FVIII 1/6 ϕ163

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23rd August, 1983.

Dear Jim,

Many thanks for the note on your filtration developments. I'm not fully up-to-date with the latest in filtration news here, but I will chase it up and let you know.

I can bring you up-to-date with the FVIII work. You might remember that our second pilot-scale run resulted in about half of the FVIIIC, FVIIICAg and total protein being lost. We felt that this could have been due to a problem with concentration polarisation leading to general protein precipitation within the ultrafilter. We have now tested this theory by running the lab. unit (DC2) with a wider fibre bore to try and simulate the velocity profile of the pilot run. In contrast to our earlier lab work we consistently saw a poor performance with about 50% loss (as per the pilot run). To overcome this problem we need a higher velocity inside the fibres and probably a higher ionic strength to increase solubility. We increased the ionic strength in further lab. experiments by using 60 mM NaCl in addition to the tris/citrate. FVIII recovery improved from 50% to 80%, so we seem to be going in the right direction. We are now ready to try again with the DC10 using the higher ionic strength and a higher volume throughput. This has been scheduled for 30th August.

We have also tidied up our citrate/calcium titration profiles and now have a lot of batch-to-batch data. It is fairly consistent and we are now settling for a calcium addition of 2.5 mM/l (to compensate for 20 mM/l citrate).

On August 29/30 we will still heat for 10 hours at 60°C but this will probably complete our work under these conditions. Our time/temper study of vaccinia/FVIII is virtually complete and we have found that FVIIIC survives fairly well for up to 1 hour at 70°C and that 8 logs of vaccinia can be killed in about 30 minutes at 70°C. We have therefore chosen a regime of 9.25 hours at 60°C followed by 0.75 hrs. at 70°C. This will:-

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(i) give at least a 3 log (60°C) + 8 log (70°C) vaccinia kill

(ii) provide 10 hrs. at 60°C or higher; in case there is any virus which has a peculiar time/temper. profile

(iii) give a FVIII yield similar to 10 hrs. at 60°C.

Bruce is still looking at other viruses but has yet to find anything as stable as vaccinia.

Because of the difficulties in scaling-up ultrafiltration we have also begun to look at precipitation as an alternative. We have had very encouraging results from a few small-scale experiments using NaCl salting-out (i.e. 90% recovery of FVIII in the precipitate). This may become very attractive and I intend to set Ida a detailed programme of work before I disappear on holiday (most of September). The only problem would seem to be the higher risk of cross-contamination; what do you think?

Best wishes

Peter R. Foster