

temperature. All flasks were incubated for 15 minutes at 75-85 rpm. The floc was allowed to settle at ambient temperature.

The flocculated solutions were then transferred into 1 L glass graduated cylinders to determine flocculated packed settling rate. Readings were taken at various intervals for 240 minutes and the relative flocculation volume was calculated as described above.

As shown in FIG. 7, the combination of PDADMAC and PEG 3,000 either added simultaneously or sequentially decreased the flocculent settling time over that of PDADMAC alone.

A large scale culture was next prepared. CHO cells expressing a recombinant monoclonal antibody were grown in an 80 L bioreactor in a perfusion culture for 19 days. Cell culture broth was cooled to 21° C. PDADMAC at a concentration of 45 pg/Total cell density and PEG 3,000 at a final concentration of 15% (w/v) were added simultaneously at ambient temperature, addition rate was 21 minutes, followed by 5 minute incubation at 100 rpm. The floc was allowed to settle at ambient temperature.

Once the flocculent has settled (primary settling), the clarified supernatant was harvested by pumping the fluid from the bioreactor followed by filtration through a depth filter containing diatomaceous earth followed by a 0.2μ cut off membrane filter.

The floc was washed in an equal volume of 9% sucrose solution to remove any residual recombinant protein and allowed to settle for 16 hours. Once floc settled (secondary settling) the clarified secondary supernatant was harvested as described above.

Clarified harvested cell culture supernatants from the above flocculations (small scale and large scale) were purified using Protein A chromatography followed by a product quality determination. The Protein A eluate was not neutralized prior to product quality determination. Protein A eluate product quality attributes measured were molecular variants as measured by SEC, host cell proteins as determined by ELISA.

The Protein A purified material was then passed over a CEX column at pH 7.5.

TABLE 4

Harvest	CEX @ pH 7.5							
	SEC			CHOP (ppm)	Post-			
	HMW	Monomer	LMW		Pre	Main	Shoulder	Post
Control	11.6%	87.2%	1.2%	9,602	8.7%	82.9%	6.8%	1.6%
Small Scale 2 Step Method	9.2%	89.6%	1.2%	3,950	8.2%	83.5%	6.8%	1.6%
Small Scale 1 Step Method	9.0%	89.8%	1.2%	3,602	8.2%	83.5%	6.8%	1.5%
Large Scale Primary Settle	9.6%	89.2%	1.2%	5,056	8.1%	83.5%	7.0%	1.4%
Large Scale Secondary Settle with Sucrose Wash	11.7%	86.9%	1.5%	11,268	8.4%	83.8%	6.4%	1.5%

Product quality is similar between the control and the primary flocculate harvest for both scales. The PDADMAC/PEG primary harvest tends to remove the higher order aggregate which is reflected by the low HMW levels in the Protein A pool. A reduction in the host cell protein level for PDADMAC/PEG harvest was observed. The resuspension with sucrose resulted in slightly higher levels of CHOP and HMW

compared to the primary PDADMAC/PEG harvest and is believed to be the resolubilization of these impurities.

Example 7

This experiment looks at the impact on settling time by adding a surfactant along with PDADMAC and PEG.

CHO cells expressing a recombinant monoclonal antibody were grown in an 80 L bioreactor in a perfusion cell culture for 15 days. Cell broth from day 14 was cooled to 30° C. for testing. Two spin flasks were set up with 1 L of cell culture broth in each flask. To one flask PDADMAC and PEG 3,000 were added simultaneously, PDADMAC was added at a concentration of 25 pg/Total cell density (molecular weight 400,000-500,000) and PEG 3,000 at a final concentration of 3% (w/v). To the other flask Triton X-100 at a final concentration of 0.05% (v/v) was added in addition to PDADMAC and PEG at the above concentrations. (Triton X-100 stock solution was at 10% (v/v) from an original stock solution of 20% (v/v), Sigma Aldrich, St. Louis, Mo.) The three components were added simultaneously. The addition rate was ~1 minute, with incubation for 15 minutes at 75-85 rpm. All flasks were spun as described in earlier examples. The floc was allowed to settle at ambient temperature.

The flocculated solutions were then transferred into 1 L glass graduated cylinders to determine flocculated packed settling rate. Readings were taken at various intervals for 240 minutes and the relative flocculation volume was calculated as described above.

As shown in FIG. 8, the addition of Triton X-100 along with PDADMAC and PEG 3,000, decreased the flocculent settling time over that of PDADMAC and PEG 3,000 alone.

What is claimed is:

1. A mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding a cationic polymer selected from the group consisting of: a polymer of diallyldimethylammonium chloride and a polymer of polydiallyldimethylammonium chlo-

ride, and a non-ionic polymer selected from the group consisting of: polyethyleneglycol and dextran to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, and recovering the primary clarified supernatant.