Special aspects in the pharmaceutical development of protein formats beyond mAbs

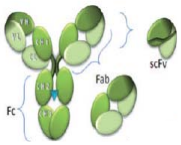
MIBio2012 –
Molecular Interactions in Biopharmaceutical Formulations

Dr. Susanne Jörg, Novartis Pharma AG, Basel

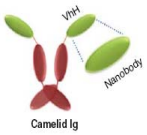
BPRD Non-Platform Molecules and Technology, Pharmaceutical Developoment

NOVARTIS

New molecule formats and their challenges



Antibody fragments[1]



Nanobodies[2]

Therapeutic proteins[3]

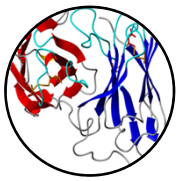
Fusion proteins[4]

Main stability challenges

- Increased aggregation
- Decreased solubility, precipitation
- Protein degradation
- Viscosity challenges
- Material incompatibility / adsorption
- ...

[1] *P. Chames. British J Pharmacol. 2009, 157: 220-33*
[2] *N. Nicolaides. Expert Opin. Drug Discov. 2010, 5(11):1123-40*
[3] *www.pdb.org*
[4] *Novartis, IBP*

Agenda



Molecule Stabilisation

Case study on stabilisation of single chain antibody fragment

- pH and buffer screen
- Lyophilisate screening study
- Surfactant optimisation study

Administration challenges

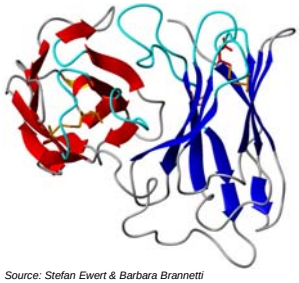
Case studies on administration of biopharmaceutcial formulations

- Drug-drug combinations
- Challenges due to material incompatibilities

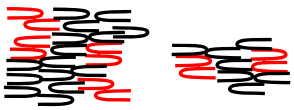
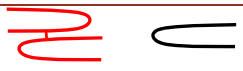
Molecule characteristics

Single-chain variable human-derived monoclonal antibody fragment (scFv)

- Prone to covalent and non-covalent aggregation
- High molecular weight aggregates, resulting in a not adequately controlled aggregate size distribution (batch to batch variability).
- Limited solubility at physiological pH range



Source: Stefan Ewert & Barbara Brannetti

Parameter	Structural units (examples)	Methods
Overall aggregation (non-covalent and covalent)		• SEC, native conditions • DLS, turbidity, subvisible and visible particlulate matter
Covalent aggregation		• SEC, denaturing conditions • SDS-PAGE, red./non-red.

Objectives

Development of a stable formulation as lyophilisate in vial for iv infusion

- reduced formation of non-covalent aggregation and sub-visible and visible particulates
- batch-to-batch consistency
- local tolerability of the formulation

pH and buffer screen (liquid)
- Target pH range based on solubility
- Selection of buffers suitable for target pH

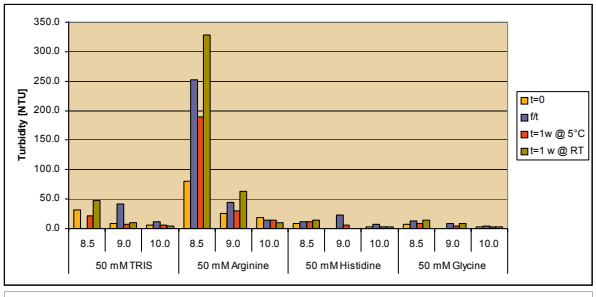
Lyophilisate screening study
- Evaluation of stabilizers
- Evaluation of surfactants
- Evaluation of antioxidants

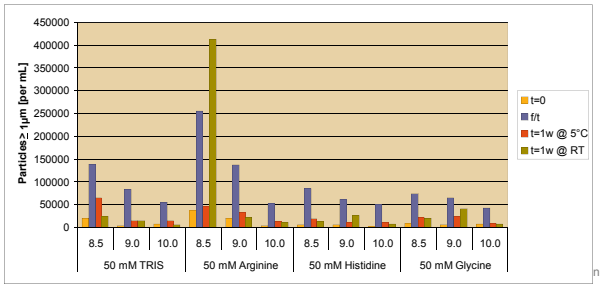
Optimisation study
- Establish pH range
- Optimise surfactant concentration

NOVARTIS

pH and Buffer Selection Study

Results - non covalent aggregates, particulate analytics





Buffer type

- TRIS, glycine and histidine with comparable non-covalent aggregation behaviour
- Arginine with highest turbidity, subvisble and visible particulate matter

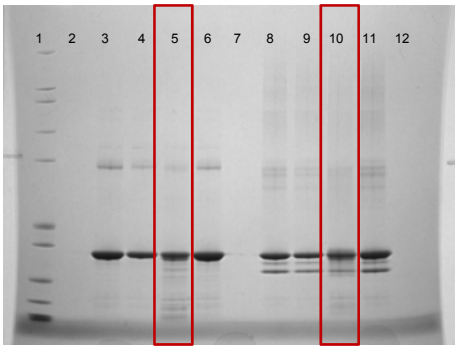
pH value

- pH 8.5: increased turbidity, subvisble and visible particulate matter
- pH of 10.0 brings no significant improvement over pH 9.0 in non-covalent aggregation behaviour

NOVARTIS

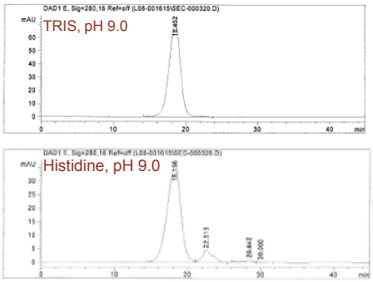
pH and Buffer Selection Study
Results –degradation products

SDS-PAGE



Lane 1: MW Marker
Lane 2, 7, 12: Blank
Lane 3-6: non-reduced SDS-PAGE
(3: Tris; 4: Arginine; 5: Histidine; 6: Glycine; all pH 9.0 and after 1wk storage @ RT)
Lane 8-11: reduced SDS-PAGE
(8: Tris; 9: Arginine; 10: Histidine; 11: Glycine; all pH 9.0 and after 1wk storage @ RT)

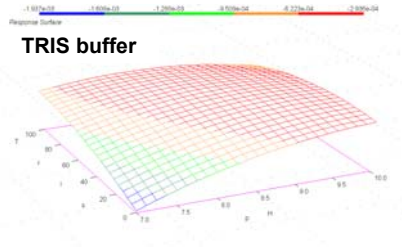
nSEC

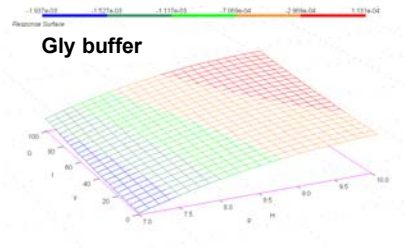


- Histidine with degradation products
  - SDS PAGE: additional bands under reduced and non-reduced conditions
  - nSEC: Histidine with 2nd peak, corresponding to a MW of ~5000 Da

NOVARTIS

pH and Buffer Selection Study
Results – Determination of B_{22}-values by SIC





pH value

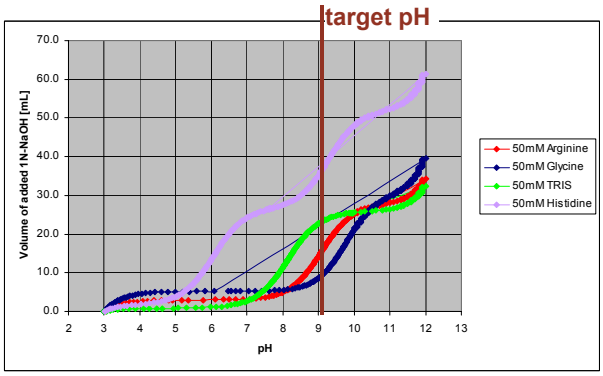
- B_{22} values are quite negative below pH 6
- Colloidal stability is maximal when the pH is at 8.5 or above. Above that pH, the response surface for B_{22} values is relatively flat.

Buffer type

- Tris and glycine as best buffers, providing comparable increase in B_{22} values
- As Tris seems to provide better colloidal stability for a wider pH range, it is recommended to use Tris

NOVARTIS

pH and Buffer Selection Study
Buffer titration curves



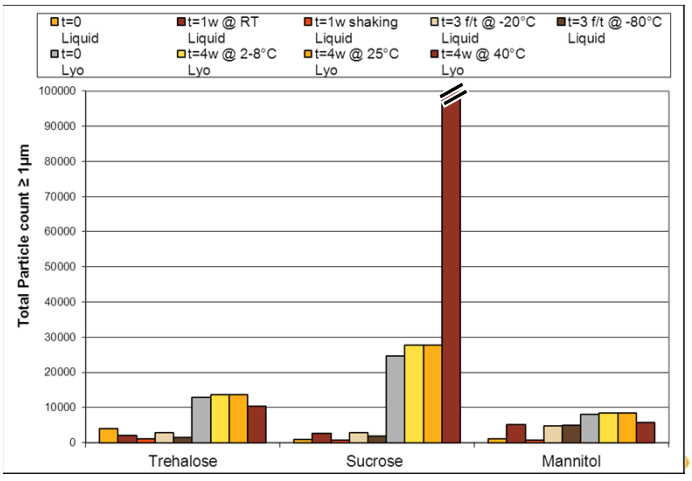
- Tris (pKa 8.3) and arginine (pKa 9.0) offer a better buffering capacity towards pH values below pH 9.0 than glycine (pKa 9.6) and histidine (pKa 9.2).
- Histidine with high buffering capacity which is disadvantageous at pH value out of physiological range.

NOVARTIS

Lyophilisate Screening Study
Results - turbidity

Stabilizer

- Trehalose superior over other stabilizers tested.



NOVARTIS

Lyophilisate Screening Study

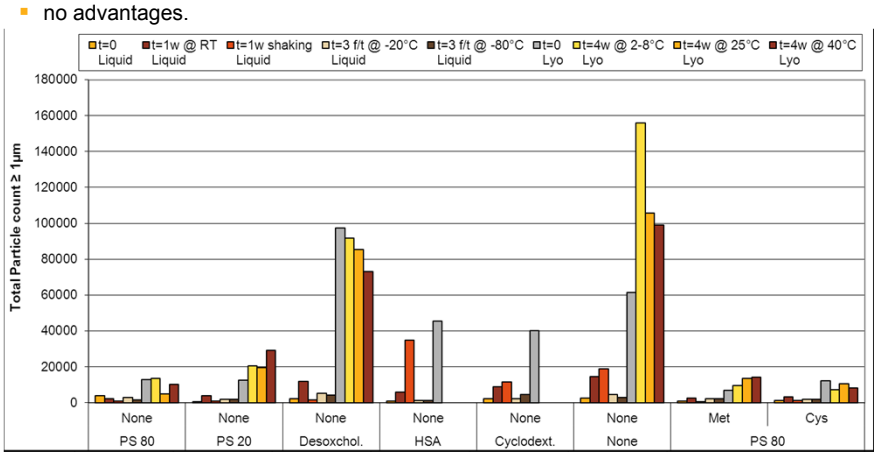
Results - non covalent aggregates, particulate analytics

Surfactant

- Presence of surfactant prevents increase in turbidity, subvisible particulate matter.
- Polysorbate 80 superior over other surfactants tested

Antioxidant

- no advantages.

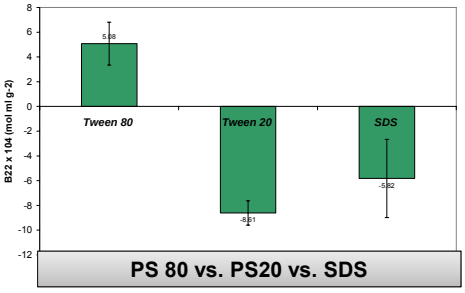


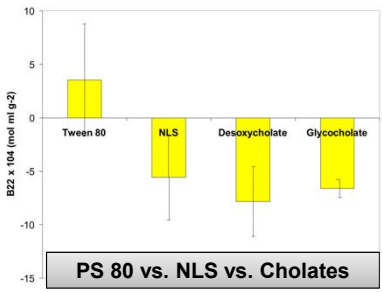
Lyophilisate Screening Study

Results – Determination of B_{22}-values by SIC

Surfactant

- Polysorbate 80 identified as most suitable surfactant to increase B_{22} values, while Polysorbate 20 was found to be destabilizing.
- Other surfactants tested did not provide any improvement in B_{22} values.
- In addition, combinations of surfactants also do not appear to be promising for increasing B_{22} values (not shown)

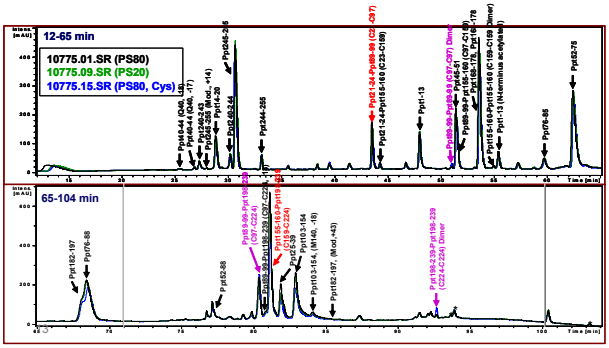




NOVARTIS

Lyophilisate Screening Study
Results – covalent aggregates

Reconstituted Lyo T=0	Monomer [%]	Dimer [%]	Agg. [%]
TRIS Drug substance	88.6	9.7	1.7
Polysorbate 80	90.2	9.2	0.0
Polysorbate 20	88.9	11.1	0.0
Na-desoxycholate	86.8	13.3	0.0
PS80 / methionine	88.0	12.1	0.0
PS80 / cysteine	67.9	20.3	9.8



dSEC

- Cysteine: increase in covalent dimers
- Constant pattern for all other surfactants

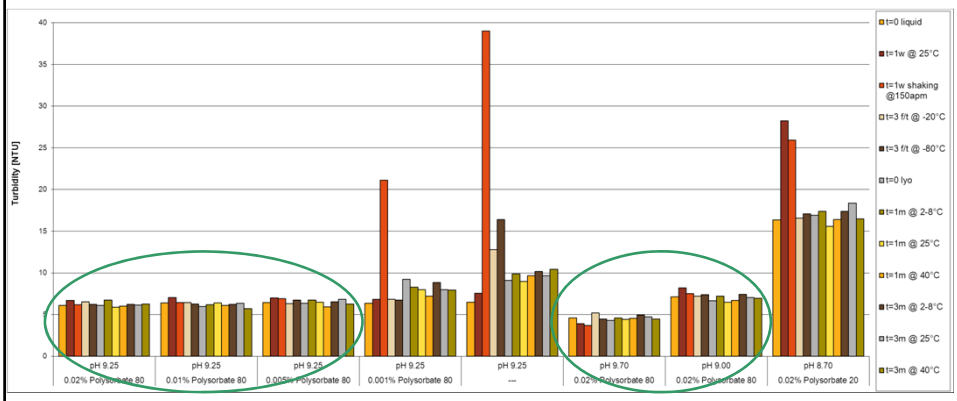
Peptide Map

- Cysteine: formation of non-expected intermolecular disulfide bridges

NOVARTIS

Optimisation Study
Results: turbidity

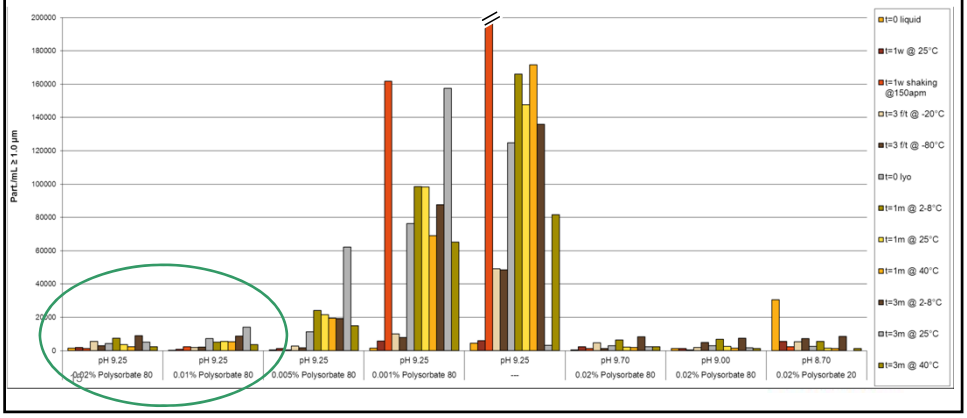
- Turbidity stays constant around 6-7 NTU for formulations with 0.005 – 0.02% polysorbate 80. Increased turbidity in formulations with 0.001 and no surfactant, particularly upon shaking and freeze-thaw..
- Turbidty constant for pH 9 – 9.7. Higher turbidity observed for pH 8.7.



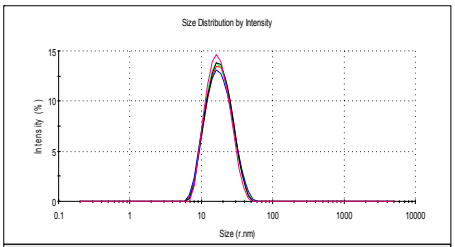
NOVARTIS

Optimisation Study
Results – subvisible particulate matter

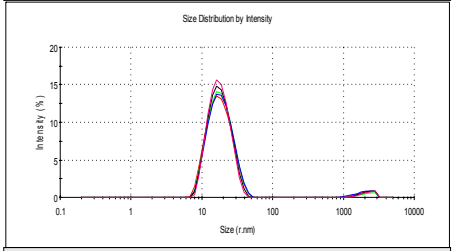
- Particulate matter increase upon lyophilisation. 0.01 – 0.02% PS80 sufficient to keep number of particles ≥ 1.0µm/ml below 10,000.
- Particle sizes ≥ 10 and 25 µm within Pharmacopoeial limits
- Formulation with 0.005, 0.001 and no surfactant show increase in subvisible particles upon shaking, freeze-thaw and lyophilisation.



Optimisation Study
Results – DLS



pH 9.25, 0.02% PS80



pH 9.25, no PS80

	Lyo T=0		
	Peak 1 [nm]	Peak 2 [nm]	PDI[1]
Drug Substance	19.7	> 1000	0.22
pH 9.25, 0.02% PS80	16.1	n.a.	0.15
pH 9.25, 0.01% PS80	16.7	> 1000	0.18
pH 9.25, 0.005% PS80	16.5	> 1000	0.16
pH 9.25, 0.001% PS80	17.0	> 1000	0.18
pH 9.25, no PS80	17.3	> 1000	0.20

[1] *Polydispersity Index*

NOVARTIS

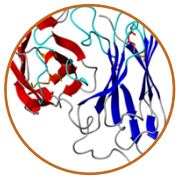
Summary

- Selection and optimisation of well-known formulation conditions allow keep ing challenging molecule properties under control.
- Variety of analytical methods applied to thoroughly investigate critical quality attributes, such as covalent and non-covalent aggregation and degradation.

Selected formulation shows desirable quality upon lyophilization and short-term accelerated stability - reduced non-covalent aggregation as well as sub-visible and visible particulate matter formation.

- **pH 9.0 – 9.5** identified as optimal pH range. At lower pH increased molecular weight, turbidity, subvisble and visible particulate matter load.
- **Tris and Trehalose** superior over other buffers / stabilizers tested.
- **Presence of surfactant** prevents aggregate formation. **Polysorbate 80** significantly superior over other surfactants tested.

Agenda



Molecule Stabilisation

Case study on stabilisation of single chain antibody fragment

- pH and buffer screen
- Lyophilisate screening study
- Surfactant optimisation study

Administration challenges

- Case studies on administration of biopharmaceutcial formulations
  - Drug-drug combinations
  - Challenges due to material incompatibilities

Administration challenges

Drug-drug combination products

Goals

- Establish drug-drug combination products with enhanced efficacy
- Ease of use as a combined single administration

Challenges

- Incompatibilities during storage and use
- Analytical challenges in mixtures - distinguishing between 2 similar molecules and degradants
- Each combination project equals effort of 3 single API projects- individual molecules and combined

Status

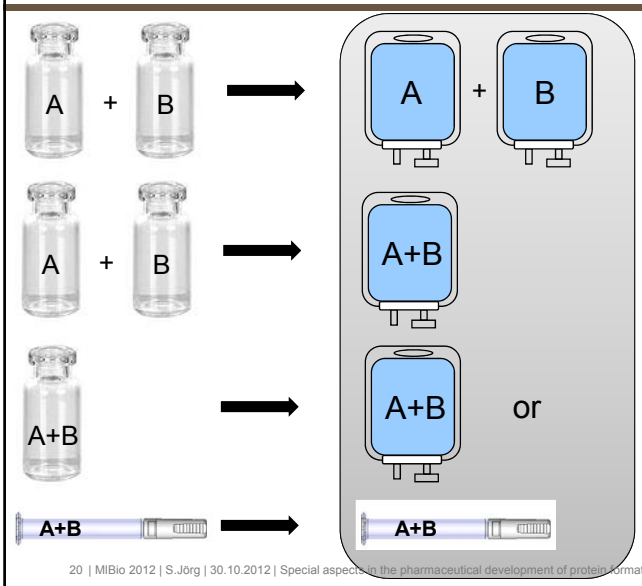
- No single unit combo-biologic on the market to date
- Several in clinical evaluation

NOVARTIS

Courtesy of T. Serno

Drug-drug combination products

Delivery options



Sequential administration

- easiest option for PoC
- potentially long infusion times

Mix at point of use

- Prerequisite: in-use stability and combo compatibility

Final DP is mixture

- Best commercial option
- Prerequisite: fixed dose ratio to be known

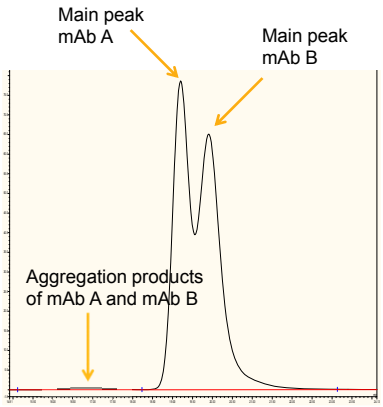
NOVARTIS

Courtesy of T. Serno

Drug-drug combination products

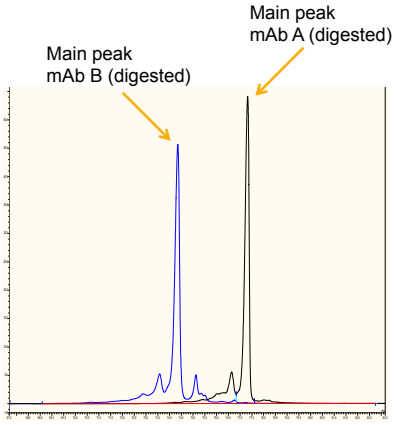
Analytical challenges

mAb mix in **HP-SEC**-chromatogram



Insufficient peak resolution for quantification (separation by size), still useful for ***purity*** assessement

Overlay of **CEX** chromatograms



CEX provides improved peak resolution (separation by charge), suitable for ***quantification***

NOVARTIS

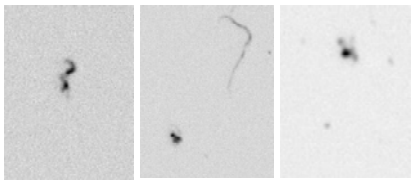
Courtesy of T. Serno

Compatibility issue of mAb derived protein

Description and interim solution

Compatibility issue

- Compatibility testing of mAb derived protein with i.v. administration sets
  - At very low doses adsorption is problematic (observed at 0.3 mg/kg);
  - Sub-visible particulate levels formed at a dose ≤ 1.0 mg/kg during infusion through i.v. line.
- Sub-visible particles being identified as proteinaceous particulates.



MFI screenshots of subvisibleparticles

Interim solution

Specific release of two i.v. sets which were found compatible.

- No pattern observed with respect to line or filter materials
  e.g. Supplier 1 passed, Supplier 2 failed for PVC/DEHP infusion line
- Use of smaller infusion bags to achieve conc. of 0.8-18mg/ml

Protein dose [mg]	Infusion volume [ml]
0 – 900	50
80 – 1800	100
200 - 4500	250

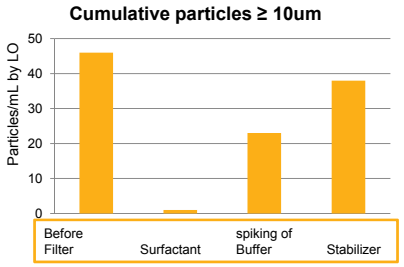
NOVARTIS

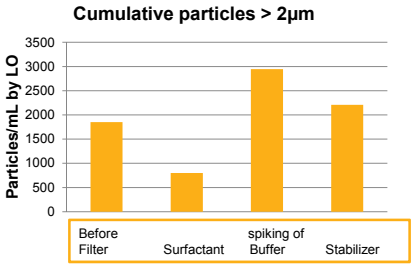
Compatibility issue of mAb derived protein

Courtesy of T. Serno

Root cause investigation

- Spiking of Surfactant (at a conc.above the CMC of the surfactant) significantly reduced particle formation
- Spiking of Buffer / Stabilizer without effect
- Dilution of Surfactant was likely the reason for problems encountered during compatibility testing





NOVARTIS

Administration challenges

Mitigation options – alternative i.v. infusion modes

Mitigation options	Description
1) Partial infusion	• Higher protein concentration, but smaller infusion volume • Bag is emptied until defined infusion volume is remaining • Limited by minimal infusion time clinically required for infusion
2) Syringe pump infusion	• Higher protein concentration and low infusion rate • Limited by minimal. clinically acceptable infusion rate
3) Bolus injection	• Use of higher protein concentration and small injection volume • Limited by minimal infusion time clinically required for infusion
4) Piggyback infusion	• High concentration drug mixed with diluent using «three-way stopcock» just prior infusion into patient • Requires infusion pump for diluent and syring pump for drug
5) Spiking of excipient	• Excipient spiked into infusion bag prior to dilution of drug

NOVARTIS

Conclusions

New biologics molecules– such as mAb derived formats, nanobodies, therapeutic and fusion proteins – come along with new challenges.

- Case Study 1:
  - Selection of optimised formulation conditions ensures to keep challenging molecule properties under control.
  - Variety of analytical methods has been applied to thoroughly investigate critical quality attributes, such as covalent and non-covalent aggregation and degradation.
- Case Study 2:
  - Drug-drug combination product development need to trade-off between complexity during clinical development and ease of use for commercial applications.
  - Compatibility of the protein with materials used during administration to the patient needs to be assured. Creative solutions might be required to ensure clinical dosing.

Thorough pharmaceutical development is key to enable integrity and stability of biologics compounds upon storage and administration.

Acknowledgements

Case Study 1:

- Jürgen Sigg, Roland Düblin, Stefan Rapp
- Hui Zhao, Kurt Forrer, Manuel Diez
- Legacy Biodesign

Case Study 2

- Tim Serno, Marco Lang

... and all BPRD Pharmaceutical Development Colleagues