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p.c/w 2.167

24 May 1984

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Dear Jim,

Here is a copy of the run sheet for our last FVIII pasteurisation experiment. This should cover most of the points that you have raised.

1. The heparin that we use is Pularin manufactured by Evans Medical and supplied locally by Vestric.
2. We have found polypropylene to be the best material for pasteurisation (ie better than glass and other plastics) in terms of visual clarity of the solution after heating (9.5 hours at 60°C and 0.5 hours at 70°C). There was little visual difference at 60°C but it became marked after heating at 70°C. This phenomenon was also influenced by calcium concentration. We have not tested stainless steel yet but I plan to do this when Ida returns from holiday.
3. The buffer to redissolve the final precipitate was 10mM citrate and 1.25 mM calcium chloride (step 17). However we are still losing activity at the subsequent filtration (step 18) and I want to raise the citrate to 20mM to try and increase the solubility of whatever may be coating the filter. If this is not successful we may also look at alternative filters.

The final buffer also needs to be tidied up, just to get the right osmolality.

We are also still losing about 30% over the ultrafiltration step and we will be looking at the Pelicon cassette in our next experiment.

4. I haven't designed the helical stirrer yet but my inclination is to keep it similar to the existing stirrer but perhaps with a somewhat broader blade. Otherwise the design should be similar to that given in the Vox paper on thawing. The stirrer speed is critical though. If it is too low the sorbitol does not get mixed-in properly but if it is too high, air becomes entrained with the usual consequences (ie 30-40% loss

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of activity). We are using 70 rpm but my advice would be to evaluate each stirrer, setting the speed at the minimum commensurate with reasonable mixing.

5. In our last experiment we managed to improve our recovery of the FVIII precipitate. The feed rate to the Sharples was 40 l/hr with a 1.5mm jet. We recovered 87% of the activity but there was still evidence, from the distribution on the liner, that particles are being carried through. We will probably cut the flow to 35 l/hr next time and hopefully the 3-wing will help to improve this (when it arrives).

We have had a look for the Kabi patent but we have not come across it (our retrieval system is working but there is still a lot of material still to be put into the system). I have enclosed a list of affinity references which you prepared (with Sarah) just before you left PFC.

Best wishes

PETER R. FOSTER