SOLVENT-DETERGENT TREATMENT OF IgM-ENRICHED IMMUNOGLOBULIN


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ABSTRACT
Viral safety of human plasma products plays a key role in their safe uses. Solvent-detergent (SD) virus inactivation method has gained widespread popularity in the manufacture of biological products. This treatment which inactivates lipid-enveloped viruses effectively consists of incubation of a plasma protein solution in the presence of a non-volatile organic solvent and a detergent. In this study, IgM-enriched immunoglobulin was incubated at 24 °C for 6 h under slow stirring in the presence of tri(n-butyl) phosphate (0.3% w/w) as solvent and tween 80 (1% w/w) as detergent. After completion of the inactivation process and removal of the solvent-detergent, the ability of SD-treatment to remove Infectious Bovine Rhinotracheitis (IBR) virus (a lipid-enveloped virus) and Foot-and-Mouth Disease virus (a non-enveloped virus) were evaluated by "virus spiking studies" using a scaled down process. Reduction factor of 4 log was obtained for the SD-treatment of IgM-enriched immunoglobulin spiked with IBR virus. No virus inactivation was observed in the SD-treated IgM-enriched immunoglobulin, spiked with Foot-and-Mouth Disease virus. It was concluded that treatment of IgM-enriched immunoglobulin with TNBP-TWEEN 80 may be considered as an efficient lipid-enveloped virus inactivation step in the manufacture of this product.

Key words: Solvent-detergent, Virus inactivation, IgM, Tween 80, Bovine rhinotracheitis virus

INTRODUCTION
Plasma derivatives which comprise an increasing variety of therapeutic products such as polyvalent immunoglobulins are obtained by pooling plasma of donors. Hence a manufacturing approach that comprises specific measures to ensure very high margin of viral safety is required (1). In other words, viral safety of plasma products plays a key role in their safe uses (2).

Fractionation of plasma by ethanol precipitation (3) and protein purification by chromatography (4), at least in part, contribute to the partitioning of viruses and are important tools in optimizing plasma product viral safety(5). However, manufacturers no longer depend on Cohn-Oncley cold ethanol fractionation as the only viral elimination step (6). They have implemented rigorous donor screening plasma testing, good manufacturing practices and additional viral inactivation and validation steps (7) in the manufacturing processes in order to provide viral safety of the plasma products (1,5,8).

The most commonly used viral inactivation or elimination treatments are solvent-detergent incubation (9), pasteurization (10), nanofiltration (11), specific dry heat and steam heat treatments, acid pH incubation, combination of beta propiolactone and UV irradiation (8) and photochemical inactivation (1). Since the introduction of modern viral inactivation treatments by solvent-detergent or pasteurization in industry, risks of transmission of the highly pathogenic plasma-borne lipid-enveloped viruses (HIV, HBV and HCV) by plasma products have essentially been eliminated (12).

The solvent-detergent virus inactivation method has gained widespread popularity in the manufacture of biological products. This popularity arises from several advantages of the treatment such as: its high virucidal action, high protein compatibility, ease of insertion into virtually any pre-existing or newly developed purification process, and the availability of extensive documentation (13). Solvent-detergent treatment consists of incubation of a plasma protein solution in the presence of a non-volatile organic solvent such as tri-(n-butyl) phosphate (TNBP) at a concentration of 0.3% (w/w) and a detergent such as tween 80 at
a concentration of 1% (w/w) (10). This treatment which leads to an efficient viral inactivation by disruption of the structural integrity of lipid-enveloped viruses (14) without alteration in recovery or physiological functions or increase in immunogenicity of plasma proteins (14-16), is generally performed at temperatures between 24 °C and 30 °C over a maximum of 6 hours under mild stirring (13,17). SD treatment of plasma or immunoglobulin solution followed by virucidal reagents removal by oil extraction and column chromatography, has a proven safety record for Factor VIII products (13) and intravenous immunoglobulins (16). However in most cases SD method is combined with pasteurization at 60°C which ensures inactivation of a wide range of the SD-resistant viruses without lipid envelopes (18). Dichtelmuller et al used beta-propiolactone treatment for inactivation of viruses in IgM-enriched immunoglobulin (8). In this study Solvent-detergent treatment and pasteurization were used for inactivation of enveloped and non-enveloped viruses in IgM-enriched immunoglobulin. The primary target in the fractionation process of each plasma derivatives is inactivation of highly pathogenic plasma-borne lipid-enveloped viruses. An appropriate level of safety against these viruses can be achieved by introduction of techniques involving the use of solvent-detergent treatment and pasteurization (18).

MATERIALS AND METHODS
IgM-enriched immunoglobulin was produced by cold ethanol fractionation followed by octanoic acid treatment (8). Schematic representation of the manufacturing processes is shown in Fig.1. Reagents:
Tri-(n-butyl)phosphate, tween 80, n-hexan, perchloric acid and heptanoic acid were obtained from Merck. Ricinus oil was obtained from Pharm. Raffiniert. Sephadex DEAE A-50 was obtained from Pharmacia Biotech.
Viruses:
Infectious Bovine Rhinotracheitis (IBR) virus, a member of Herpesviridae family, as a lipid-enveloped DNA virus and Foot-and-Mouth Disease Virus (a member of Aphthovirus generation and Picornaviridae family), as a non-enveloped RNA virus, were used for validation of the extent of virus removal during processing. Solvent-detergent treatment
The mixture of IgM-enriched immunoglobulin, tri-(n-butyl)phosphate (0.3% w/w ) and tween 80 (1% w/w) was kept at room temperature (24±2°C) for 6 hours under slow stirring. After completion of the inactivation process, Ricinus oil (5% v/v) was added gradually under moderate stirring during 45 min. Then the stirring was stopped and the mixture was allowed to stand for 30 min in order to assure a complete separation of the oil and plasma layers and removal of tri-(n-butyl) phosphate. The plasma layer was withdrawn and cleared using 0.45 μ m filter (15). Thereafter, the clear IgM-enriched immunoglobulin was passed over a sephadex DEAE A-50 column for the removal of tween 80.
Validation of virus inactivation
The ability of solvent-detergent treatment to remove relevant viruses was evaluated by “virus spiking studies” using a scaled down process. IBR virus and Foot-and-Mouth Disease Virus of high titer were added to the IgM-enriched immunoglobulin separately and the manufacturing process was carried out on a laboratory scale. Then ten-fold serial dilutions of samples were made in culture medium and the infectivity titres of cytopathic effects (CPE) inducing viruses were calculated according to Spearman Kaerber (8), which provides the preferred detection system for infectivity determination and virus quantification (8,19).
RESULTS AND DISCUSSION

Table 1 shows results of the process validation studies. In this table, reduction factors (RI) expressed in logarithmic scale which were determined by subtracting the log of the total virus found at the end of the process from which was present initially (9,19), and calculated according to the following equation (20):

\[10^{RI} = \frac{(V \times 10^a)}{(V \times 10^b)}\]

In which, \( V \) = Volume of starting material, \( 10^a = \) virus titre in starting material, \( V = \) Volume of final material, and \( 10^b = \) virus titre in final material.

The level of virus reduction which is considered acceptable for a single step is \( \geq 4 \) log (19). Achievement of a reduction factor of 4 for IBR virus by solvent-detergent treatment of IgM-enriched immunoglobulin, proved that the method which was used in this study may be considered as an efficient virus inactivation step in manufacturing of this product. TNBPTween 80 treatment of IgM-enriched immunoglobulin inactivates lipid-enveloped viruses but allows retention of the biologic activity of the proteins highly (15,16). Time-dependent inactivation of IBR virus by tri-(n-butyl) phosphate (0.3% w/w)-tween 80 (1% w/w) treatment is shown in fig.2. The expression of reduction factors (RI) as logarithmic reductions in titre imply that residual virus infectivity may be greatly reduced, but it will never be reduced to zero (20).

Table 1. Inactivation of IBR virus in IgM-enriched immunoglobulin by solvent-detergent treatment (TNBP-Tween 80)

<table>
<thead>
<tr>
<th>Treatment Duration (min)</th>
<th>Virus Titre*</th>
<th>Reduction Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(10^5)</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>(10^2)</td>
<td>3</td>
</tr>
<tr>
<td>240</td>
<td>(10^1)</td>
<td>4</td>
</tr>
<tr>
<td>360</td>
<td>(10^1)</td>
<td>4</td>
</tr>
<tr>
<td>480</td>
<td>(10^1)</td>
<td>4</td>
</tr>
</tbody>
</table>

* These values are means of three tests in each step.

Although ethanol contributes to elimination of viruses both by partitioning and a direct virucidal effect (14), it does not appear to be sufficient to ensure the virus safety of IgM-enriched immunoglobulin. However, by incorporation of the additional viral security methods such as solvent-detergent treatment, pasteurization and octanoic acid treatment, this product would appear to be safe and efficacious.
The use of almost any virus, animal or otherwise, can provide useful information in "virus spiking studies". Animal viruses can be selected because of the relevant virus properties (19), e.g. presence or absence of an envelope, size and physico-chemical properties. In this study, the process validation studies utilizing spiking experiments with IBR virus and Foot-and-Mouth Disease virus were performed to give a quantitative estimate virus inactivation's degree by Solvent-detergent treatment of IgM-enriched immunoglobulin.

IgM-enriched immunoglobulin, spiked with Foot-and-Mouth Disease virus, was treated with tri-(n-butyl)phosphate (0.3% w/w) -tween 80 (1% w/w) and as it was expected, no virus inactivation was observed. Viral validation studies have demonstrated that the processes differ in their capabilities to inactivate lipid and non lipid-enveloped viruses (6). Besides, the International Association of Biological Standardization (IABS) recommends that at least two independent steps for virus inactivation are to be carried out. Therefore, the solvent-detergent method which leads to efficient inactivation of lipid-enveloped viruses need to be combined with pasteurization to ensure inactivation of a wide range of SD-resistant viruses without lipid-envelopes (18).

REFERENCES


