Highly concentrated formulations of Biotheurapeutics

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MabDelivery – Labex MabImprove
Tours

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Global Biotherapeutics

Reasons of the trend towards highly concentrated formulations (HCF) of biotherapeutics

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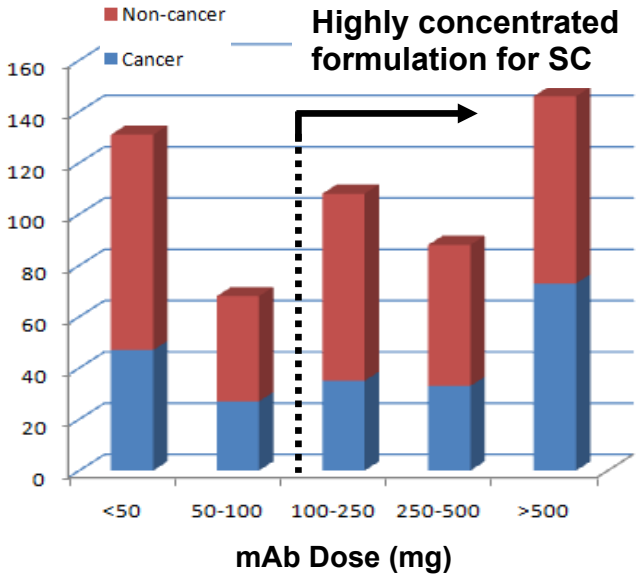
- Increase demand for subcutaneous (SC) route of administration - as opposed to the conventional intravenous (IV) route

- The assets of SC route of administration
  - Patient-friendly presentation, especially coupled with a device
  - Allows home administration: reduce treatment cost + increase patient compliance

- The constraints of SC formulations
  - Limited in the injection volume (a few mL)
  - Biotherapeutics often need high doses (several 100 of mg)



Source: PharmaCircle Database, 500+ Mabs, from phase 1 to launch and SNY-101: Large Volume Injection Analysis, Jan 2012

→ ***The development of highly concentrated formulations (>100 mg/mL) is a major requirement for SC injection***

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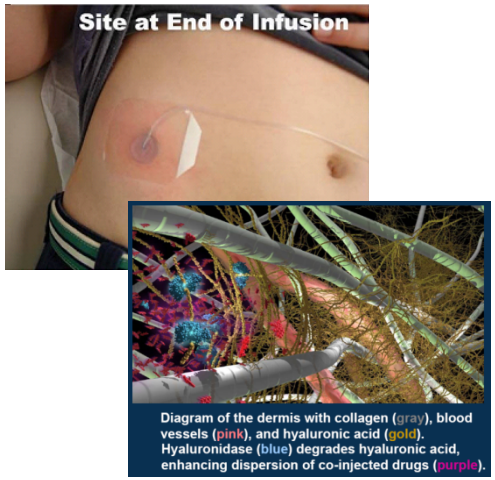
The increased demand for SC formulations



Halozyme, Enhanze™ technology, Bio 2007.

- Indications that require frequent and chronic administration (arthritis, asthma…)
- SC injection is actually not limited to 0.5-1 mL
  - Manual injection of volumes up to ~2 mL is possible
  - The use of large volume devices is a promising approach for specific product requirements and possibly in the case of very high volumes (2-10 mL)
  - The use of hyaluronidase (Halozyme's techno) significantly enhance the volume of injection
    (Herceptin: 5 mL)

Commercial biotherapeutics products injected SC: a few examples

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Name of the Drug	Year	Company/ Developer	Indication	Concentration (mg/mL)	Presentation
Enbrel (etanercept)	1998	Amgen/ Wyeth	Rheumatoid Arthritis, Psoriatic Arthritis	50	Lyophilized vial PFS* Autoinjector
Humira (adalimumab)	2002	Abbott	Rheumatoid Arthritis, Psoriatic Arthritis	50	PFS*
Xolair (omalizumab)	2003	Roche/ Novartis	Asthma, Chronic Idiopathic Urticaria	125	Lyophilized vial
Simponi (golimumab)	2009	Janssen	Rheumatoid Arthritis, Psoriatic Arthritis Ankylosing Spondylitis, Ulcerative Colitis	100	PFS* SmartJect Autoinjector
Ilaris (Canakinumab)	2013	Novartis	Cryopyrin-Associated Periodic Syndromes, Active Systemic Juvenile Idiopathic Arthritis	150	Lyophilized vial
Herceptin (trastuzumab)	2014	Roche/ Genentech	Metastatic Breast Cancer	120	PFS*

*PFS: Pre-Filled Syringe

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Overview of the presentation

- Challenges associated to highly concentrated formulations (HCF)
- Case study of a Bi-specific antibody (Bi-mAb): aggregation induced by protein concentration and impact on viscosity
- Case study of a monoclonal antibody (mAb1): viscosity impact on subcutaneous delivery

Overview of the presentation

- Challenges associated to highly concentrated formulations (HCF)
  - **Aggregation**
  - **Viscosity**
  - **pH shifts**
  - **Appearance of the drug product**

Challenges associated to highly concentrated formulations: aggregation (1/2)

- The Lumry-Eyring aggregation model among others described the different aggregation pathways
- The apparent reaction order (n) gives insight on the elementary step limiting the rate of monomer consumption:

$$\frac{dM}{dt} = -k_{app} M^n$$

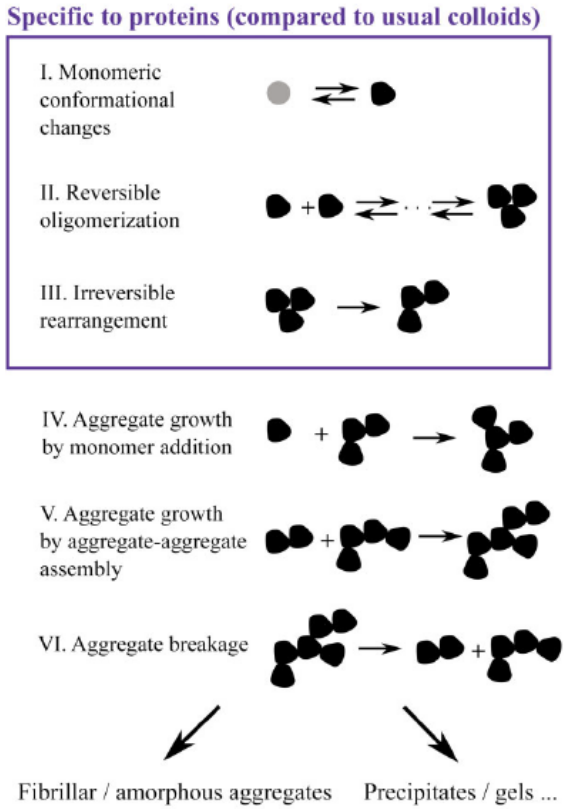
M: monomer concentration

k_{app}: apparent reaction rate

n: apparent reaction order

t: time

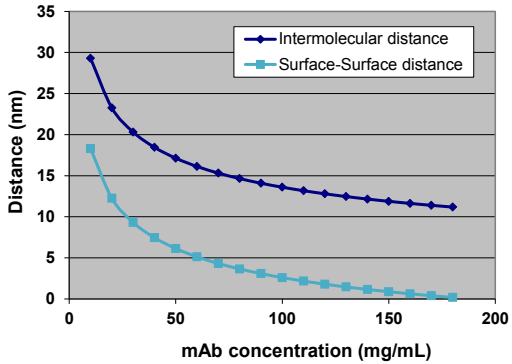
  - n~1: Order 1 reaction → Step 1 is limiting
  - n~2: Order 2 reaction → Step 2 is limiting



Nicoud et al. 2015. A multiscale view of therapeutic protein aggregation. Biotechnol J. 10, 367-378

Challenges associated to highly concentrated formulations: aggregation (2/2)

- Aggregation requires bi-molecular collisions and thus has a strong dependence on mAb concentration
  - Aggregation phenomena are generally enhanced in HCF
- Molecular crowding effect shifts thermodynamic equilibrium to a greater amount of aggregates such as dimers, trimers…
  - Generally reversible however might lead to further irreversible aggregation
- Reducing inter molecular distance changes the prominent interactions in between mAbs
  - New aggregation mechanisms might appear in the concentrated solution that was not present in the diluted one

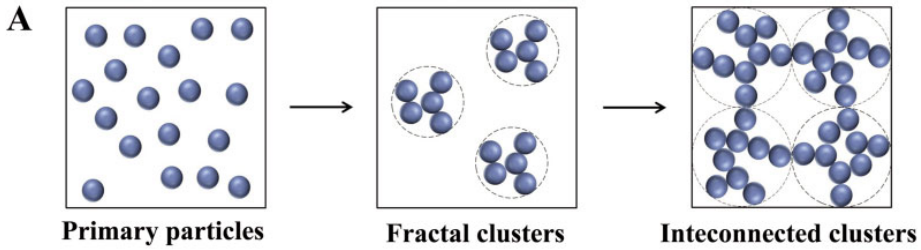


Theoretical calculation using spherical molecules of 150 kDa and 11nm diameter

→ Aggregation is a particular topic when dealing with HCF as complex aggregation mechanisms are at stake

Challenges associated to highly concentrated formulations: viscosity (1/5)

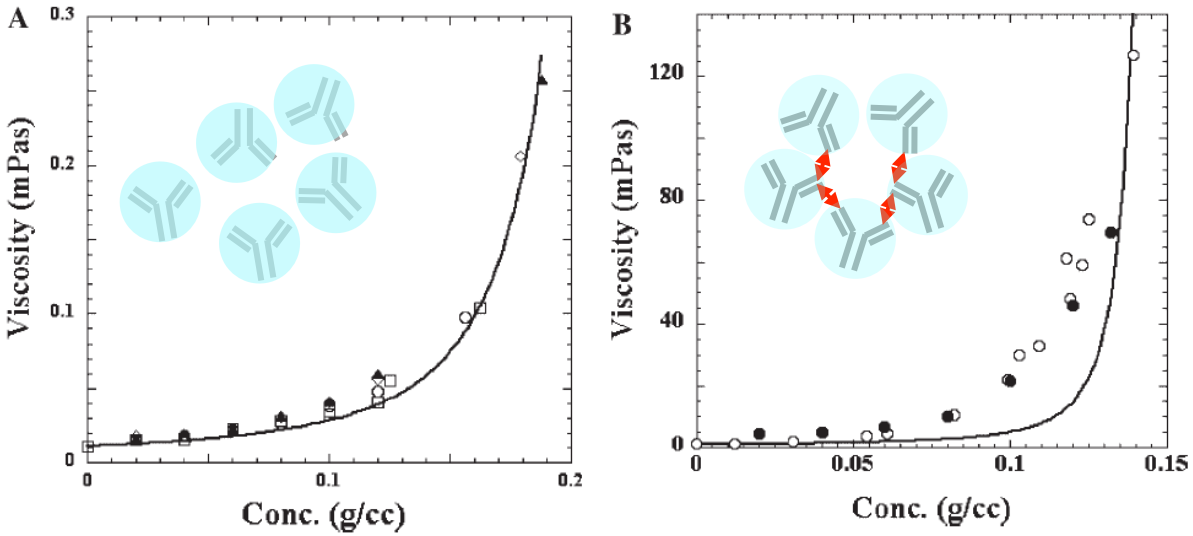
- At high concentration, an increased in solution viscosity coming from both the increase in volume fraction and intermolecular interactions
  - Increase in volume fraction described by the hard quasispherical model developed by Ross and Minton (see next slide)
  - Intermolecular interactions lead to reversible self-association and/or to irreversible aggregation
    - The resulting viscosity is higher than in solution comprising of non interacting molecules
    - According to colloidal science, phenomenon explained by the increase of volume fraction occupied by the “aggregates”



Nicoud et al. 2015. A multiscale view of therapeutic protein aggregation. Biotechnol J. 10, 367-378

Challenges associated to highly concentrated formulations: viscosity (2/5)

- To model viscosity increase in function of mAb concentration, the “hard quasispherical model” developed by Ross and Minton is widely used
  - Account for the excluded volume effect
  - Does not take into account the possible intermolecular interactions



Liu et al. 2005. Reversible self-association increases the viscosity of a concentrated monoclonal antibody in aqueous solution. J Pharm Sci. 94, 1928–1940

Mooney equation:

$$\eta_r = \exp\left(\frac{[\eta]c}{1-\frac{K}{S}[\eta]c}\right)$$

$\eta_r=\eta/\eta_0$

η_0: solvent viscosity

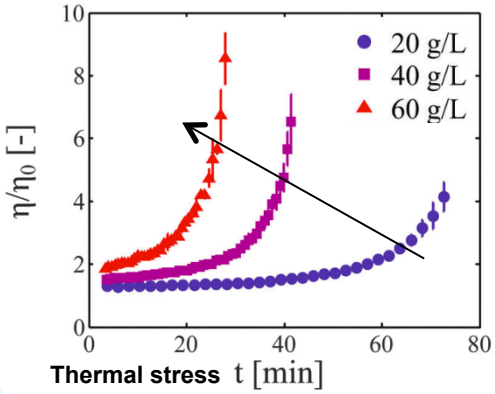
$[\eta]$: protein intrinsic viscosity

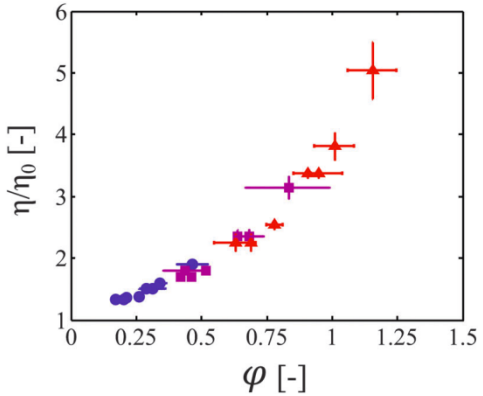
K: crowding factor

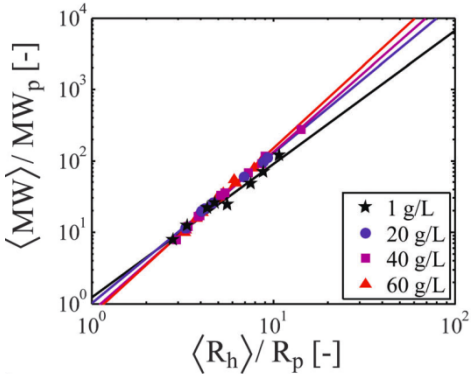
S: shape factor

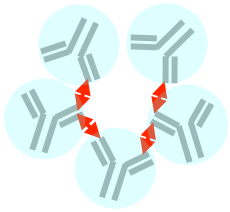
Challenges associated to highly concentrated formulations: viscosity (3/5)

- To better understand viscosity increase in function of mAb concentration, the volume fraction (ϕ) occupied by aggregates has been evaluated
  - Takes into account measured aggregates concentration, size and morphology
  - Nature of aggregates changes with increasing concentration (more compact)
  - Polydispersity of the aggregate distribution seems to play a role: minimizing the viscosity increase







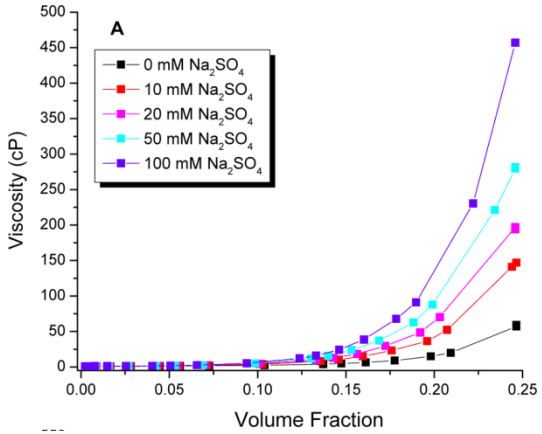


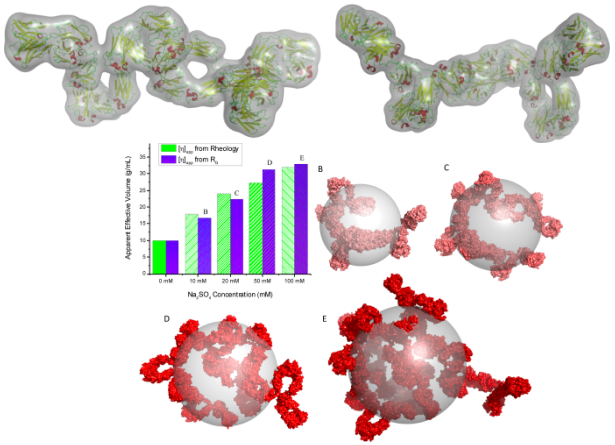
Nicoud et al. 2015. Impact of aggregates formation on the viscosity of protein solutions. Soft Matt. , DOI: 10.1039

→ Impact of irreversible aggregation on viscosity increase of HCF

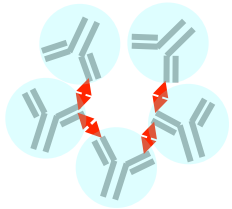
Challenges associated to highly concentrated formulations: viscosity (4/5)

- The use of excipients in the formulation (amino acids, polyols, salts) may impact the flow properties of mAb solutions
  - The increase of salt (Na_2SO_4) decrease the repulsive electrostatic interactions in between mAbs, thus increase self association into oligomers
  - Computational model of oligomers can be determined (Fab-Fc or Fab-Fab)





Lilyestrom et al. 2013. Monoclonal Antibody Self-Association, Cluster Formation, and Rheology at High Concentrations. J. Phys. Chem. B. 117, 6373-6384



→ Contribution of reversible interaction on viscosity increase of HCF

Challenges associated to highly concentrated formulations: viscosity (5/5)

- The increase of the solution viscosity complicates the manufacture of the Drug Product
  - Filtration, mixing and filling steps require dedicated studies during process development

- The delivery of the product by SC administration is also impacted
  - Viscosity limits the injection of the product
    - The selection of needle type and size is a compromise between reducing patient pain at the injection site while keeping a low injection time/force
      - Injection force below 15-30N to be able to inject the product manually
      - Injection time is important for patient compliance and/or device implementation

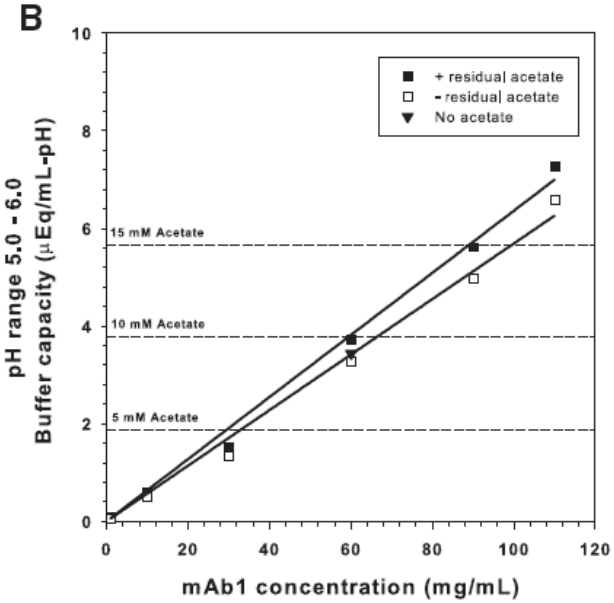
    → Syringeability tests are performed

  - Small concentration/temperature variations impact the dose delivered to the patient

→ Viscosity increase is often what limits the development of a higher concentrated formulation for a given mAb

Challenges associated to highly concentrated formulations: pH shifts

- Protein may have a significant buffering capacity at high concentration which may shift the solution pH
  - Protein buffer capacity increase linearly with protein concentration
  - By 60–80 mg/mL, mAb buffer capacities surpassed that of 10mM acetate, commonly employed for buffering in the pH 4–6 range



Gokarnet al. 2008. Self-Buffering Antibody Formulations. J PharmSci97:3051–3066

- Self-buffered formulations as an alternative for the development of HCF?
  - Certain process steps engender pH shifts (Tangential Flow Filtration, freezing/thawing)
  - Unless the buffering system has a negative impact on the protein stability, self-buffered formulations are not preferred

→ Need a careful selection of the buffering system concentration

Challenges associated to highly concentrated formulations: other topics

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- Appearance of the Drug Product: potential change in color and increase in turbidity of the solution
  - Not an issue for product stability if no variation during storage
  - Bring challenges in clinical studies design when double blinded
  - Placebo containing the same amount of excipients as the active may not match the active:
    - Slightly diffusing/yellow solution, viscous aspect, presence of foam…

Active at 150 mg/mL | Placebo

- Tangential Flow Filtration (TFF)
  - Donan effect impacting buffer concentration and target pH
  - Higher concentration achieved at membrane boundary
- Analytical development
  - Several analytical techniques require high dilutions
  - High viscosity/turbid solutions can introduced analytical artefacts

Overview of the presentation

- Challenges associated to highly concentrated formulations (HCF)
- Case study of a Bi-specific antibody (Bi-mAb): aggregation induced by protein concentration and impact on viscosity
- Case study of a monoclonal antibody (mAb1): viscosity impact on subcutaneous delivery

Case study of a Bi-specific antibody (Bi-mAb): introduction (1/5)

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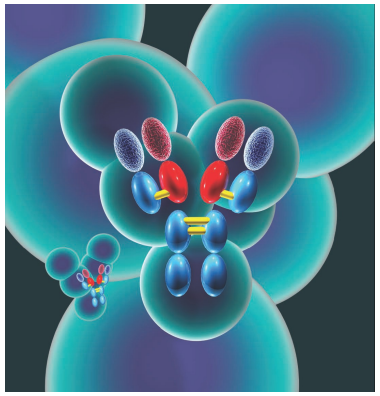
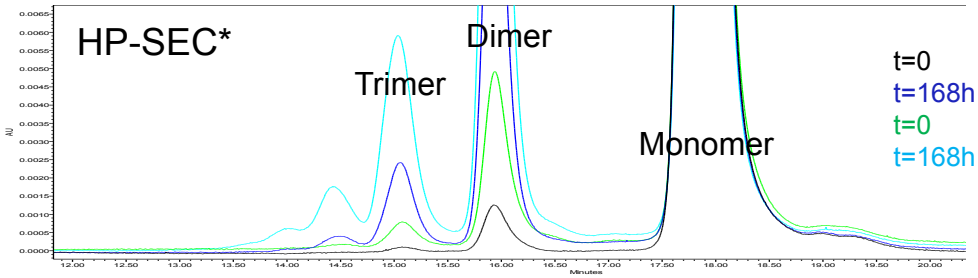
- IgG4 bi-specific antibody format
- 200 kDa
- Route of administration SC
- The drug product is a lyophilisate at 100 mg per vials
- The formulation after reconstitution is:
  - 100 mg/mL Bi-mAb, Phosphate/Tris, Sucrose, Proline, PS80



- Prone to aggregation in liquid form:
- Dimers, trimers… formation



*HP-SEC: High Pressure Size Exclusion Chromatography

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Case study of a Bi-specific antibody (Bi-mAb): aggregation increase with concentration (2/5)

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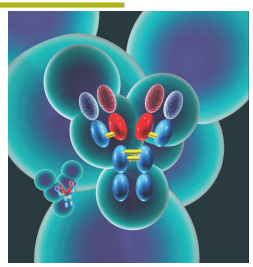
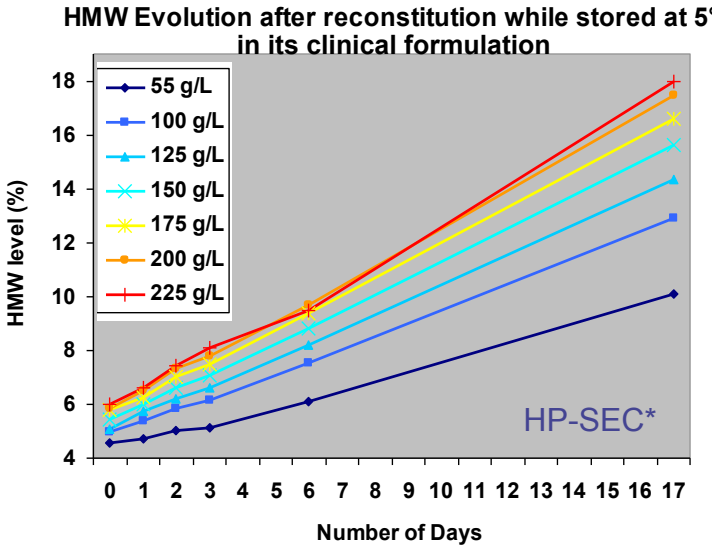
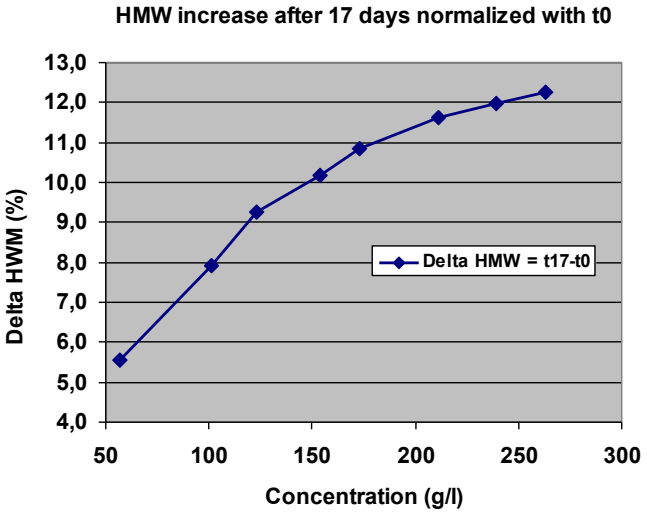


- The rate of High Molecular Weight (HMW) formation increase with Bi-mAb concentration
  - +5.5% for 55 mg/mL *vs* +12% for 225 mg/mL
  - Rate increase non linearly with concentration: effect of viscosity increase





→ ***The aggregation rate increase with concentration due to bi-molecular collisions higher probability***

*HP-SEC: High Pressure Size Exclusion Chromatography

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Case study of a Bi-specific antibody (Bi-mAb): aggregation pathway (3/5)

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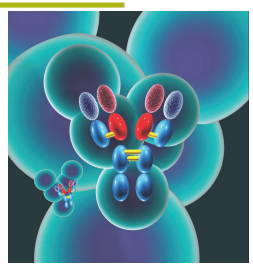
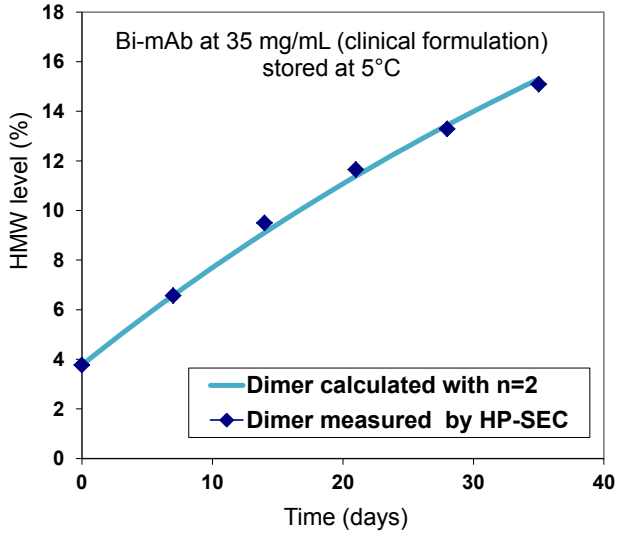
- The apparent reaction order n is equal to 2 which gives an insight on the aggregation mechanism: M + M → D
- Determination of driven interactions type (hydrophobic, columbic…) and aggregate structure (covalent…) are under study





$$\frac{dM}{dt} = -k_{app} M^n$$

M: monomer concentration

k_{app}: apparent reaction rate

n: apparent reaction order

t: time

Case study of a Bi-specific antibody (Bi-mAb): aggregation impact on viscosity (4/5)

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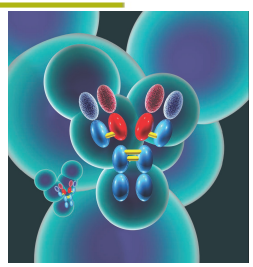
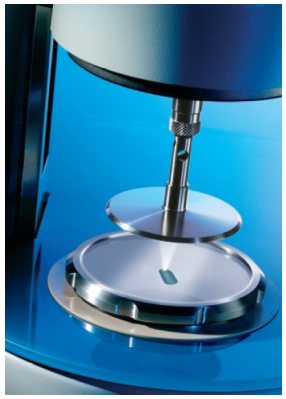
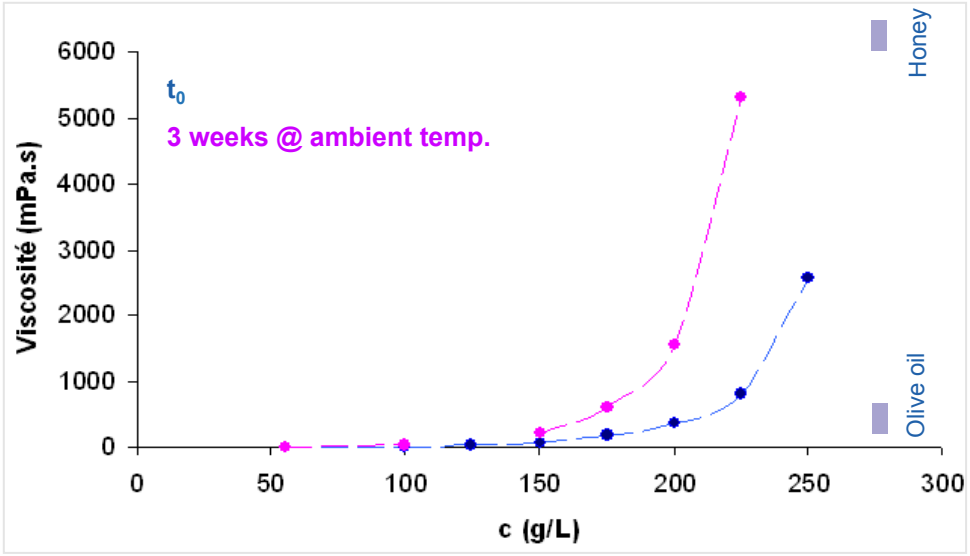
- Viscosity increase after 3 weeks storage at ambient temperature of Bi-mAb in its clinical formulation
  - Increase of viscosity for all tested concentrations (55 → 225 mg/mL)
  - Higher increase for higher concentrations
  - Attributed to aggregation which is concentration dependent itself







→ Viscosity can be a relevant indicator of the solution state evolution upon storage

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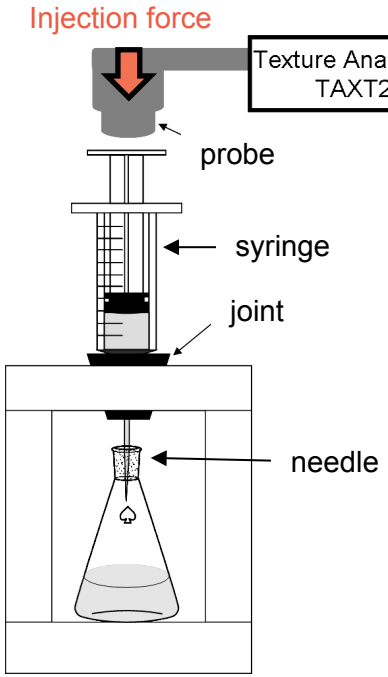
Case study of a Bi-specific antibody (Bi-mAb): syringeability tests (5/5)

- Syringeability studies determine the maximum concentration to be injected subcutaneously following a given drug product profile



Needle gauge: 25-27G
Syringe volume: 1 mL
Injection time: 1 min
Force applied max: 10N

Concentration	Needle	Time (s)	force N
55 g/L	27G	11	3,5
100 g/L	27G	11	4,5
125 g/L	27G	11	7
150 g/L	27G	14	12
175 g/L	27G	28	13
	27G	55	8
210 g/L	27G	180	7,5
	25 G	31	12,5
	25 G	61	7,5
240 g/L	25 G	138	13
260 g/L	25 G	138	15

→ Viscosity increase limits the development of higher concentrated formulation

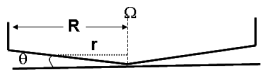
Overview of the presentation

- Challenges associated to highly concentrated formulations (HCF)
- Case study of a Bi-specific antibody (Bi-mAb): aggregation induced by protein concentration and impact on viscosity
- Case study of a monoclonal antibody (mAb1): viscosity impact on subcutaneous delivery

Case study of a monoclonal antibody (mAb1): introduction (1/2)

- Humanized IgG4 monoclonal antibody
- 150 kDa
- Route of administration SC
- The drug product is a liquid at 225 mg per vials
- The formulation is:
  - 150 mg/mL mAb, Histidine, proline, sucrose, PS80

- Viscosity measured at 25°C, 7 mPa.s
  - Rheometer: shear rate applied from 0.01 to 5000 s^{-1}

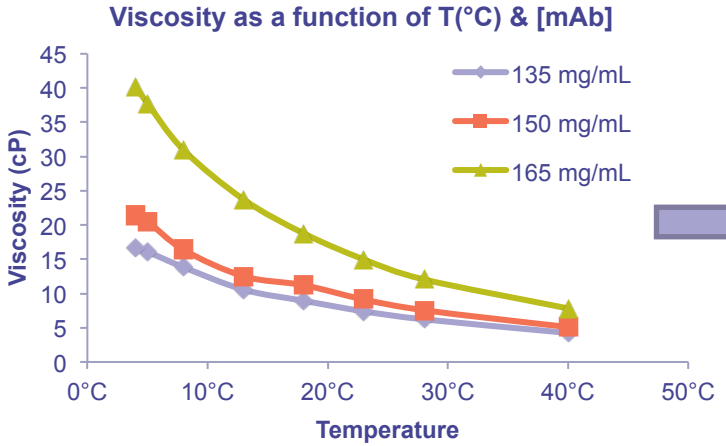


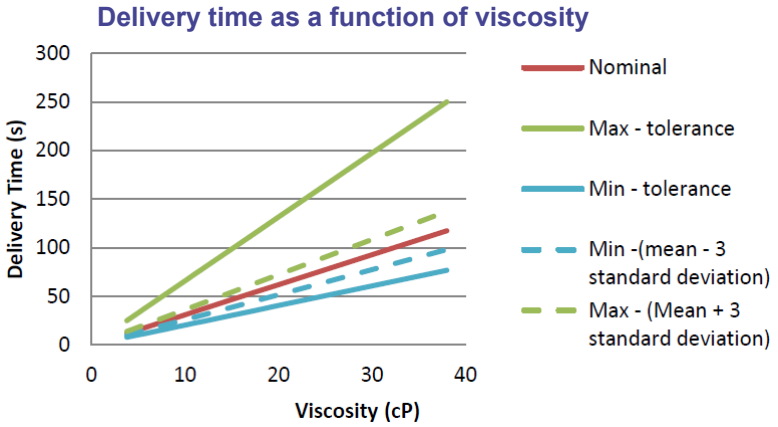
*HP-SEC: High Pressure Size Exclusion Chromatography

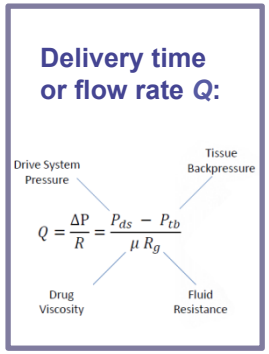
Case study of a monoclonal antibody (mAb1): viscosity variation (2/2)

- Small concentration/temperature variations impact the viscosity values of the solution and hence the dose delivered in a certain time (flow rate *Q*)
  - 10% variation on the mAb concentration results in an increase of solution viscosity 9 → 19 mPa.s at 18°C

  → ~ Double the delivery time







→ Viscosity variation must be taken into account during process development and delivery system definition

Take home messages

- The major challenges while developing HCF are:
  - **Aggregation as complex mechanisms are at stake when short intermolecular distances are achieved**
  - **Viscosity increase, especially regarding delivery of the drug product which often limits the development of a higher concentrated formulation**

- Viscosity and aggregation phenomena are linked:
  - **Understanding intermolecular interactions is key to identify viscosity reducer**
  - **Viscosity can be a relevant indicator of the solution state evolution**

- The development of highly concentrated formulation stable in liquid form and the increased in SC injection volumes, mainly thanks to devices, offers IV-to-SC conversion opportunities to the majority of mAbs and other scaffolds in development

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Till Bussemer

BioTherapeutics – Framingham

Bernado Perez

Sanofi Pasteur – Marcy l'étoile

Didier Clenet

Thank you

Enhance™/ Halozyme

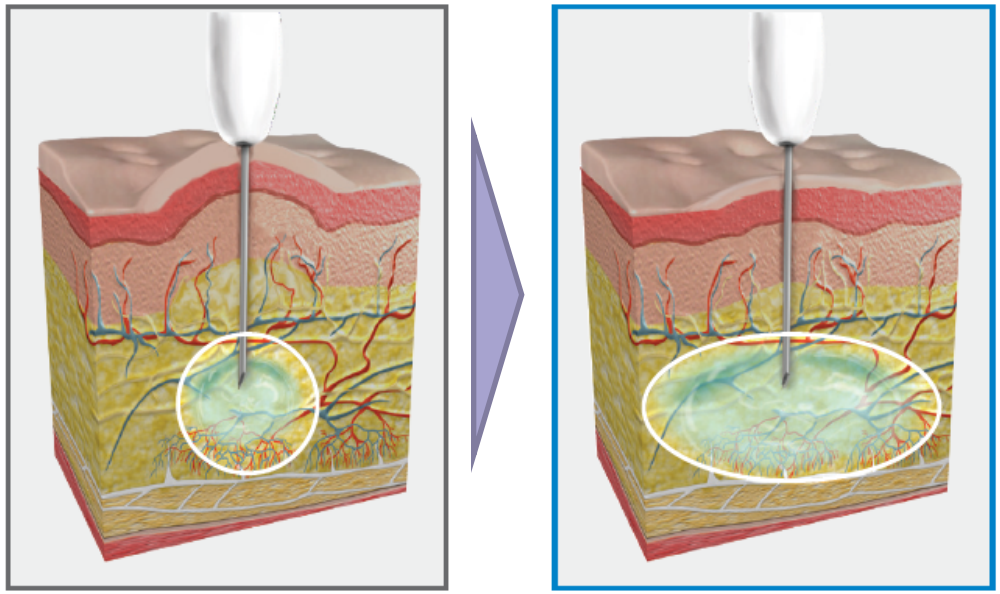
Global Biotherapeutics

- Traditional SC injections limited to ~2ml
- Hyaluronan (HA) is a gel-like substance than stops fluid flow in the SC space
- PH20 degrades HA and allows fluid flow
- 1/3rd of total body pool of HA turns over every day
- After 24hours HA has returned and SC space is normal

Additional comment from F. Grams:

- *The pores formed through the hyaluronic acid digestion are big enough for the penetration of antibodies but too small for bacteria to enter. Therefore there is no infection risk. There is also* no systemic exposure of the enzyme as *the t1/2 is very short*
- *Halozyme has done a trial with 400ml per arm, i.e. 800ml s.c. administration per patient. This is a lot, but not unlimited*

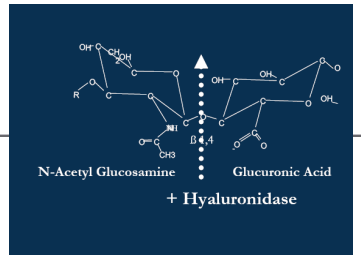
Normal SC Injection ***SC Injection with PH20***



Feasible subcutaneous volume limited

Any subcutaneous volume now feasible

Exclusive: IP protection through 2027 in U.S. and 2024 in ROW



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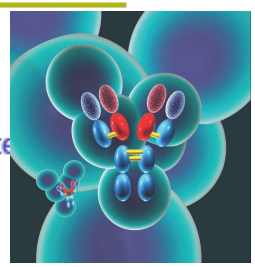
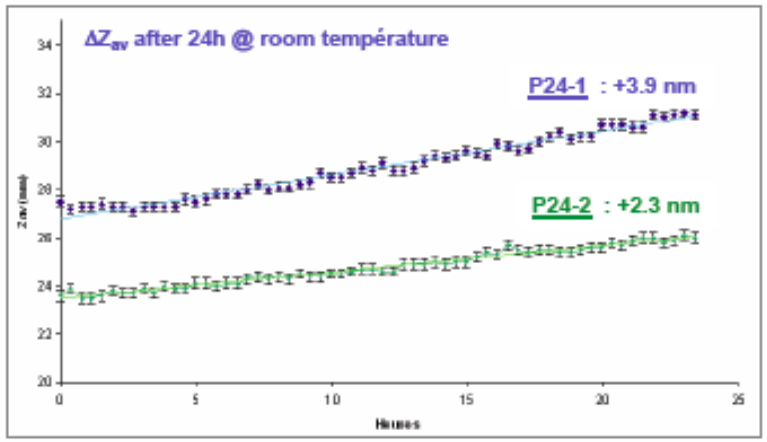
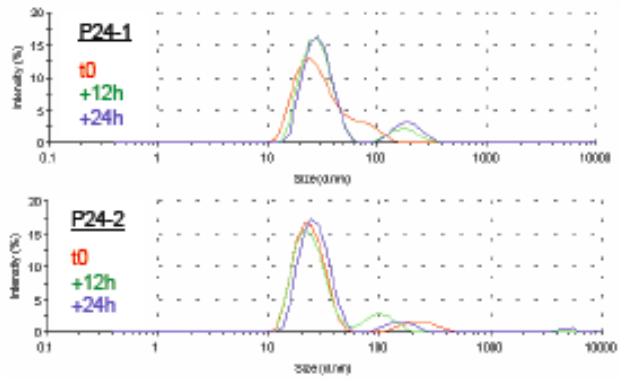
Case study of a Bi-specific antibody (Bi-mAb): aggregation characterization

Global Biotherapeutics

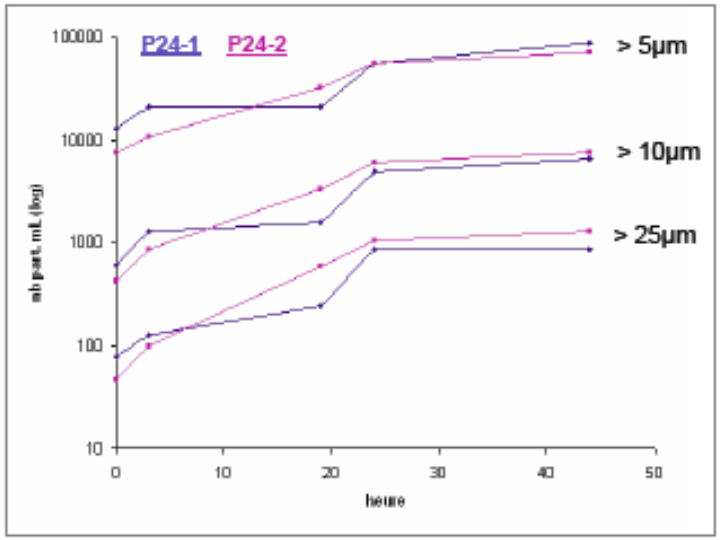


- DLS (nm - µm): increase of the Zav value





- FCM (> µm): increase of the amount of large aggregate



Increase of the amount of particles after 24h @room temperature

	> 5µm	> 10µm	> 25µm
P024-1	43648	4376	783
P024-2	48155	5639	1017

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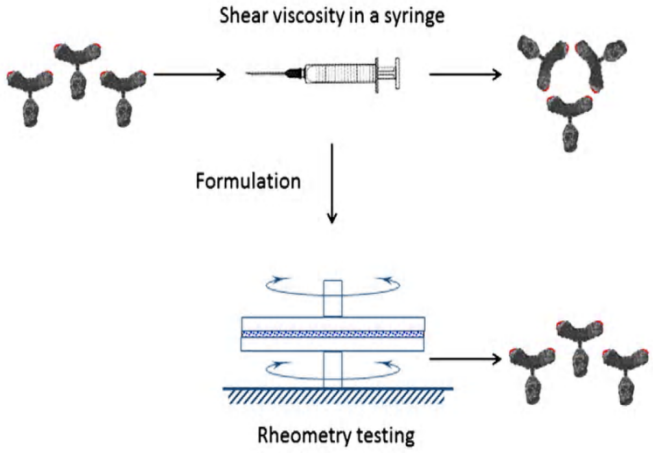
Fundamentals and nomenclature of rheology



Adapted from Bernardo Perez-Ramirez et al.
Advanced Drug Delivery Reviews 63 (2011) 1107-1117

Fundamentals and nomenclature of rheology:

- Dynamic (shear) visco: $\eta = \frac{\sigma}{\dot{\gamma}}$ / Kinematic viso: $\nu = \frac{\eta}{\rho}$
- Reduced viscosity: $[\eta] = \lim_{c \to 0} \left(\frac{\eta - \eta_o}{\eta_0 c} \right)$
- Temperature dependence (Newtonian and non-Newtonian fluids): $\eta = K_o \dot{\gamma}^{n-1} e^{k/\theta}$
- Macromolecular interactions: $\eta = \eta_o \left(1 + k_1 c + k_2 c^2 + \ldots\right)$
- Poiseuille's equation (pressure / flow rate relationship): $\eta = \frac{\Delta P \pi R^4}{8QL} \left(\frac{4n}{3n+1} \right)$ (N=1 for Newtonian)