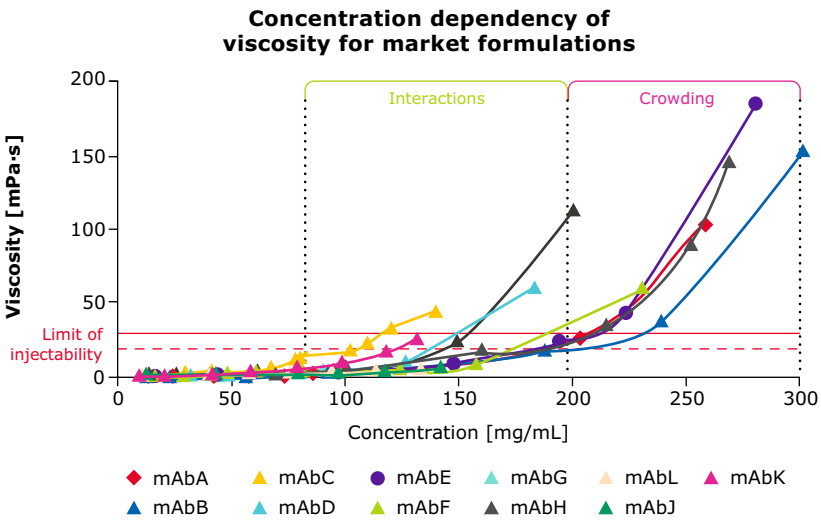

Figure 1.

Dependency of viscosity on antibody concentration and likely underlying causes. Red lines show injectability limit.

When characterizing protein viscosity behavior, one can differentiate two different concentration regimes as shown in Figure 1. At concentrations below 75 mg/mL, proteins are rarely viscous. When increasing the concentration to between 100 and 200 mg/mL, some proteins exhibit elevated viscosity exceeding the limit of injectability, which is typically between 20 and 25 mPa·s. At this concentration regime, several proteins exhibit an affinity for self-interaction, i.e. forming transient clusters that give rise to elevated viscosity. At concentrations above 200 mg/mL, the nearest neighbor distance between the protein molecules shrinks so that without a specific affinity for self-interactions, said protein-protein interactions take place. While viscosity-reducing excipients can affect proteins exhibiting either of these interaction patterns, they are likely to be more efficient at protein concentration regimes below 200 mg/mL.[3,4]

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These intermolecular interactions between proteins have the same molecular origin as the intramolecular interactions that structurally stabilize the proteins. This means viscosity-reducing excipients that affect protein-protein interactions can potentially also destabilize proteins. As such, it is essential to balance an excipient's viscosity-reducing ability against its potential to destabilize a protein. For some excipients, a concentration-dependent effect on protein stability is well-documented. At lower concentrations, the excipients act as stabilizers, but this behavior changes as concentration increases, often with an adverse effect on protein stability.[5] Excipient concentration is thus a critical factor in managing protein stability.

These two aspects can be better balanced by using an excipient combination of an amino acid and an anionic excipient. When used in combination, excipients are more efficient in reducing viscosity and may even do so in an over-additive manner. Consequently, lower concentrations of the individual excipients can be used, which is more favorable for protein stability.

This white paper evaluates the viscosity-reducing capacities of excipients and excipient combinations. It shows the over-additive effect of using two excipients together and addresses how excipients' viscosity-reducing ability depends on pH. The results show the effect of protein viscosity on injection force and highlight the platform's ability to balance viscosity reduction with protein stability. The case studies presented demonstrate that using a combination of two excipients at lower concentrations instead of a single excipient at a higher concentration enables balancing protein viscosity and protein stability in a favorable way.

Table 1: Excipients and abbreviations

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

Results & Discussion

Table 1 summarizes the excipients that are part of the Viscosity Reduction Platform. For clarity reasons, abbreviations mentioned in Table 1 are used in the following. L-Arginine (Arg) is the industry standard for viscosity reduction using a single excipient and consequently serves as the benchmark excipient.

Single excipients often reduce viscosity but may impact protein stability

A single excipient is often used to reduce the viscosity of a protein formulation. Figure 2 shows two model proteins, infliximab and evolocumab, where each component of the Viscosity Reduction Platform has been used individually. Infliximab has a viscosity of about 40 mPa·s at a concentration of 120 mg/mL in its concentrated marketed formulation (see Figure 2A). Adding 75 mM of the single excipients reduces the viscosity by anywhere from 10 to 80%. A similar viscosity reduction is observed when doubling the excipient concentration to 150 mM. Comparing the performance of an excipient at 75 and 150 mM shows that the greatest difference in viscosity reduction between the two concentrations is seen with excipients that are not particularly effective. Excipients able to halve the viscosity of infliximab do not show a proportionally strong viscosity-reducing effect when their concentration is increased. Used individually, Arg and Orn do not reduce infliximab viscosity effectively. However, we will show that these two excipients can indeed be valuable when used in excipient combinations.

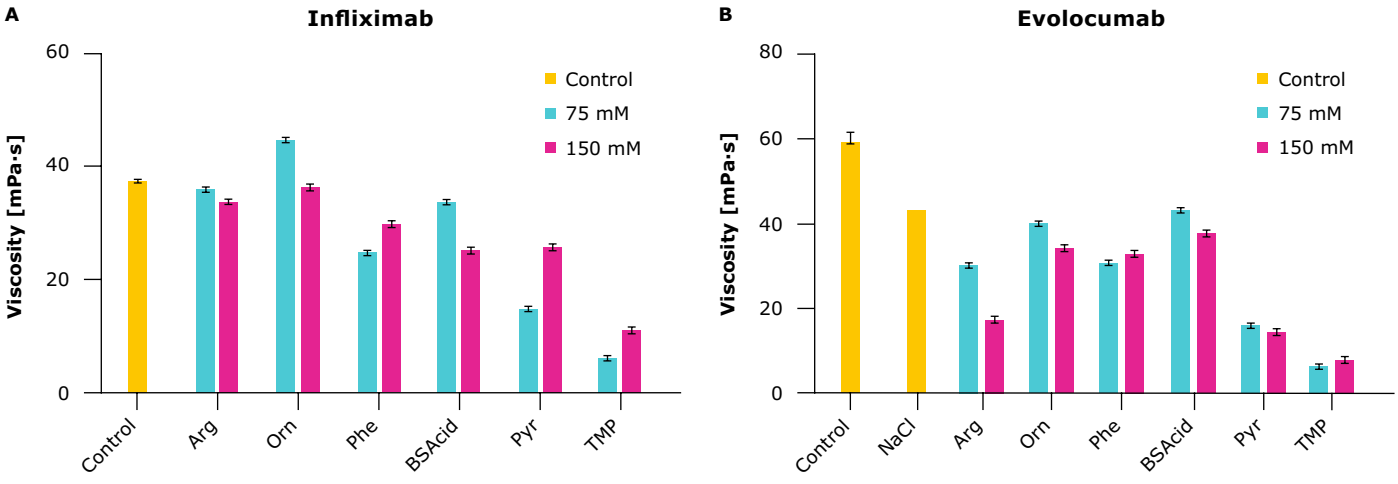

Figure 2.

Influence of increased excipient concentrations on protein formulation viscosity.

Similarly, Figure 2B shows that many excipients lead to an improved viscosity reduction for 170 mg/mL evolocumab when used at higher concentrations. By contrast, Phe actually increases protein viscosity when its concentration is increased. Overall, for both model antibodies, it was observed that doubling the concentration of an excipient does not typically lead to improved viscosity reduction.

Balancing viscosity reduction and protein stability is crucial to successfully develop a stable, highly concentrated protein formulation. A forced degradation study was thus conducted to evaluate the effect of elevated excipient concentrations (125–150 mM) on protein stability. Figure 3 summarizes the monomer content of infliximab and evolocumab formulations after 28 days at 40 °C and 75% relative humidity. Infliximab was formulated at a concentration of 120 mg/mL, while evolocumab was formulated at a concentration of 170 mg/mL. The amino acids do not show an adverse effect on protein stability, with the exception of Phe, which is the most effective viscosity-reducing amino acid for infliximab. Phe's observed destabilizing effect highlights the importance of balancing protein stability and protein viscosity.

The three anionic excipients show a clear destabilizing effect on both proteins, as can be seen in Figure 3. With TMP, a substantial loss of monomer content is seen, likely due to the known instability of the vitamin derivate itself at high temperatures.[6] In summary, highly efficient viscosity-reducing excipients used at concentrations between 125 mM and 150 mM can destabilize a protein. In contrast, amino acids typically allow protein stability to be maintained.

To conclude, while increasing the excipient concentration may allow for improved viscosity reduction, some excipients can destabilize proteins when used at high concentrations. Furthermore, even these increased excipient concentrations may not be able to lower viscosity sufficiently to reach the targeted formulation viscosity.

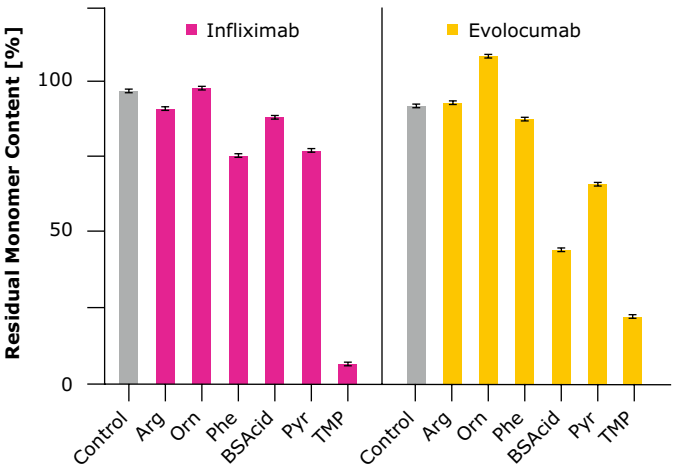

Figure 3.

Effect of single excipients at concentrations between 125–150 mM on monomer content of infliximab and evolocumab stored at 40 °C/ 75% rH for 28 days.

Effect of protein formulation pH on excipient performance

As demonstrated, excipients' viscosity-reducing ability can differ depending on the protein they are used for. As a next step, it is important to consider the formulation conditions. Figure 4 shows the viscosity of 170 mg/mL evolocumab formulated at pH 5 (acetate buffer) and pH 7.2 (phosphate buffer). The materials used to prepare the base buffer are listed in Table 2.

Table 2: Materials used for base buffer preparation

Buffer	Buffer Components
Acetate buffer	Acetic acid (glacial) 100% EMPROVE® EXPERT Ph Eur,BP,JP,USP
	Sodium hydroxide solution 32% EMPROVE® EXPERT
Phosphate buffer	Sodium dihydrogen phosphate monohydrate EMPROVE® EXPERT BP,USP
	di-Sodium hydrogen phosphate heptahydrate EMPROVE® EXPERT DAC,USP
	Optional addition: Sodium chloride EMPROVE® EXPERT Ph Eur,BP,ChP,JP,USP

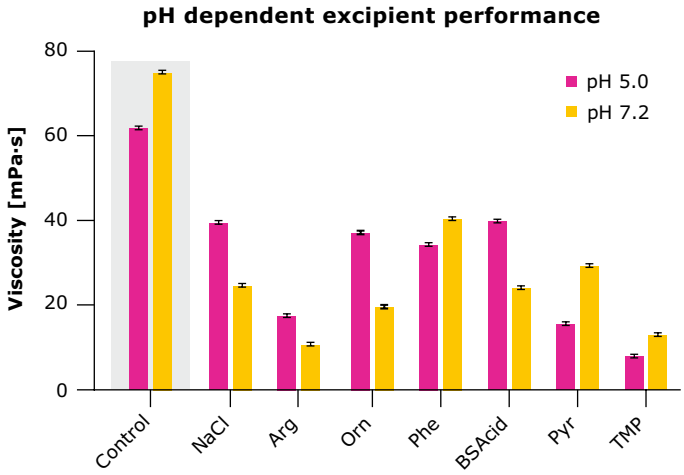

Figure 4.

pH dependency of evolocumab formulation at 170 mg/mL: Comparison using pH 5 acetate and pH 7.2 phosphate buffers.

Formulated in the respective base buffers, the viscosity is much higher than 20 mPa·s. At pH 5.0, it is 59 mPa·s, and at pH 7.2, it is 72 mPa·s. Adding sodium chloride has a stronger effect at pH 7.2 than at pH 5, potentially due to the lower number of charges present on the protein at pH 7.2, which is closer to the protein's isoelectric point of about 7.6. The Viscosity Reduction Platform excipients (see Table 1) show differing trends. The performance of Phe is stable with respect to the pH condition. The excipients Arg, Orn, BSAcid, Pyr, and TMP exhibit changes in performance at different pH levels. Computational chemistry techniques were used to calculate a selection of relevant excipient properties across a pH range of 4 to 8.[9–12] These parameters were used to determine whether this difference in viscosity reduction could be explained by changes in excipient or protein properties. The underlying molecular pK_a values significantly impact these properties and were confirmed experimentally by titration studies. A summary is given in Table 3.

Table 3: Physical properties of excipient molecules at pH 5 and pH 7

	Charge [atomic units]		Dipole moment [Debye]		Solvent-accessible surface area (SASA) [Å²]		MolLogP	
Excipient	pH 5.0	pH 7.2	pH 5.0	pH 7.2	pH 5.0	pH 7.2	pH 5.0	pH 7.2
L-Ornithine hydrochloride	1.0	1.0	25.5	25.5	304	304	−5.3	−5.3
L-Phenylalanine	0.0	0.0	11.3	11.3	275	275	−1.4	−1.4
Benzenesulfonic acid	−1.0	−1.0	13.7	13.7	231	231	0.6	0.6
Thiamine phosphoric acid ester chloride	−0.2	−1.7	29.6	26.2	442	469	−3.7	−4.9
L-Arginine	1.0	1.0	9.0	9.1	290	289	−5.5	−5.5
Pyridoxine	0.8	0.0	3.3	3.7	276	274	−0.4	0.1

Only in the case of TMP a change in protonation state was found when the pH was reduced to 5. Accordingly, changes were observed in dipole moment, accessible surface area, and the water-octanol partition coefficient indicating the molecule's hydrophobicity. TMP was nevertheless a highly efficient viscosity-reducing excipient for evolocumab under both formulation conditions. As there is no pH-dependent change for the other excipient molecules, the difference in viscosity-reducing performance with evolocumab likely has a protein origin. Evolocumab's hydrophobicity is pH-independent, leading to an increased charge on the protein at a lower pH, which affects protein-excipient interactions. This case study suggests that different excipients may be required to formulate a protein under different conditions. An excipient toolbox would thus allow formulation scientists to find the right excipients for the desired formulation conditions.

Using excipient combinations to reduce protein viscosity

As individual excipients may not be powerful enough to reduce the viscosity of a highly concentrated protein formulation on their own, the Viscosity Reduction Platform is based on the use of excipient combinations. An amino acid – i.e. Arg, Orn or Phe – is combined with an anionic excipient. Being able to vary excipient combinations in this way gives formulation scientists a high degree of flexibility when it comes to balancing viscosity reduction against protein stability and other considerations like route of administration, which may determine the pH of the formulation that is to be developed.

Figure 5A shows the formulation viscosity of infliximab at a concentration of 120 mg/mL with a variety of excipient combinations. The grey control bar is the unmodified marketed formulation concentrated to the given protein concentration. The resulting viscosity of about 40 mPa·s is too high for subQ administration. The purple bar represents arginine as the benchmark excipient, which by itself is only able to slightly reduce the viscosity. In several cases, combining Orn, Arg or Phe with an anionic excipient leads to a more substantial reduction in viscosity – including below the injectability limit, most importantly. With each amino acid, there are multiple combinations that would allow for injectability of infliximab. Orn is particularly effective with Pyr. Arg can be combined with TMP. Phe is best combined with BSAcid or TMP. In summary, different excipient combinations are efficient for infliximab. However, not all excipients may be suitable for every route of administration due to potential tissue-specific reactions, which is why using an excipient portfolio is beneficial.[8]

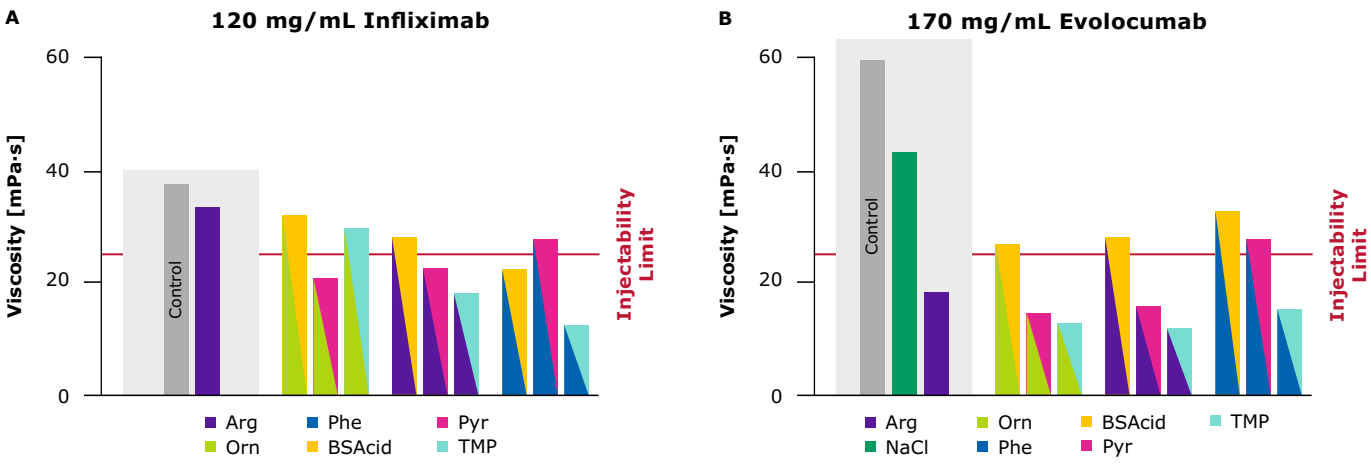

Figure 5.

Combinations of Viscosity Reduction Platform excipients compared to experiments without a viscosity-reducing excipient (grey bar), with sodium chloride as control (green bar) and the industry standard L-Arginine (Arg) (purple bar). The color codes of the split bars indicate the excipient combinations used. **A)** Model antibody infliximab. **B)** Model antibody evolocumab.

Figure 5B shows the same approach using evolocumab as a model protein. Here, 150 mM of sodium chloride was included as a control to monitor ionic effects. In contrast to infliximab, evolocumab is marketed in a low-salt formulation. Evolocumab's viscosity can be managed well with arginine. However, there are conditions where arginine is not desirable because of the route of administration or a local reaction to the excipient. The Viscosity Reduction Platform presented here provides a range of alternatives.

In summary, the data with these two model antibodies shows that using excipient combinations can reduce viscosity more effectively than the leading industry standard.

Combined excipients are also more efficient than highly concentrated single excipients and can even perform synergistically. Moreover, an excipient portfolio gives formulation scientists greater flexibility. Depending on the nature of the antibody, the desired pH, or the route of administration, having a variety of options at hand can be beneficial when developing the final formulation. The most suitable choice of excipients will depend on the type of protein and the formulation conditions.

Impact of reduced protein viscosity on syringeability

To highlight the impact of viscosity and the Viscosity Reduction Platform on syringeability, the following case study investigates two relevant factors: aspiration time and extraction force. First, the aspiration time of infliximab and evolocumab was tested at high concentrations (120 mg/mL and 170 mg/mL) with and without the most effective viscosity-reducing excipients (Figure 6A). Aspirating infliximab into a 1 mL syringe through a 27-gauge needle takes 75 s. With Orn/TMP this time can be reduced by 19%, and with Phe/TMP by 44%. For evolocumab, it takes 116 s to aspirate a highly concentrated solution into the same syringe. With Orn/Pyr this time can be reduced to 46 s, and with Arg/TMP to 37 s.

Figure 6B shows the syringe extraction force required for different formulations of infliximab and evolocumab using a 1 mL syringe through a 27-gauge needle (BD Plastipak™ 1 mL syringe, 27G, 13 mm needle). The syringe extraction force is very sensitive to the type of syringe used, its dimension, the needle length, and the inner needle diameter. In the present study a flow rate of 0.2 mL/s is used to showcase the impact of the Viscosity Reduction Platform on the injection force. Flow rates of 0.15 mL/s and 0.45 mL/s are described in literature.[7] Evolocumab is supplied by the manufacturer in a pen to self-inject using a flow rate of 0.2 mL/s. Therefore this flow rate was chosen as an example. An extraction force of about 20 N was observed for 120 mg/mL infliximab in its marketed formulation. Viscosity-reducing excipients can reduce this to about 15 N. For 170 mg/mL evolocumab, the difference is even more pronounced. In the standard buffer, an extraction force of 30 N was measured. Both excipient combinations are able to reduce the extraction force by about 50%. These examples highlight the practical impact that reduced formulation viscosity has on the syringeability of highly concentrated protein solutions.

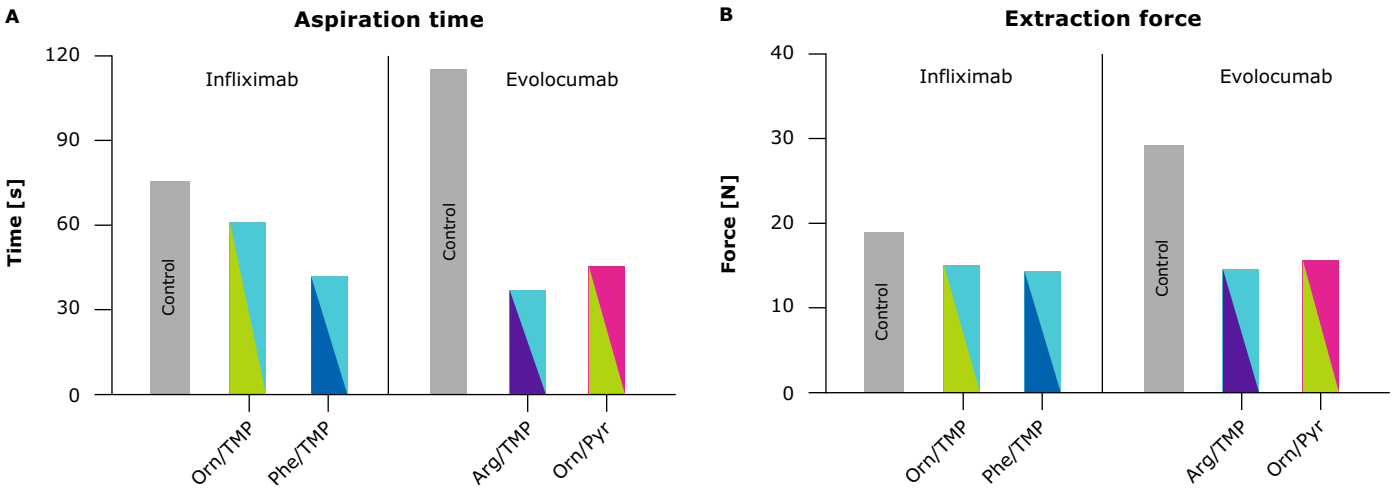

Figure 6.

A) The aspiration time of infliximab and evolocumab in their reference buffers versus formulated with the best-performing viscosity-reducing excipient combinations, and **B)** the extraction force of the two molecules in the same formulation.

Addressing protein stability with the Viscosity Reduction Platform

As previously discussed, the balance between protein viscosity and protein stability is rather delicate.

Focusing on protein stability, a forced degradation study was performed using combinations of excipients with varying concentrations of the individual components. As shown in Figure 7, excipient combinations can overcome the adverse effect of using an anionic excipient alone. The formulations used were not optimized further after addition of the viscosity-reducing excipients. Instead, the stability of the two model proteins was investigated over a longer period at 2–8 °C and 25 °C/60% relative humidity.

Figure 8A shows for all selected excipient combinations that infliximab and evolocumab were able to retain a high monomer content after 24 weeks at 2–8 °C. This high stability was achieved without further optimization of the formulation and could thus be potentially improved even more if the antibody were to undergo thorough formulation development. It is particularly noteworthy that the combination of Phe and TMP is able to maintain a high monomer content at 2–8 °C. At 25 °C, formulations containing TMP showed a strong destabilizing effect up to a total loss of monomer. This further supports the hypothesis that the decrease in protein stability is due to the decomposition of the excipient molecule. When stored under accelerated conditions, i.e. 25 °C/60% rH, a high monomer content (even above 95% in some cases) was observed for selected excipient combinations. Overall, it was shown that using viscosity-reducing excipients in combination with each other can maintain formulation stability under relevant storage conditions.

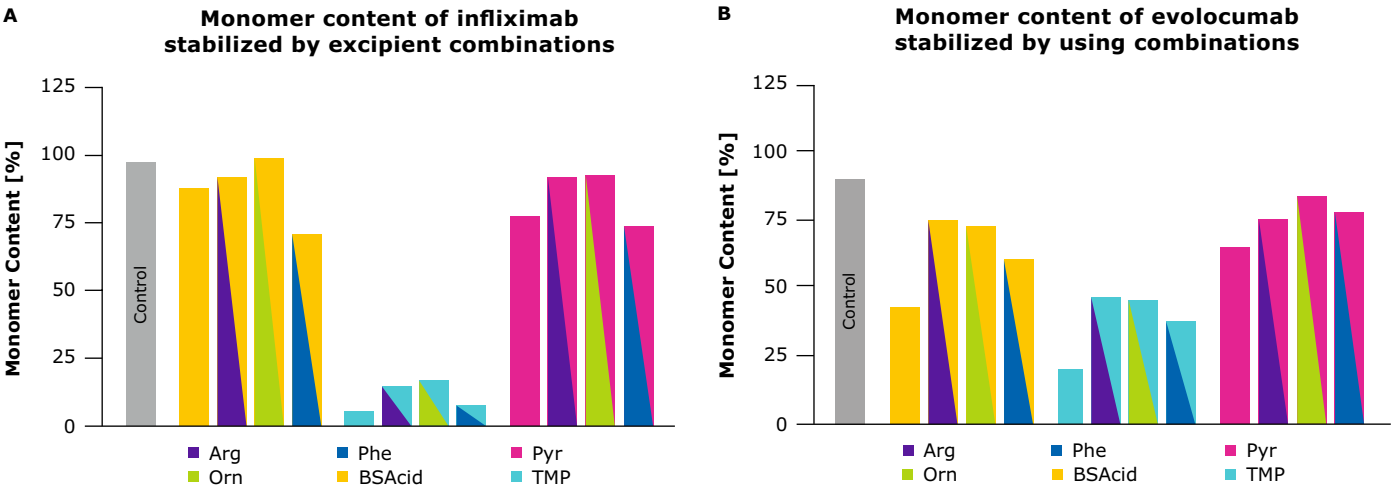

Figure 7.

Monomer content of **A)** infliximab and **B)** evolocumab formulations after a forced degradation study of 28 days at 40 °C/75% rH. Solid bars represent data with only one excipient, split bars represent excipient combinations, where the color code indicates which excipients were used.

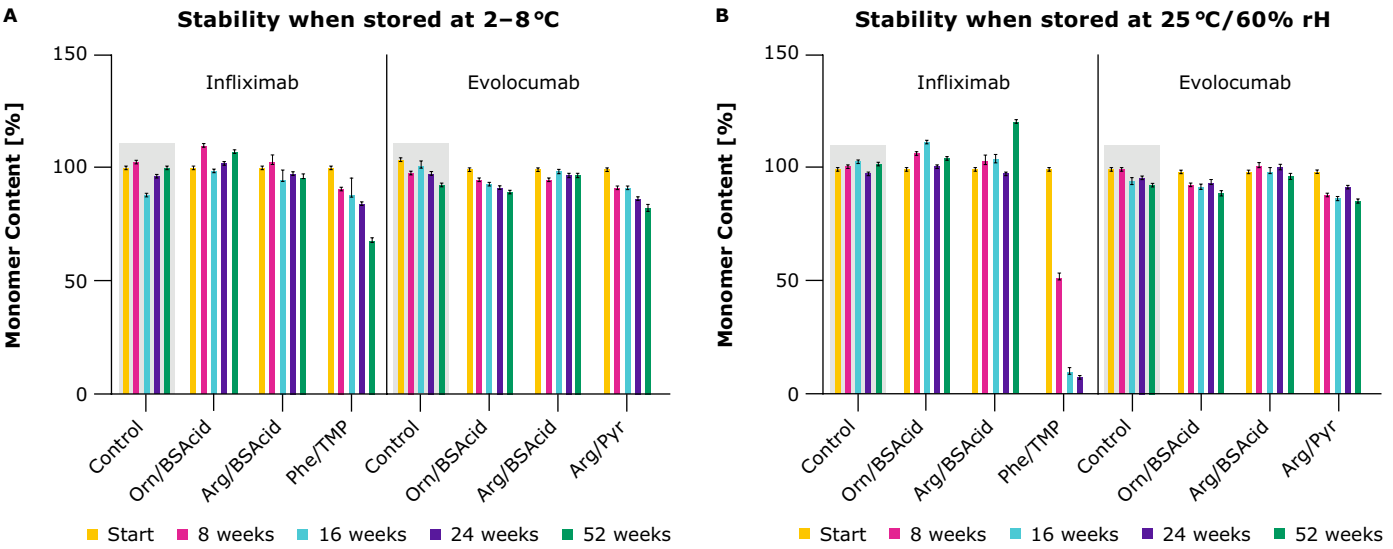

Figure 8.

Long-term stability of selected formulations with excipient combinations that successfully reduced viscosity. **A)** Stability at 2–8 °C and **B)** stability at 25 °C/60% rH.

Use of Viscosity Reduction Platform on plasma-derived protein therapeutics

Plasma-derived proteins (PDPs) are effective therapeutics for various forms of diseases. Applications can range from viral infections such as cytomegalovirus and rabies, over treating chronic diseases affecting the immune or nervous system, to antibody deficiency syndrome.[13–15] For therapy induction, i.v. administration is beneficial due to the rapid rise in IgG levels in the blood stream. For subsequent maintenance doses, subQ formulations of plasma IgGs can have a profoundly positive effect on the patient's treatment, since higher and more stable IgG plasma levels can be reached.[16,17] Additionally, highly concentrated subQ PDP products could lower the total medication volume required or reduce the number of required administrations per week. Alternatively, viscosity-reducing excipients could help to deliver a higher amount of protein per volume or enabling a faster delivery by reducing the force required to inject. These could be important factors to reduce the burden of treatment, which should not be greater than the burden of the disease itself. Hence, the Viscosity Reduction Platform was utilized to overcome application limitations of highly concentrated PDPs related to viscosity. The results are presented in this section.

Viscosity of PDPs is driven by molecular crowding

Octagam®, Pentaglobin® and Berirab® were used as model drug products to highlight the impact that viscosity-reducing excipients can have on PDP formulations. These three drug products represent different classes of PDP therapeutics (for base buffer compositions, see Table 4). Octagam® is an IgG formulation that is used to treat congenital and acquired immunodeficiencies.[18,19] It comprises of different types of IgGs that are circulating in the general donor population. Berirab® is applied during post exposure prophylactics for bites of rabite animals.[20] Like Octagam®, it is an IgG formulation, but it is enriched with anti-rabies antibodies. Pentaglobin® is a drug product used to treat bacterial infections in comprises of different types of immune globulins, IgG IgM and IgA antibodies.[21]

At first, viscosity of PDPs was measured to profile the concentration range at which PDPs adopt high viscosity levels beyond injectability (25 mPa·s). The results showed that all PDPs tested cross the threshold of injectability at protein concentrations of approx. ≥200 mg/mL (Figure 9). To compare, infliximab and cetuximab crossed this threshold already at lower concentrations (~120–150 mg/mL). To demonstrate the impact of aforementioned heterogeneity in viscosity, diffusion interaction parameters (kD) of different PDP formulations were compared to the kD of infliximab (Figure 10). kD is a measure for self-interaction of proteins and to some extent correlates with the solution viscosity. mAbs such as infliximab have a particularly strong tendency to attractive PPIs as highlighted by the strongly negative kD. Remaining formulations exhibit less negative to neutral kD changes and accordingly become viscous at higher protein concentrations compared to those of mAbs.

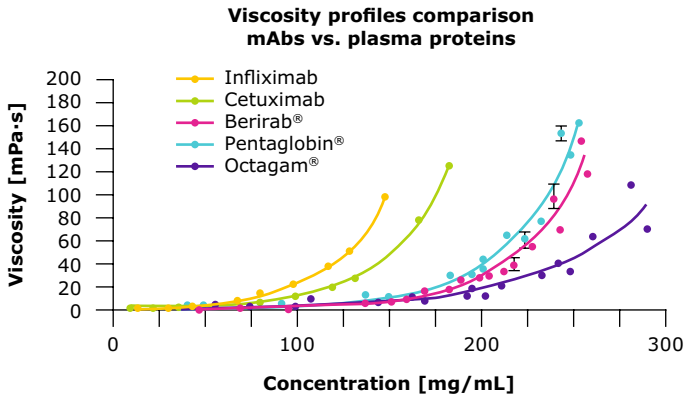

Figure 9.

Dependence of formulation viscosity on protein concentration for mAbs and PDPs.

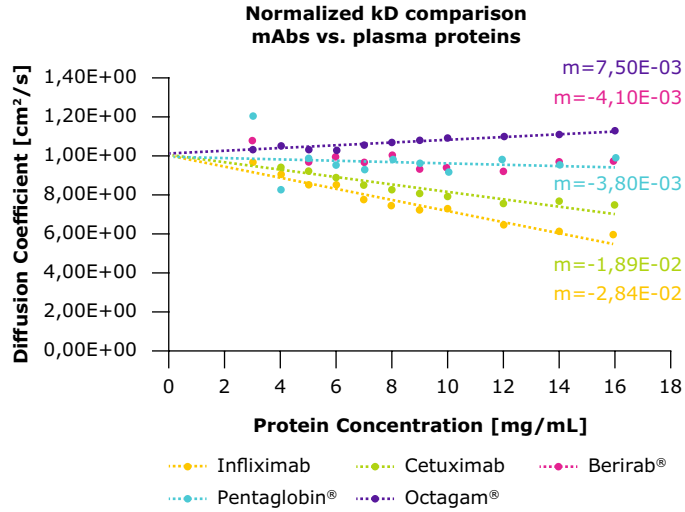

Figure 10.

Comparison of kD mAbs and PDPs.

Table 4: Buffer components for different PDP base buffers

PDP	Buffer Components
Octagam®	Maltose monohydrate EMPROVE® ESSENTIAL
	Sodium chloride EMPROVE® EXPERT Ph Eur,BP,ChP,JP,USP
Pentaglobin®	Sodium chloride EMPROVE® EXPERT Ph Eur,BP,ChP,JP,USP
	D-(+)-Glucose, anhydrous EMPROVE® EXPERT Ph Eur,BP,USP,ACS
Berirab®	Glycine granulated EMPROVE® EXPERT Ph Eur,BP,ChP,JP,USP

The reason for this difference is the higher tendency of inter- and intramolecular interactions of homogeneous (mAbs) over heterogeneous (PDPs) bioformulations as well as molecular crowding.[22] Compared to these mAbs, plasma proteins have a lower tendency to self-interact. As highlighted earlier, at concentrations ≥200 mg/mL, protein crowding takes the upper hand over intramolecular PPIs leading to non-specific PPIs and destabilization of protein structure as well as a rapid increase in solution viscosity.

Viscosity Reduction Platform excipient combinations efficiently reduce viscosity at highly concentrated PDPs

As discussed previously, the protein-protein interactions that give rise to the high viscosity of PDPs are driven by molecular crowding. Nevertheless, said protein-protein interactions can still be weakened in strength and/or reduced in number using excipients. In order to confirm this hypothesis, viscosity measurements were performed in concentrated market formulation of Octagam® as a model PDP (250 mg/mL) with the Viscosity Reduction Platform (concentration at market formulation 50 or 100 mg/mL). The concentration was selected due to the favorable concentration-viscosity correlation at 250 mg/mL as can be seen in Figure 9. The results of viscosity measurements are depicted in Figure 11 and show that single excipients Orn and BSAcid exhibit higher viscosity values compared to that of market formulation, whereas other single excipients resulted in viscosity reduction (5–15%). Interestingly, Arg did reduce solution viscosity only marginally (~5%).

Using Viscosity Reduction Platform combinations, this reduction could further be increased by up to 38% (75 mM Arg + 75 mM TMP). The results with the Phe/TMP combination are even more intriguing (>40%), however the protein concentration in this formulation was approx. 10 mg/mL lower than that of the control sample, preventing a direct comparison. Hence, Viscosity Reduction Platform combination Arg/TMP is considered as the best lead and was evaluated for a concentration-based lead profiling study as outlined below.

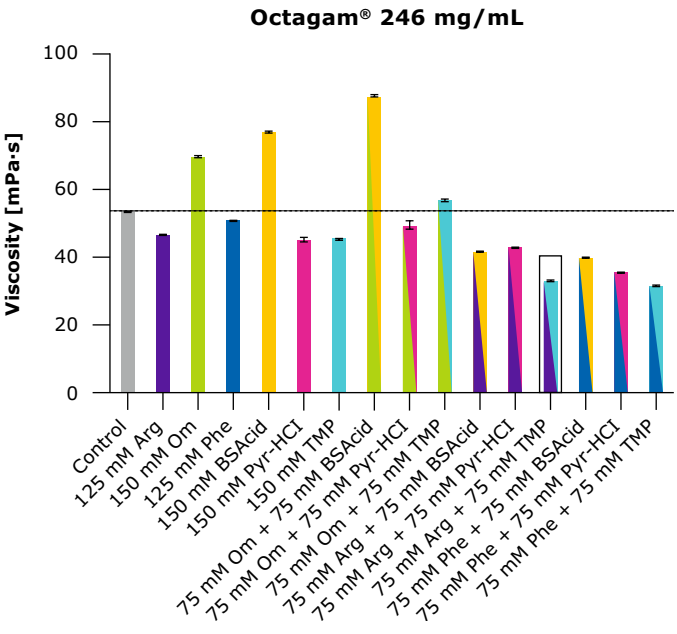

Figure 11.

Viscosity screening for Octagam® at 246 mg/mL. Combinations of Viscosity Reduction Platform excipients compared to experiments without a viscosity-reducing excipient (grey bar), with the industry standard Arg as benchmark (purple bar). The color codes of the split bars indicate the excipient combinations.

Concentration ratios of excipient combinations do not have a critical impact on viscosity reduction of Octagam® in solution

Typically, excipient concentrations are optimized using a Design of Experiments (DoE) approach. It is therefore conceivable that by changing the ratio of the two viscosity-reducing excipients, an additional improvement could be achieved. To test this hypothesis, Arg and TMP were combined in various ratios and the viscosity was measured. The respective viscosities of the concentrated market formulation (246 mg/mL) and formulations comprising one excipient, either Arg or TMP, were used as a reference. While single excipient-containing solutions delivered a moderate reduction in solution viscosity (12–15%), all Viscosity Reduction Platform combinations exhibited ≥29% viscosity reduction, with 75 mM Arg/75 mM TMP showing the highest relevant reduction in solution viscosity (~38%).

Even though the viscosity for PDP formulation with Viscosity Reduction Platform combinations are still slightly beyond injectability (>25 mPa·s), the objective of reducing viscosity for PDPs is not to create an injectable formulation that can otherwise not be made but to improve patient convenience. Hence, lowering the number of repetitive applications with higher protein loads is the first step to enable this.

In conclusion, the Viscosity Reduction Platform is a strong viscosity reduction tool not only for utilization in highly concentrated mAb products but also for other biologics formulations. We have demonstrated that using the Viscosity Reduction Platform, a higher protein concentration for PDP formulations compared to market formulations could be achieved. Furthermore, Viscosity Reduction Platform combinations performed better than formulations containing only one Viscosity Reduction Platform excipient.

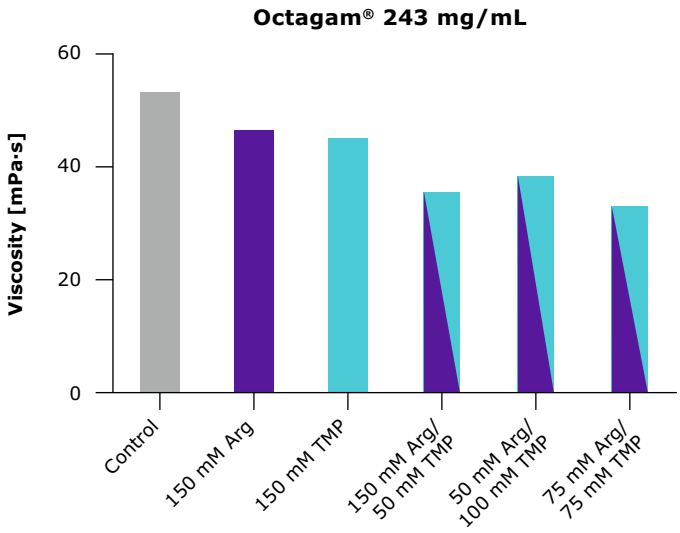

Figure 12.

Profiling of Viscosity Reduction Platform lead combination for Octagam® (243 mg/mL) at different excipient ratios. Samples were compared to control experiments without reducing excipient (grey bar) and to the single excipients of the lead combination Arg (purple bar) and TMP (blue bar), respectively. The color codes of the split bars indicate the excipient combinations used.

Conclusion

The Viscosity Reduction Platform contains a portfolio of excipients and is based on combining an amino acid with a second viscosity-reducing excipient. The latter excipients are ones that often adversely affect protein stability when used individually at high concentrations but combining them with an amino acid circumvents this and improves viscosity-reducing capacity. The Viscosity Reduction Platform allows for a better balance of protein stability versus protein viscosity. This platform therefore enables subcutaneous delivery while preserving long-term stability. It also makes the application through a device both more patient-friendly and more economical. The Viscosity Reduction Platform provides formulation scientists with a variety of options for formulation development that take the route of administration and the requirements of the protein into account. Beyond monoclonal antibodies, also formulations of plasma-derived proteins can be improved by the application of the platform.

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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