Excipient combinations to manage protein viscosity for highly concentrated formulations

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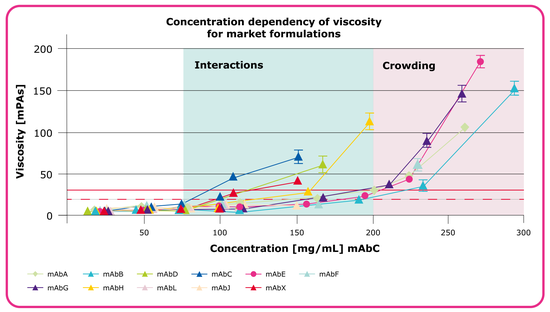
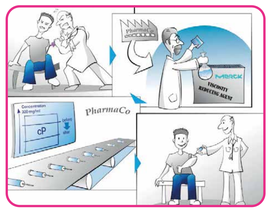
Purpose

When delivered via the subcutaneous route of application the volume of a drug is restricted to approx. 2 mL. For therapeutic proteins, such as mAbs or plasma proteins, this restriction requires the use of high protein concentrations. As can be seen by Figure 1 some proteins begin to become highly viscous at concentrations as low as 100 mg/mL. At such concentrations the formulation could potentially not even be forced through a syringe any longer. We investigate the effect of excipient combinations to manage protein viscosity.



Objectives

To provide best in class excipients to reduce viscosity and enable patient friendly administration.



Methods

Monoclonal antibodies were obtained in their respective FDA or EMA registered formulation.

For excipient studies, a chimeric monoclonal anti-TNF-α antibody at pH 7.2 was selected (mAbC). All excipients & buffer components were purchased from MilliporeSigma.

For buffer exchange and achieving high protein concentrations we used Amicon® Ultra-4 Ultracell-30k centrifugal filter units. For excipient testing five diavolumes were exchanged via centrifugation at 2,000 xg with these spin columns. Volume was also reduced by centrifugation at 2,000 xg. Protein concentration was determined according to Lambert-Beer's law and extinction measurement at 280 nm in a BioSpectrometer® Kinetic (Eppendorf, Hamburg, Germany). Dilutions were prepared with the respective buffer and concentration verified again by the same method.

Viscosity was measured after equilibrating samples to 20 °C using a m-VROC™ viscometer at a shear rate of 1,000–3,000 /s. 200 µL of sample were loaded into a 500 µL gastight syringe (Hamilton, Reno, USA) and measured in triplicates after a priming step.

Particle diffusion D_t was measured on a Dynapro PRIII (Wyatt Technology, Santa Barbara, USA) using Dynamic Light Scattering (DLS) with 10 acquisitions of 5 seconds each at 25 °C.

The diffusion at infinite dilution D_0 was derived from the equation $D_t = D_0 (1+ k_D*c)$ by fitting the diffusion linearly over a range of 3 to 14 mg/mL mAbC. To determine the diffusion interaction parameter k_D a normalized form given by D_t/D_0 was plotted.

The syringe glide force is calculated according to the following equation:

Injection force = $8\eta lnQ \frac{R_b^2}{R_n^4}$ + piston friction.

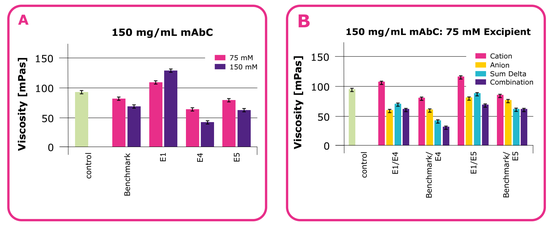
Typically, needles thinner than 27G are used for sc administration.*

n: viscosity [mPa*s]
l: length of needle [mm]
Q: flow rate/injection rate [mL/s]
R_b: radius of syringe/barrel [mm]
R_n: inner radius of needle [mm]

* Garindel & Presser, Lyophilization of High Concentrated Protein Solution, Book Chapter: Lyophylization of Pharmaceuticals and Biologics, Springer Protocols 2019

Results

Use of Excipients to manage protein viscosity

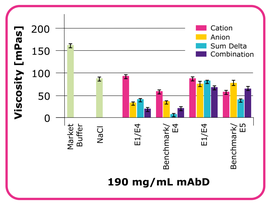


A: Upon the addition of 75 mM excipient, we observe a light reduction in protein viscosity, however the effect is not strong enough to allow for subcutaneous administration. Even when the excipient concentration is of doubled, the viscosity remains too high for this purpose.

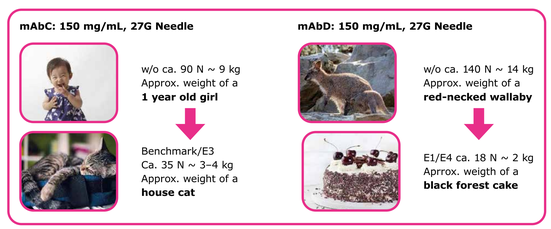
B: Synergistic reduction viscosity by excipient combinations. Cationic and anionic excipients were measured separately, shown in red and blue. The yellow bars visualize the expected viscosity if the effect of the individual excipients were additive. The pink bars represent actual experiments.

Using Excipient combinations on an additional Antibody

Also for mAbD we observe synergistic viscosity reduction for several excipient combinations. In some cases the additive effect would yield to an expected viscosity of near zero. However, since the protein molecules in the solution occupy space, a zero viscosity is not possible even in the complete absence of protein interactions.

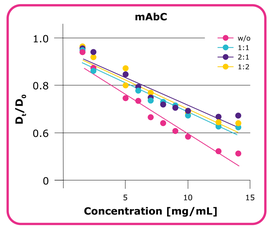


Calculated effect of viscosity reduction on injection force



Use of Excipients to nage protein viscosity

This figure shows a normalized k_D Plot, where a protein-protein interaction parameter is measured in lieu of protein viscosity. A slope of zero would indicate the absence of interactions. By adding excipients 1 and 5 the slope becomes less negative indicating a reduced viscosity. However, the effect is most predominant, when both excipients are used in a 2:1 ratio.



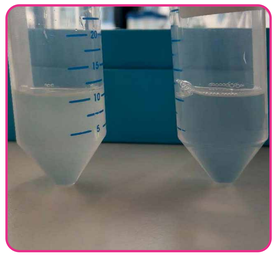
Forced degradation study I: Concept

Design of the forced degradation study

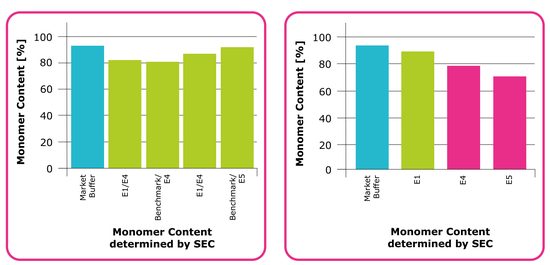
- Use of mAbC that is inherently unstable to measure changes towards the better or worse
- 150 mM single excipients
- Compare two combinations comprising of 75 mM of cation and 75 mM anion

Conditions

- 80 mg/mL mAbC
- Marketed formulation + excipients
- 40 °C / 75% relative humidity
- Time points 0, 14, and 28 days



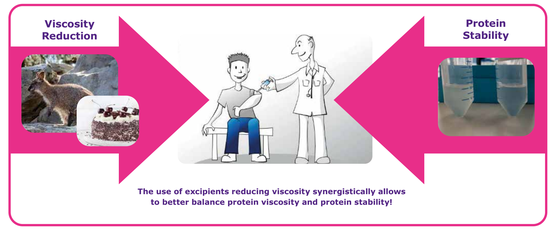
Forced degradation study II: Results



After 28 days under thermal challenge formulations comprising combinations of viscosity reducing excipients show a monomer content similar to a formulation without said excipients. While Excipient 1 does not have an adverse effect on the stability of mAbC, excipients 4 and 5 destabilize the mAb and reduce the monomer content, when used by themselves.

Conclusions

1. Protein viscosity can be a serious obstacle for sub-cutaneous drug administration
2. Use of excipient combinations shows synergistic viscosity reduction
3. Optimizing the ratio of viscosity reducing excipients offers an additional parameter to manage protein viscosity
4. A reduced viscosity may allow for injectability of a drug or improve patient convenience due to less pain during application
5. A reduced viscosity may also reduce back pressure during filtration steps and shear forces during downstream processing and therefore improve process economics
6. By using excipient combinations the amount required of the individual molecule is reduced



References

[1] Liquid Formulation R&D, MilliporeSigma

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Lit. No. MS_PS7018EN
11/2020

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