Accepted Manuscript

Title: An Integrated Precipitation and Ion-exchange
Chromatography Process for Antibody Manufacturing:
Process Development Strategy and Continuous
Chromatography Exploration

Author: Steffen Großhans Gang Wang Christian Fischer
J*ü*rgen Hubbuch

Please cite this article as: Steffen Grosshans, Gang Wang, Christian Fischer,
J*ddotu*rgen Hubbuch, An Integrated Precipitation and Ion-exchange Chromatography
Process for Antibody Manufacturing: Process Development Strategy and Continuous
Chromatography Exploration, *<![CDATA[Journal of Chromatography A]]>* (2017),
https://doi.org/10.1016/j.chroma.2017.12.013

1 An Integrated Precipitation and Ion-exchange Chromatography
2 Process for Antibody Manufacturing: Process Development Strategy
3 and Continuous Chromatography Exploration

4   Steffen Großhans*, Gang Wang*, Christian Fischer, Jürgen Hubbuch**

5   *Karlsruhe Institute of Technology (KIT), Institute of Process Engineering in Life Sciences, Section IV:*
6   *Biomolecular Separation Engineering, Karlsruhe, Germany*

7 Abstract

8 In the past decades, research was carried out to find cost-efficient alternatives to Protein A chro-

9 matography as a capture step in monoclonal antibody (mAb) purification processes. In this work,

10 polyethylene glycol (PEG) precipitation has shown promising results in the case of mAb yield and

11 purity. Especially with respect to continuous processing, PEG precipitation has many advantages,

12 like low cost of goods, simple setup, easy scalability, and the option to handle perfusion reactors.

13 Nevertheless, replacing Protein A has the disadvantage of renouncing a platform unit operation

14 as well. Furthermore, PEG precipitation is not capable of reducing high molecular weight impurities

15 (HMW) like aggregates or DNA. To overcome these challenges, an integrated process strategy

16 combining PEG precipitation with cation-exchange chromatography (CEX) for purification of a

17 mAb is presented.

18 This work discusses the process strategy as well as the associated fast, easy, and material-

19 saving process development platform. These were implemented through the combination of high-

20 throughput methods with empirical and mechanistic modeling. The strategy allows the development

21 of a common batch process. Additionally, it is feasible to develop a continuous process. In the

22 presented case study, a mAb provided from cell culture fluid (HCCF) was purified. The precipitation

23 and resolubilisation conditions as well as the chromatography method were optimized, and the

24 mutual influence of all steps was investigated. A mAb yield of over 95.0% and a host cell protein

25 (HCP) reduction of over 99.0% could be shown. At the same time, the aggregate level was reduced

26 from 3.12% to 1.20% and the DNA level was reduced by five orders of magnitude. Furthermore,

27 the mAb was concentrated three times to a final concentration of $11.9\,mg/mL$.

28 *Keywords:* Antibody manufacturing, Integrated processing, Protein precipitation, Mechanistic

29 modeling, Periodic counter-current chromatography

*These authors contributed equally to this work.
**Corresponding author
E-mail address: juergen.hubbuch@kit.edu
Telephone: +49 721 608 47526