OPTIMIZATION OF MAMMALIAN CELL CULTURE CLARIFICATION PROCESS BY CENTRIFUGATION IN THE MANUFACTURING OF MONOCLONAL ANTIBODIES

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Introduction. Centrifugation is one of the more used processes in the biopharmaceutical industry for primary clarification of mammalian cell cultures. There are other technologies that are also useful like sedimentation, tangential flow microfiltration and depth filtration, but centrifugation is in some cases the best option. That was the case of the clarification of the monoclonal antibody, MAb, evaluated in this study.
The purpose of this study was to find the best operation conditions in the centrifugation process for the primary clarification of a selected MAb. The higher flow and the lower particle level that could be easily clarified in the next step the secondary clarification were required as result of this study. Such problems are not typically considered in the small-scale purification, but are of vital importance in the development of manufacturing processes.

Methods. The optimization of centrifugation was performed in a manufacturing large-scale tubular bowl centrifuge. Process conditions G force (or RPM) and residence time (or flow) were changed following a design of experiments, DOE, of response surface. The response variable in study was the turbidity of the clarified product. Turbidity is measure of the content of particles. The criteria for the selection of the process conditions were (1) "Lower turbidity is better" and (2) "Higher flow is better". As turbidity is a relative measurement of particle content and we tested a manufacturing process the optimization of costs, process times and installation surface was very important. To define the better centrifugation conditions the evaluation of secondary clarification was added to the study. Secondary clarification consisted of depth filtration with some selected filtration media. The characteristics of the centrifuged product had a very important effect in the required filtration area and filter pore size, so another selection criterion was added, (3) "Lower filtration area is better".

Results. Figure 1 shows the centrifugation response surface for the MAb in study. The turbidity was higher at lower residence time (higher flow). As the results were the opposite of the desired, the inclusion of the third criterion was indispensable to a good selection, "lower filtration area is better". Some constant flow filterability tests were run, using some selected filtration media. Results with better filtration media are shown in table 1. When the filtration medium changes, the better centrifugation conditions change. Filtration medium A gave lower turbidity with the three centrifugation conditions that were tested, but a higher filtration area was required. The criteria of turbidity and filtration area were covered with the three selected filter media, but with different centrifugation conditions. As the study is the design of a manufacturing clarification process to choose between the three better conditions the selection should include costs analysis and operative feasibility.

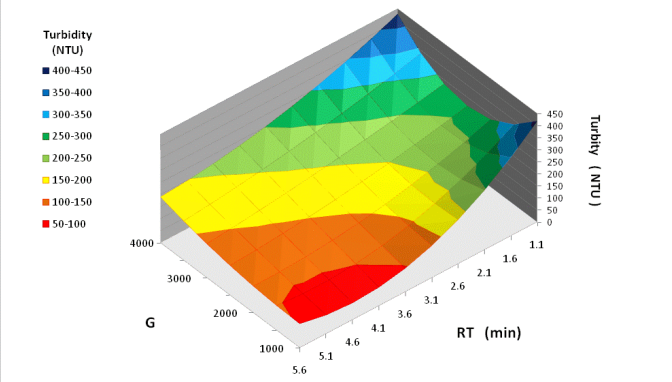

Fig.1 Response surface of turbidity vs. residence time and G force.

Table 1. Summary of secondary clarification results with different selected filtration medias (area for 1 L).

Centrifugation	**G**		**2000**	**2000**	**4000**
	RT (min)		1.39	1.11	1.11
	Turbidity (NTU)		148	375	327
Filtration	**Filter media A**	Area (cm^2)	97	100	76
		Turbidity (NTU)	5.4	5.6	7.3
	Filter media B	Area (cm^2)		68	72
		Turbidity (NTU)		9.6	23.0
	Filter media C	Area (cm^2)	58	48	49
		Turbidity (NTU)	19.7	43.4	97.2

XX Selected condition

Conclusions. With the analysis of response surface it is possible to know the behavior of the primary clarification by centrifugation of mammalians cell culture, but the turbidity is not the only manufacturing parameter that should be considered in the selection of operation conditions, the following process steps must be taken into account, in this case secondary clarification. In this study some different centrifugation conditions can be chosen, that depends on the preferred criteria, turbidity or filtration area. Integration of evaluation of centrifugation and filtration allows a better decision-making.

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References.

1. Yavorsky, D., Blanck, R., Lambalot, C., Brunkow, R. (2003). *PharmTech.* vol. (27): 62-76.
2. Lander, R., Daniels, C., Meacle, F., (2005). *BioProcInternational.* vol. (3): 32-40.