290d Enveloped and Non-Enveloped Virus Clearance by Flocculation Prior to Microfiltration

Binbing Han and Ranil Wickramasinghe

Validating virus clearance is a major concern in the biotechnology industry. New unit operations are frequently added to the purification train simply to validate virus clearance resulting in reduced product recovery and increased manufacturing costs. There is therefore a great need to validate virus clearance in existing unit operations. Clearance of murine leukemia virus (MLV) and minute virus of mice (MVM) from CHO cell suspensions by flocculation and microfiltration has been investigated here. MLV is a retrovirus while MVM is a parvovirus. These viruses are recommended by the US Food and Drug Administration for validation of retrovirus and parvovirus clearance. MLV is an enveloped virus while MVM is non-enveloped. MLV particles are between 80-130 nm in size while MVM particles are much smaller being 18-24 nm. Feed streams consisted of CHO cells spiked with the viruses. Two cationic flocculants were used to flocculate the feed suspension prior to microfiltration using hollow fibre membranes. The level of virus clearance in the permeate was determined using the TCID50 assay.

MLV clearance was studied using membranes with 0.1 and 0.65 fÝm nominal pore sizes. About 1,000 fold clearance of MLV for 0.1 fÝm pore size membranes is obtained. Virus clearance is due to rejection of the virus particles by the membrane. Addition of the flocculant however leads to an increase in the permeate flux. On the other hand for 0.65 fÝm pore size membranes, little virus clearance in the permeate occurs in the absence of the flocculant. However for flocculated feed streams, about 1,000 fold clearance of MLV is observed. The cationic flocculant aggregates the negatively charged virus particles by forming bridges between the particles.

MVM clearance was studied using 0.1 fÝm pore size membranes. Clearance of MVM in excess of 1,000 fold was obtained in the bulk permeate for flocculated feeds streams. However only about 10-100 fold clearance was obtained for un-flocculated feed streams. Here results for clearance of enveloped and non-enveloped viruses will be compared.