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Virus inactivation in albumin by a combination of alkali conditions and high temperature

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ABSTRACT

Non-enveloped viruses such as HAV and B19 are of potential concern in plasma products. In the case of albumin, pasteurisation at 60 °C for 10 h is generally used for virus inactivation. However this procedure is only partially effective against some non-enveloped viruses. Using a range of non-enveloped viruses i.e. HAV, SV40, CPV, treatment at a high pH of about 9.5 and a temperature of 60 °C for 10 h was found to be effective for virus inactivation. These extreme conditions caused no increase in aggregate composition of the albumin. In addition the albumin composition was stable over a period of at least 6 months. The ligand binding properties of the albumin, as determined using the dye phenol red, were also not affected by this treatment. This procedure has the potential for increasing the spectrum of viruses inactivated by the 60 °C pasteurisation step.

1. Introduction

The incorporation of specific virus inactivation e.g. solvent-detergent or heat-treatment, or removal steps e.g. virus filtration, which are each able to significantly reduce virus levels have greatly contributed to the virus safety of plasma derived plasma products in general [1–4]. However, non-enveloped viruses such as hepatitis A (HAV) [5] and human parvovirus B19 (B19V) [6,7] still pose a potential threat to plasma products. This is a result of the known properties of these viruses [8] i.e. their small size and relative resistance which makes them difficult to remove or inactivate by conventional plasma fractionation and virus reduction procedures. The recent incorporation of PCR testing [9] for the range of viruses of potential concern in plasma, including HAV and B19V, has further enhanced the safety of products with regard to these viruses. However, it still remains preferable to include a specific virus reduction step that is able to cope with these more refractory viruses. This is particularly true in the case of B19V which may exist at particularly high levels of up to about 10^{12} genome equivalents/ml in human plasma [6]. In the case of coagulation factors and immunoglobulins, it has generally proved necessary to include up to 2 virus reduction steps in order to obtain adequate levels of virus reduction for both enveloped and non-enveloped viruses [3,10,11].

In the case of human albumin, pasteurisation at 60 °C for 10 h is included as a dedicated virus inactivation step [12,13]. This product has a very long history of safe use and this specific step, in combination with virus reduction brought about by the ethanol fractionation process itself, has generally been considered sufficient to assure the virus safety of this particular product [3]. However the pasteurisation step itself is likely to be less effective against non-enveloped viruses such as HAV and parvoviruses including B19, given the general properties of these viruses [8]. Indeed HAV [14–17] and the model parvoviruses generally used [17,18], have been shown to be relatively resistant to pasteurisation in plasma products. However, while there have been possible cases of HAV transmission by cellular blood products and coagulation factors, there have been no reports of the transmission of this virus by albumin. Thus it might be considered necessary to increase this level of inactivation in the future to bring this product into line with the safety expectations for other plasma products such as coagulation factors [3], and immunoglobulins [3,11]. While the addition of a second specific virus reduction step to albumin is one possibility that can be considered, a simpler approach might be to try to increase the effectiveness of the well established pasteurisation step for the inactivation of non-enveloped viruses.

Many non-enveloped viruses are relatively resistant to conditions of low pH [8]. This is true of HAV, which normally infects humans by the oral route and is resistant to conditions of low pH. Also parvoviruses are known to be generally resistant to a wide range of factors including low pH. However, this resistance may not

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Table 1
Virus inactivation in albumin treated at high pH and 37 °C.

Virus	pH	Exp No	Virus Titre/Inactivation (log/ml)[a]						
			Initial	2 min	10 min	1 h	2 h	5 h	24 h
HAV	9.4	1	4.2	4.4/0[b]	4.2/0.0	3.9/0.3	3.6/0.6	3.5/0.7	nd
		2	5.0	nd	nd	4.4/0.6	4.6/0.4	4.4/0.6	3.1/1.9
	10.0	1	4.5	4.4/0.1	4.6/0	4.0/0.5	3.9/0.6	3.4/1.1	nd
		2	4.8	nd	nd	4.4/0.4	4.3/0.5	4.0/0.8	1.3/3.5
BPV	9.4	1	6.1	5.9/0.2	5.7/0.4	5.3/0.8	5.0/1.1	3.5/2.6	nd
		2	6.2	nd	nd	4.5/1.7	4.0/2.2	2.0/4.2	<-0.1/>6.3
	10.1	1	6.0	5.5/0.5	4.7/1.3	3.2/2.8	1.8/4.2	<-0.1/>6.1	nd
		2	6.2	nd	nd	2.4/3.8	0.9/5.3	<-0.1/>6.3	<-0.1/>6.3

[a] Albumin (10%) was used. Inactivation relative to virus spiked albumin at pH 7.0 and 20 °C sampled at zero time.
[b] Virus titres followed by inactivation values are given.

necessarily be true for high pH conditions. Indeed, alkali treatment commonly forms the basis of the cleaning regimes used for decontaminating and sanitizing equipment and chromatographic media in the biopharmaceutical industry [19]. With this in mind the possibility of using high pH as an inactivation step with these viruses has been tested. In addition, in an attempt to increase the level of virus inactivation, the process was performed in combination with a high temperature (i.e. standard pasteurisation conditions of 60 °C for 10 h). The possibility that the severe conditions evaluated might have a detrimental affect on the albumin itself, has also been considered. Thus the aggregation state and stability of the product on storage has been tested. In addition the ligand binding properties of the albumin have been tested by dye-binding studies.

2. Materials and methods

2.1. Product

Human albumin (Zenalb®) manufactured from fraction V by the Kistler & Nitschmann process [20], was supplied by the BPL Production Department. Various concentrations of between 4.5 and 20% were used after the addition of sodium octanoate at 23 g/kg of albumin, unless otherwise noted. The formulated albumin was adjusted to various pH values using 1M sodium hydroxide where necessary.

2.2. Virus

The following viruses were used: Simian virus 40 (SV40; strain A2875), hepatitis A (HAV; Strain HM175A2), canine parvovirus (CPV; strain VR953) and bovine parvovirus (BPV: strain Haden). SV40 was propagated in Vero cells maintained in 199 medium supplemented with 1% foetal-calf serum (FCS) and 4% newborn-calf serum. HAV was propagated in BSC-1 cells maintained in minimal essential medium (MEM) supplemented with 5% FCS. CPV was propagated in A72 cells maintained in Dulbecco's Modified Eagles medium supplemented with 5% heat inactivated foetal-calf serum. BPV was propagated in MDBK maintained in MEM supplemented with 5% FCS.

2.3. Virus inactivation

Samples of albumin (ca 20 ml) were adjusted to various alkali pH values with sodium hydroxide and heated to a temperature of 60.0 ± 0.5 °C in a water bath. The heated albumin was then spiked with virus, at a dilution of 1 in 20 or greater, and samples taken after various time periods of up to 3 days depending on the experiment. The samples were then diluted 1 in 10 in cell culture medium, previously adjusted with 1M HCl in order that the resulting sample had a neutral pH. Controls consisting of virus spiked albumin and cell culture medium, at neutral pH, were also included.

2.4. Virus infectivity assays

Virus infectivity was determined by plaque assay on confluent monolayers of BSC-1 (HAV) or Vero (SV40) cells. In the case of BPV and CPV, 1/4 confluent MDBK or A72 cells were used respectively. Serial 10-fold dilutions were prepared and aliquots of 0.5 ml assayed on cell monolayers in 3.8 cm^2 tissue culture wells. The most concentrated samples assayed i.e. a 1 in 10 dilution was confirmed to have no cytotoxic effect on the cells and not to interfere with the virus infectivity assay. In order to increase the dynamic range and sensitivity of the assay, volumes of 7 ml were assayed on cells in 3–5 Petri dishes (58 cm^2). An overlay of 0.4% carboxymethylcellulose was applied and the plates incubated for 2 days, or 8 days in the case of HAV. After incubation, plates were fixed and stained with napthol blue-black. Virus titres in pfu/ml were calculated from the sample dilution, assay volume and plaque count. Where virus was undetectable, the titre was calculated assuming one plaque was present in the total volume assayed and the titre expressed as a 'less than' value. Inactivation values were calculated by subtracting the $\log_{10}$ virus titre after treatment from that determined before treatment. Where virus was undetectable after treatment, inactivation is given as a 'greater than' value.

2.5. Effect of high pH treatment on the albumin

For these studies, unless otherwise stated, samples treated at high pH and high temperature were adjusted back to pH 7 with 1M HCl and subjected to a standard terminal pasteurisation cycle of 60 °C for 10 h before analysis.

2.5.1. Composition

Samples of albumin, were adjusted to various pH values and treated at 60 °C for 10 h. Samples were then analysed directly by FPLC size-exclusion chromatography, using Superose 12 (GE Healthcare), and the proportions of monomer, dimer and aggregate determined from the peak areas.

Samples were also analysed by electrophoresis on a 1% agarose gel. In this case the treated samples were returned to neutral pH with 1M HCl and subjected to a standard terminal pasteurisation cycle of 10 h at 60 °C before analysis.

2.5.2. Stability

The effect of storage on the composition of the alkali treated albumin was also tested. Treated samples were held at 37 °C for either up to 27 h or up to 26 weeks in order to give some indication of the stability of the treated albumin over various time periods. The proportion of albumin in the form of monomer and dimer, was then determined by HPLC size-exclusion chromatography using TSK3000 media.