ZINC FRACTIONATION OF CRYOPRECIPITATE

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A more satisfactory factor VIII concentrate, other proteins by zinc, as a potential method for the preparation of
We have therefore investigated the precipitation of fibrinogen and
Reconstitution time.
However, some clinical difficulties may occur, such as fibrinogen
particulary where self-sufficiency is being attempted.
Because of this lower quality products are often preferred,
the manufacture of intermediate-purity concentrates.
VIII concentrates tend to give a lower yield than those used for
The standard methods used for the preparation of high-purity factor

INTRODUCTION
Glycine at std. dilutions.

WII solution was also investigated, using sorbitol and
Pasteurization (60°C, 10 hrs) of the resultant factor

pH and temperature

The addition of heparin and changes in

Time allowed for precipitation

Zinc concentration

Parameters studied included the effect of:

For 10 minutes,
30ml (1/10th plasma) at pH 7.0 and 20°C
1.5, 2.0 and 2.5% CRYPRECIPITATE extracted in 20ml TISS

tesily taken from a routine manufacturing process (1)

Experiments using aliquots of CRYPRECIPITATE extracted
Precipitation was carried out in SMAL-scale (20ml)

METHODS
Fibronectin concentration was measured by the method of Ware (5).

Proase et al. (4).

Method and the reduced method (VIII R:Ag) of Immunoelectrophoresis (3) using the standard Factor VIII Related Antigen (VIII R:Ag) by a 2-stage immunoradiometric assay (2).

Factor VIII clotting Antigen (VIII C:Ag) by a antigen assays were used to measure:

With calibration based on British Plasma Standards.

The 2-stage method, and the 1-stage method, and clotting assays were carried out by:

Factor VIII

The following assay methods were used:

Assays
Zinc concentration (mM/litre)

Fibrogen

Total Protein

FVII C

Mean ± S.D. (N=5) in supernatant (%) protein remaining
quantity of sodium citrate (10-20 mM) to the supernatant.

This behaviour could be prevented by the addition of a small

supernatant.

slight precipitation continued to be thrown down in the

However, after removal of the precipitate by centrifugation, a

change was observed (see opposite). The degree of precipitation was monitored at maximum time but little

Heavy precipitate formed almost immediately.

Added dropwise with gentle stirring at pH 7.0 and 20°C, A

In the experiments above (tables 1-3), zinc acetate (10mM) was

RATE OF PRECIPITATION
These conditions to be attractive.

Resulted in losses of factor VIII which were too high for however the precipitation at lower temperatures (15°C + 10°C).

Achieved by changes in pH (see opposite) and temperature, further increase in precipitation but some effect could be increasing the heparin concentration (up to 10 u/ml) gave no 

Fibronectin.

This second precipitation resulted in the removal of more occur.

After zinc precipitation caused further precipitation to The addition of heparin (1 u/ml) to the supernatant remaining
<table>
<thead>
<tr>
<th>% Fibronectin</th>
<th>% FVIII</th>
<th>% Total Protein</th>
<th>PH</th>
<th>Zinc (mm/l)</th>
<th>Heparin (u/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.7 ± 0.6 (n = 2)</td>
<td>58.4 ± 5.8</td>
<td>6.0 ± 5.6</td>
<td>7.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>52.1 ± 5.1</td>
<td>2.0</td>
<td>6.8</td>
<td>1.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>9.4 ± 6.3</td>
<td>1.1</td>
<td>6.0</td>
<td>1.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>92.6 ± 7.3</td>
<td>2.3</td>
<td>6.6</td>
<td>1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>49.3 ± 11.7</td>
<td>1</td>
<td>6.6</td>
<td>1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>47.3 ± 15.9</td>
<td>1</td>
<td>6.0</td>
<td>1</td>
<td>1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

AT DIFFERENT PH'S

THE EFFECT OF HEPARIN ADDITION
Results from a pilot-scale experiment are shown opposite.

The fractionation conditions:

Solution

By washing of the cryoprecipitate or by dilution of the extract was in the range 2-3 M/l. However, this can be adjusted either residual citrate content of the cryoprecipitate extract used here. Citrate has a marked effect on the degree of precipitation. The be precipitated with little loss of Factor VIII.

Laboratory experiments

Comment on results from
<table>
<thead>
<tr>
<th>Stage</th>
<th>% Recovery in Supernatant</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>47.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8.3 Iltres</td>
<td>0.75 kg</td>
</tr>
<tr>
<td>6</td>
<td>8.0 Iltres</td>
<td>1.64 kg</td>
</tr>
<tr>
<td>7</td>
<td>1.5 Iltres</td>
<td>151.5 kg</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Zinc Precipitation at Pilot-Scale**
Factor VIII recoveries after heating for 10 hours at 60°C are tabulated opposite.

Sorbitol, 1850 g/litre FVIII solution
glycine, 50 g/litre FVIII solution

Fractionation (7)
selected for use with a factor VIII solution prepared by zinc adequate protein stabilization. The following conditions were mixtures of sorbitol and glycine have been studied to provide to reduce the risk of viral contamination.

Heat treatment of coagulation factor concentrates is being developed

PASTEURIZATION OF FACTOR VIII
## Mean FVIII C Recovery = 79%

<table>
<thead>
<tr>
<th>Volume of Solution (liters)</th>
<th>FVIII C after Heating (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>1.70</td>
</tr>
<tr>
<td>2.2</td>
<td>2.21</td>
</tr>
<tr>
<td>1.3</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Experiment No. 1

## Mean FVIII C Recovery = 72%

<table>
<thead>
<tr>
<th>Stage</th>
<th>FVIII (IU/mL), Mean &amp; S.D., N = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>After Heating (10 hrs, 60°C)</td>
<td>1.76 ± 0.25</td>
</tr>
<tr>
<td>Before Heating</td>
<td>2.47 ± 0.82</td>
</tr>
</tbody>
</table>

2. Pilot-Scale Experiments

Laboratory Experiments

FVIII C Recovery after Pasteurization
Add sorbitol slowly with gentle mixing and heating. Heating to 60°C. Hold pH above 7.0 then adjust to 7.5 before maintaining CaCl₂ constant by adding CaCl₂.

 Dilute or wash so that residual citrate < 3 mM/L.

COMMENT

STAGE

should be noted:

An outline of the process is shown opposite. The following points (see poster 2.11):

Control of lonsed calcium concentration

Heat treatment using sorbitol and glycine

Zinc Fractionation or Croprecipitate

Based on:
The design of a full-scale process is now being carried out. This is

PROCESS DEVELOPMENT
Outline of a possible process:

1. Fresh Frozen Plasma (4°C)
2. Fresh Frozen Plasma (-10°C)
3. Crush & Thaw Plasma (2°C)
4. Recover Cryoprecipitate
5. Tris
6. HCl
7. Zn Acetate/Heappalin
8. Supernatant
9. Citrate/Calcium

Centrifuge

(10 mins, 20°C)

Zinc Precipitation

Adjust pH to 6.7

Extract Cryoprecipitate

Recover Cryoprecipitate

Solids

Supernatant
FREEZE DRYING

FILTRATION (0.2 µm)

(DIFFILTRATION)
ULTRAFILTER
(CONCENTRATION)
ULTRAFILTER

DILUTION
(60°C, 10 hrs)
HEAT

ADD 2ND STABILIZER

ADD STABILIZER

SUEPFERIANT

PROCESS (continued)

18
17
16
15
14
13
12
12
11
10

BUFFER SOLUTION

BUFFER SOLUTION

Sorbitol

HCl

Glycine

STAGE REAGENT
Appropriate stabilizers can be pasteurized (60°C, 10 hrs) in the presence of Factor VIII solution produced by Zinc Fractionation.

Fibronection and Fibrinogen.

The precipitate removed may be a convenient source of fibrinogen.

Loss in yield.

The quantity of Factor VIII from cryoprecipitate with little Zinc Fractionation may provide a simple technique for improving

CONCLUSIONS
REFERENCES
Acknowledgements

References (continued)