EVALUATION OF DIFFERENT CLARIFICATION PROCESSES IN MANUFACTURING OF MONOCLONAL ANTIBODIES FROM MAMMALIAN CELL CULTURE HARVESTS

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Introduction. In the manufacturing of biopharmaceutical products the clarification of cell culture harvests is really important as it could have a large effect in global process yield. Therefore the investment of resources and time in the selection and design of the clarification process is essential. The processes that are mainly used in the clarification of cell culture harvests of biopharmaceuticals are: Sedimentation, Centrifugation, Tangential Flow Microfiltration and Depth Filtration. Although this kind of studies is not generally a problem to be considered in small-scale purification laboratory trials, they are very important in the development of manufacturing processes. The purpose of this study was the selection of the better clarification processes for mammalian cell culture harvests in the manufacturing of a selected monoclonal antibody, MAb, by means of experimental laboratory tests.

Methods. Clarification of cell culture harvests can be done by means of several processes. The clarification processes that are typically used are shown in figure 1. These processes were tested in this study, and run for mammalian cell culture harvests directly from the bioreactor. Process yield and product quality were tested in order to know the better clarification process. Process yield was obtained from cell culture volume and content of MAb. Product quality was determined by means of turbidity and purity of the clarified MAb harvest. Different process conditions of centrifugation and different kinds of filtration media were tested. Secondary clarification was tested only for those trials that produced satisfactory results in the primary clarification.

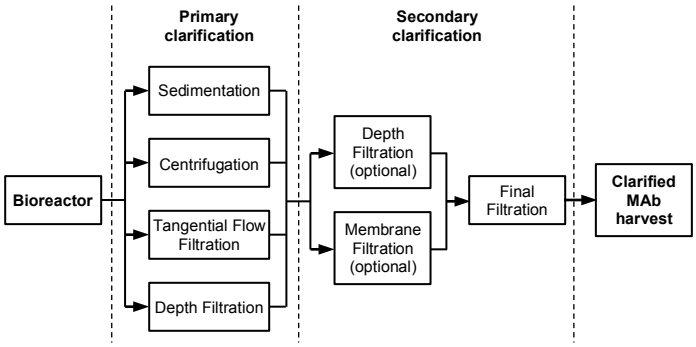

Fig.1 Clarification processes of biopharmaceuticals from mammalian cell culture harvests.

Results. A summary of the most representative results from each evaluated process is shown in table 1. For the MAb in study the primary clarification processes by sedimentation and depth filtration of the cell culture harvests directly from the bioreactor were not adequate because the required filtration area was too high. Primary clarification processes of centrifugation and tangential flow microfiltration were much better, but they needed some further optimization work.

Table 1. Results of clarification processes tested in this study for a selected MAb.

Primary Clarification	Secondary Clarification		Final Filtration	Clarification Level in Primary Clarification	Filtration Area (1)	Conclusion
Sedimentation	Membrane prefiltration		Membrane filtration	Low Turbidity is too high	Too high	Discarded Due to excessive area
Centrifugation	Membrane prefiltration		Membrane filtration	Regular Turbidity is acceptable	Regular	Good clarification process
	Depth filtration (small pore size A)		Membrane filtration	Excellent	Good	Good clarification process
	Depth filtration (small pore size B)		Membrane filtration	Good	Good	Excellent clarification process
	Depth filtration (big pore size A)	Depth filtration (small pore size B)	Membrane filtration	Regular	Good	Good clarification process
Depth filtration (big pore size A)				Regular	Too high	Discarded Due to excessive area
Depth filtration (big pore size B)				Low Turbidity is too high	Too high	Discarded Due to excessive turbidity
Tangential Flow Microfiltration			Membrane filtration	Excellent	Good	Excellent clarification process

Notes: (1) Refers to both secondary clarification and final filtration.
It was not done as it was not needed or the primary clarification was discarded.

Conclusions. For the monoclonal antibody in study sedimentation and depth filtration of cell culture harvest direct from the bioreactor are not recommended as primary clarification processes. Centrifugation, as primary clarification, followed by depth filtration, as secondary clarification, is recommended, as it gave the better results. Secondary clarification by membrane prefiltration is also possible but with higher turbidity results. Tangential flow microfiltration is also recommended as primary clarification, although a large filtration area is needed, filtration cassettes could be used several times, the expected clarification is so good that only final filtration would be needed, with excellent turbidity results.

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