Influence of UF/DF conditions on IgG aggregation at the interface of DSP and final formulation

Workshop on Protein Aggregation, 7/19-22/2010
Eva Rosenberg, Pharma Biotech Production & Development, Roche Diagnostics, Penzberg

Overview

I Introduction

II Optimization of process parameters in tangential flow filtration (TFF)
   Influence on process time, end-concentration and bulk stability

III Protein-solute interactions during TFF of IgGs
   Effect of buffer composition, pH and conductivity on protein stability during the concentration process

IV Feasible prediction of TFF solute outcome
   Utilization of the Donnan-Model
I Introduction: Aggregation during purification

- TFF: UF/DF in formulation buffer
  - elution at pH < 4.5
  - virus inactivation and conditioning for loading on ionexchange (IEX)-chromatography
  - change of buffer conditions concerning pH and conductivity during conditioning
  - change of buffer conditions concerning pH and conductivity during diafiltration (DF)
  - increase in protein concentration
  - influence of temperature and mechanical stress
  - exposure to surfaces and interfaces
  - abraded particles from process-equipment


I Introduction: obtaining highly concentrated bulks

- Challenges during TFF processing of (highly) concentrated IgG bulks
  - reaching high end-concentrations up to 100–250 g/l in optimized process time by using TFF
  - ensure stability of the bulk influenced by protein concentration, pH, conductivity, buffer solute composition
  - appropriate storage conditions encompassing temperature (-70°C/ 4 °C), containers
  - viscosity of concentrated protein solutions have influence on yield, handling, filling
II Optimization of TFF process parameters

Influence of ∆p (p_inlet - p_outlet)

- Increase in permeate flux with increasing ∆p; at ∆p=1.2 bar no further flux increase
- 20 % reduction in process time at ∆p=1.2-1.8 bar (compared to ∆p=0.7 bar)
- Influence on end-concentration, which is reduced at ∆p=0.7 bar and ∆p=3.0 bar (< 110 mg/ml)

Further parameters: T= RT, TMP=0.6 bar, 30 kDa Hydrosart UF membrane

Protein concentration [mg/ml]

- IgG: 5-140 mg/ml, 10 mM buffer pH 5.5

Influence on physical stability of the concentrates during UF (SE-HPLC, DLS, turbidity, particles):
- Lower ∆p values result in reduced aggregate levels at high protein concentration (compared at 90 mg/ml after UF)
II Optimization of TFF process parameters
Influence of $\Delta p \left( p_{\text{inlet}} - p_{\text{outlet}} \right)$

induction of protein particles during UF concentration

→ protein particles stained after 0.2 µm filtration (2 ml/18 cm²); 80-times magnification LiMi

protein particles stained with pyrogallolred-molybdate at pH < 4 (Li et al. 2007)

II Optimization of TFF process parameters
Influence of $\Delta p \left( p_{\text{inlet}} - p_{\text{outlet}} \right)$

protein particles induced by UF concentration show differences in secondary structure (2D FT-IR spectroscopy)

increase in $\Delta p$ did not result in pronounced differences in secondary structure, however the number of the induced protein particles increased

protein adsorbed to the hydrophilic UF-membrane did not show detectable differences in secondary structure

IgG: 5 and 90 mg/ml, 10 mM buffer pH 5.5
II Optimization of TFF process parameters
Influence of protein concentration and TMP

IgG: c = 45 mg/ml, 10 mM buffer pH 5.5

evaluation of maximum permeate flux (J) depending on protein concentration in the retentate (c = 5-180 mg/ml)

beside Δp=0.7-1.8 bar and tangential flow rate (Q_r)=150-450 l/m²/h

influence of transmembrane pressure (TMP) of 0.3-2.0 bar was investigated

II Optimization of TFF process parameters
Influence of protein concentration and TMP

permeate flux (J) decreases with increasing protein concentration

permeate flux increases at high protein concentrations only initially with increasing TMP

permeate flux increases at a given protein concentration with increasing Δp and Q_r

variation of process parameters Δp (Q_r) and TMP with increasing protein conc. to optimize permeate flux

Rosenberg et al. 2009
II Optimization of TFF process parameters

Variation depending on protein concentration

- Variation of $\Delta p$ ($Q_r$) und TMP with increasing protein concentration shows:
  - Permeate flux increases and concentration time is reduced
  - Increased physical stability of the concentrates during UF (particles, turbidity, DLS)
  - No impact on physical stability of the concentrates during storage (particles, turbidity, SE-HPLC, $T_m$ FT-IR)

$IgG$: $c = 5-90$ mg/ml, $10$ mM buffer pH 5.5

---

II Optimization of TFF process parameters

Impact on sterile filtration processability

- 0.2 μm-filtration in general adversely affected by higher viscosity at increased protein concentration
- Flow profile for concentrates of the variable method is highly improved compared to the concentrates derived from continuously operated UF methods

$IgG$: $c = 90$ mg/ml, $20$ mM buffer pH 5.5, membrane area 4.52 cm²
electrostatic interactions between macro-molecules (at non-isoelectric pH) and other ions leads to unequal partitioning of charged diffusible solutes through a semi-permeable membrane (Donnan-Effekt)

relevant during UF of IgGs to composition of formulation buffer after concentration:

IEP\(_{\text{IgG}}\) > pH for formulation buffers

10-50 kDa UF-membrane is semi-permeable (for buffer ions)

changes in the concentration of diffusible buffer species in the retentate during the concentration of IgGs can be described by using the Donnan-equation (shown for histidine-, sodium-, acetate- and chloride ions)

\[
S^+ = \sqrt{\frac{(z_e)^2 + \frac{3}{2} \left( \frac{e}{M} \right)^2}{\rho}}
\]

equation taken from C. Tanford 1967
amount of lost cationic and accumulated anionic buffer species depends on protein concentration:

at high protein concentrations (200 mg/ml) a loss in histidine-molarity of > 50% and an increase in acetate-molarity of about 100% was observed (pH 5.5)

amount of lost cationic and accumulated anionic buffer species depends further on pH of the solution:

the higher ∆[IEP-pH] the higher the higher ∆[pK_s-pH] the higher
loss and accumulation of buffer species have impact on pH and conductivity during UF processing:

- increase in pH and shift in conductivity depending on charge and concentration of the applied buffer species, as well as on protein concentration

---

### III Protein solute interactions during TFF of IgGs

#### Effect on pH and conductivity

- Histidine
- Acetate

---

### III Protein solute interactions during TFF of IgGs

#### Effect on protein stability

- Changes in buffer composition and concomitant pH changes during UF show adverse effects on physical (SE-HPLC, turbidity, particles) and chemical stability (deamidation) of the concentrates during storage in solution (histidine, pH 5.5)

- No detectable effect on secondary structure (2D FT-IR) or tertiary structure (2D UV) was observed

quant. of the Fab_d from IgG1 by CEX (UV) after papain-digestion (during storage at 40 °C)
IV Feasible prediction of TFF solute outcome
Utilization of the Donnan-Model

determination of needed buffer-ion-molarity before UF by using the Donnan-equation to compensate loss/accumulation during the concentration process and shift in pH

\[ S^+ = \frac{z e + 4(y \gamma \rho - \frac{c M}{1000})}{2} \]

evaluated stability-promoting concentration of diffusible buffer-components, e.g. 20 mM histidine
calculation of the initial buffer-ion molarity depending on protein concentration, pH and density

adjustment of buffer composition by using the Donnan-equation results in increased physical- (SE-HPLC, turbidity, particles) and chemical stability (deamidation) of the IgG-concentrates (200 mg/ml, histidine buffer) during processing and storage in solution

increased physical stability further results from avoiding pH adjustment after UF concentration
using the Donnan-Equation for systematic adjustment of buffer solute composition before UF makes pH adjustment unnecessary
concomitantly a dilution of the concentrated protein solution is circumvented

IgG aggregates formed after conditioning of the concentrated solution (200 g/l) to pH 5.5 by using 0.02-0.5 M hydrochloric acid (HCl)
Summary

<table>
<thead>
<tr>
<th>To be considered during TFF operations to reduce aggregation</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta p$ ($Q_r$) and TMP</td>
<td>on aggregate- and particle formation during UF processing, as well as on processing time and end-concentration.</td>
</tr>
<tr>
<td></td>
<td>on induction of the number of structural perturbed protein particles during UF concentration.</td>
</tr>
<tr>
<td></td>
<td>on processability during sterile filtration operations.</td>
</tr>
<tr>
<td></td>
<td>non on storage stability of the filtered concentrated solutions.</td>
</tr>
<tr>
<td>change in buffer composition and thus pH and conductivity</td>
<td>potentially compromised physico-chemical stability in solution at high protein concentrations (shown for a histidine buffered system).</td>
</tr>
<tr>
<td>during UF-concentration and -DF processes</td>
<td>systematic adjustment of buffer solute composition before UF processing by using the Donnan-Equation was shown to be suitable to circumvent post-processing adjustment of pH and buffer composition.</td>
</tr>
<tr>
<td>pH-adjustment at high protein concentrations</td>
<td>increased aggregate- and particle level in the concentrated solutions.</td>
</tr>
</tbody>
</table>

Impact

Sincerest thanks are extended to....

... you for invitation and attention

... Stefan Hepbildikler, Wolfgang Kuhne, Katrin Heinrich, Monika Schweigler, and all colleagues of the Department Pharma Biotech Development Recovery and DSP, Roche Diagnostics GmbH, Penzberg

... Prof. Gerhard Winter and Prof. Wolfgang Frieß and all colleagues at the Institute of Pharmaceutical Technology and Biopharmacy, LMU Munich