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Dear Jim

Thanks for your report on plasma conditioning. We have also confirmed our previous observations and your findings seem to fit in with our experience precisely.

We have been doing intensive work on freezing and freeze drying over the last 3 months. When we scaled up the Z8 process we came across two problems:

1. Our large production drier (Usifroid SM 600; 1800 FVIII vials) was performing differently to our small production drier (SM 200; 700 FVIII vials) and our pilot drier (SMJR; 50 vials).

2. Variations in final product total protein (because of plasma conditioning, etc) gave major batch-to-batch variations in solubility.

We were also seeing substantial within - batch variation following heating at 80°C and this was particularly marked at higher protein concentrations.

We now believe that we have overcome all of these problems (only time will tell) by means of a special freezing technique and by designing our freeze drying cycle more carefully.

Freezing turned out to be the most critical area. Poor results (solubility) were linked to the presence of a "crystalline" structure after freezing and the degree of crystal formation increased with increasing protein concentration (probably because of the increased heat capacity of the solution - resulting in slower freezing).

We found that an "amorphous" structure was needed and that some form of product supercooling was required to achieve this at the protein concentrations we were dealing with. The "warm-shelf" method of Jennings did not work for us because of the high fibronectin content of the Z8 product (i.e. cold precipitate formed between 0°C - +5°C giving insoluble lumps after drying and heating). After considerable experimentation we have ended up using a 2-stage freezing process which does much the same thing as Jennings "warm-shelf", but minimises the degree of fibronectin precipitation by passing through the 0°C - +5°C region quickly. We pre-cool the shelf to -10°C; load the drier then leave the vials with the shelf controlled at -10°C for 1 hour (the Z8 product supercools to about -6°C within 30 minutes). We then drop the shelf to -50°C 1 hour after loading and proceed as usual (a copy of the drying cycle is enclosed).

We have now prepared 4 batches this way with a perfect amorphous plug each time and no within-batch variation. Results after heating and also looking very good.

We plan to write this up as soon as we have a decent number of production batches under our belt and I will send you a copy of the early manuscript as soon as it is available.

Best wishes.

Yours sincerely

PETER R FOSTER

Enc.