GE Healthcare
Life Sciences

Purification of Interferon α-2a—a process development study

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Introduction

Biosimilar production is not required to follow the same process as the originator, encouraging the choice of new and potentially more efficient manufacturing steps during process development. Furthermore, modern methods such as high-throughput process development (HTPD) allow faster and more thorough screening, optimization, and characterization. This simplifies the selection of better methods and helps establish running conditions that assure a robust manufacturing process.

In this work, a process for Interferon α-2a (IFN α-2a) was developed, using HTPD techniques and design of experiments (DoE) methodology. IFN α-2a was expressed in *E. coli*. The molecular weight for IFN α-2a is 19241 Da and the isoelectric point 5.99.

Using the process development of IFN α-2a as an example, we want to demonstrate the benefits of using modern methods such as HTPD and suitable analysis methods in the process development of therapeutic proteins or their biosimilars.

Analysis

Determination of product concentration

- RPC, HPLC
- SPR using Biacore™ T200 processing unit (both denatured and refolded interferon)

Concentration assay

- CFCA using Biacore T200 (no standard needed)

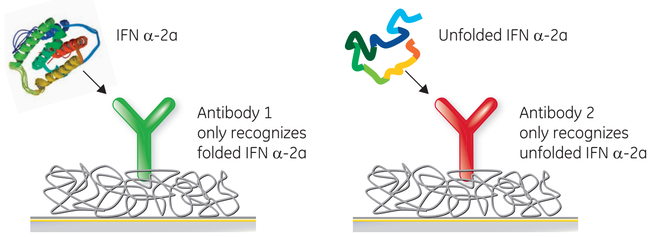


Fig 1. Methodology for Biacore concentration assay measuring folded INF α-2a and unfolded INF α-2a respectively. A protein sample is injected twice at two flow rates: 5 and 100 µL/min.

Purity

- HCP/ECP ELISA using Gyrolab™ CD technology
- SDS-PAGE and Western blotting, using Amersham™ WB system
- Gel filtration (size exclusion chromatography [SEC]), using ÄKTA™ purifier chromatography system

Similarity

- LC-MS, peptide mapping
- SPR, Biacore T200, receptor binding

Binding activity assay

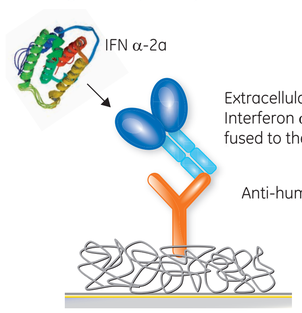
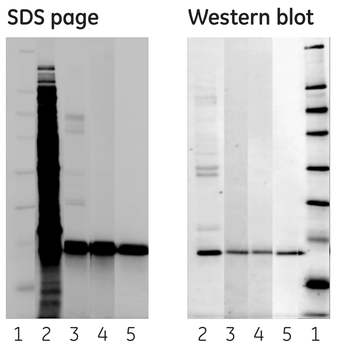


Fig 2. Methodology for binding activity assay using Biacore pocessing unit. The anti-human IgG (Fc) antibody is from the Biacore human antibody capture kit.

Process summary

Step	Yield (%)	HCP (ECP) (ppm)
Refolding UF/DF/NFF	40	8400
Capto™ S	92	440
Capto adhere ImpRes	95	15
Capto Octyl	96	Below LOQ

SDS page **Western blot**



1. Molecular weight standards
2. Cell lysis
3. Refolded
4. Capto adhere ImpRes medium
5. Originator product

Fig 3. Results from SDS page and Western blot.

Process IFN α-2a

E. coli

↓

Harvest and cell lysis

↓

Inclusion bodies harvest and wash

↓

Inclusion bodies solubilization

↓

Refolding

↓

UF/DF/NFF

↓

Capture: Capto S

↓

Intermediate

↓

Polishing: Capto Octyl

Results: screening using PreDictor™ plates

Chromatography medium (resin) screening, as well as screening for binding conditions were done in overload mode with a HTPD approach, using the PreDictor plate format. For the intermediate step three, different media based on the Capto ImpRes platform were screened; Capto Q ImpRes, Capto adhere ImpRes, and Capto MMC ImpRes medium. Sample used was IFN α-2a eluted from Capto S (capture step). Parameters tested were pH 5 to 9 (5 to 7.5 for Capto Q ImpRes) and NaCl concentrations 0 to 700 mM for multimodal media and 0 to 500 for Capto Q ImpRes. Flow-through fractions from the PreDictor plates were collected and analysed. Concentration of IFN α-2a was measured by CFCA, using Biacore T200. Experimental setup, data management, and data analysis was done using Assist software.

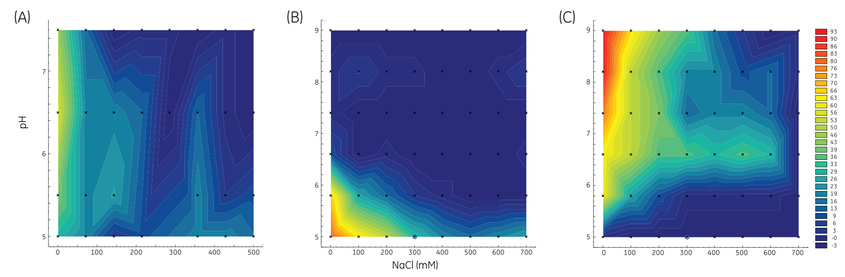


Fig 4. Static binding capacity for (A) Capto Q ImpRes; (B) Capto MMC ImpRes; (C) Capto adhere ImpRes medium obtained in PreDictor plate studies. The red areas corresponds to the highest SBC and the blue to the lowest.

Figure 4 shows the static binding capacity for each medium. Capto adhere ImpRes showed a binding of more than 80 mg INF/mL media at pH over 7 and 0 M NaCl. Capto MMC ImpRes showed a binding of more than 80 mg INF/mL media at pH 5 and 0 M NaCl, while Capto Q ImpRes showed somewhat lower binding capacity. The eluted IFN α-2a from the capture step has pH 7.8 (elution buffer Capto S: pH 7.8 + 50 mM NaCl) and with a dilution of 1:1, the material can be applied directly if using Capto adhere ImpRes without pH adjustment or UF/DF step.

Chromatography media screening in small column format

Binding conditions for each medium (Capto Q ImpRes, Capto adhere ImpRes, and Capto MMC ImpRes) obtained from the PreDictor plate study was used in column screening. Elution was done with a NaCl/pH gradient. The eluted material in the main peak was collected and analyzed by SEC and CFCA.

Figure 5 (A) shows that, with Capto adhere ImpRes, some other material was also eluted in the beginning of the gradient before the main peak. This material might be impurities. The SEC analysis showed that Capto adhere ImpRes had highest purity, as seen in Figure 5 (B). Due to high binding capacity and purity, it was decided to use Capto adhere ImpRes for further optimization.

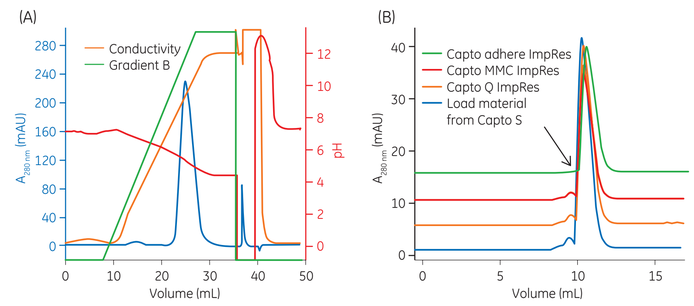


Fig 5. (A) Purification using Capto adhere ImpRes; (B) Media screening, SEC analysis.

Capto adhere ImpRes—elution optimization and robustness in small column format

A study using DoE approach, looking at pH (4.5 to 6.5) and salt concentration (0 to 300 mM NaCl) was run, using a central composite face (CCF) design with 11 experiments (three center points) using an ÄKTA avant system. The sample used was IFN α-2a eluted from Capto S (capture step). The concentration of IFN α-2a in step-eluted fractions from Capto adhere ImpRes were measured using CFCA (Biacore T200). The yield surface plot from the optimization is shown in Figure 6. The best elution conditions (high yield and low HCP levels) were found at low pH and a salt concentration of 150 to 300 mM NaCl.

The next step in the development of the intermediate step was running a robustness study. As for the optimization study, this was done using a DoE approach on an ÄKTA avant system. A full factorial (two levels) orthogonal balanced design was applied. A step-gradient elution was performed varying the salt concentration (between 230 and 270 mM NaCl) and pH (between 4.3 and 4.7), with yield and removal of HCP as responses.

Figure 7 shows that the obtained yield varied between 75% and 84%, whereas the HCP was about 15 ppm in all experiments. DoE evaluation showed no significant effects of the two parameters within the studied range. The process step is therefore considered as robust.

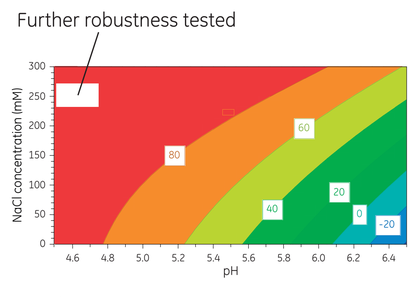


Fig 6. Capto adhere ImpRes optimization using a DoE approach. Contour plot for the response yield.

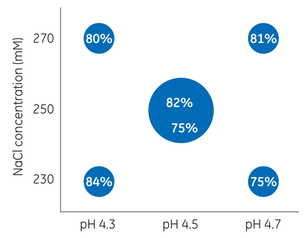


Fig 7. Robustness study using a full factorial orthogonal balanced design. The obtained yield, using Capto adhere ImpRes medium, is shown in the figure.

Discussion

Screening of several media was quickly performed using HTPD methodology. Binding capacity, yield, and purity data was compared and based on this, the medium showing the best performance in the study was chosen. Binding conditions were identified and used for further optimization. Using the HTPD approach generates a lot of samples and the need of analysis methods that are fast and can handle a large amount of samples are required. In this study a concentration and activity assay method for a Biacore processing unit was developed. The developed concentration assay can handle 96 samples during 12 h or one sample during 7.5 min, which can be compared to 15 min per sample using an HPLC method. The concentration assay was very powerful and useful during the HTPD screening experiments.

Conclusions

- The results from the PreDictor plate experiments were quickly produced and valuable for choosing binding parameters for further optimization in a small column format
- The best chromatography medium observed for the intermediate step in this study was found to be Capto adhere ImpRes
- Biacore T200 processing unit was shown to be a fast and reliable tool for concentration determination in HTPD experiments
- Approximately 70 mg Interferon was needed to estimate binding capacities , IFN α-2a behavior with three different chromatograhy media and different parameters in PreDictor plates

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Abbreviations: CFCA = calibration-free concentration analysis, ECP = *E. coli* host cell proteins, HCP = host cell proteins, HPLC= high-performance liquid chromatography, LC-MS = liquid chromatography-mass spectrometry, LOQ = limit of quantification, NFF = normal flow filtration, RPC = reversed phase chromatography, SPR = surface plasmon resonance spectrometry, and UF/DF = ultra filtration/diafiltration