Introduction

Japanese Encephalitis (JE) is a disease primarily dominant in South East Asia, caused by the Japanese Encephalitis virus (JEV, a Flavivirus, Figure 1). It is responsible for an estimated 50,000 cases of the disease every year, of which around 10,000 are fatal and approximately 15,000 result in long term neurological sequelae (WHO 2006).

Viral inactivation is a principle feature in many vaccine manufacturing processes in order not to inactivate the product itself but also any potential adventitious agents which may have been introduced during manufacture. Inactivation using formaldehyde is one of the most common methods used for inactivated viral vaccines, yet due to the relative unpredictable nature of the protein cross linking method by which inactivation occurs, antigen recovery rates -0.04% vary significantly (Franek-Conrat & Mecham 1949, Metz et al. 2003).

This poster seeks to use a design of experiments based methodology to investigate viral inactivation step of JEV. The current manufacturing conditions are non-optimal in terms of inactivation time and antigen recovery. Several key variables of the formaldehyde inactivation of JEV have been identified from the literature for investigation: temperature, pH, formaldehyde concentration and the presence of stabilisers (glycerol, sorbitol, lysine, glycine and PEG).

As a factor screening experiment, a two level factorial design will be presented involving 36 experimental runs including 4 centre points. The measured effect will be antigen recovery measured by ELISA specific to the vaccine. The data will determine which of the factors are the most significant and which factors contribute to interaction effects with regards to optimal inactivation of JEV for use in a vaccine.

Experimental Design

<table>
<thead>
<tr>
<th>Factor</th>
<th>-1</th>
<th>0</th>
<th>+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde concentration (%)</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22</td>
<td>28.5</td>
<td>35</td>
</tr>
<tr>
<td>Glycerol (%)</td>
<td>0.1</td>
<td>0.55</td>
<td>1</td>
</tr>
<tr>
<td>Sorbitol (%)</td>
<td>0.1</td>
<td>0.55</td>
<td>1</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>0.05</td>
<td>0.275</td>
<td>0.5</td>
</tr>
<tr>
<td>Glycine (%)</td>
<td>0.05</td>
<td>0.275</td>
<td>0.5</td>
</tr>
<tr>
<td>PEG (%)</td>
<td>0.1</td>
<td>0.55</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1 – Inactivation factors investigated with the high (+1), low (-1) and mid-point (0) ranges of the design for temperature, pH and final concentrations of the components added. The 32 sets of conditions will be a combination of -1 and +1 of each factor.

Results

• Formaldehyde concentration was found to have the most significant impact on antigen recovery, followed, in order of relative significance, by the interaction of pH and formaldehyde concentration, glycine concentration, and sorbitol concentration, as illustrated in Figure 2.

• Using higher formaldehyde concentrations alone would harm the vaccine quality, requiring more virus total virus per dose. It is also suggested that glycine, and to a lesser extent sorbitol, could potentially increase vaccine quality.

• 2-level factorial design with 8 factors, as described using Design Expert. 36 experimental runs conducted, including 4 mid-points and 32 different sets of inactivation conditions, run for 96 hours. 

• Antigen recovery based on ELISA was the measured response.

• Experiments performed in deep well plates with working volume of 3 ml of purified JEV.

Figure 1 – Image representing the structure of a typical Flavivirus.

• It is possible that these stabilisers protect the antigen somewhat from the damaging effect of formaldehyde. It has previously been stated that glycine bonds with peptide sites which may normally form inter- and intra- molecular bonds in the presence of formaldehyde (Metz et al. 2003).

• The software used the results to generate a model for the inactivation of JEV within the range of variables investigated. This was used to produce a 3-D surface representing the interaction between pH and formaldehyde concentration. As can be seen in Figure 3, in order to mitigate the negative effects of higher formaldehyde concentrations on antigen recovery rates the inactivation should be performed at a higher pH. This figure also illustrates the effect of using the two most significant stabilisers investigated, glycine and sorbitol, at higher concentrations, predicting optimal antigen recovery within the system investigated.

Figure 2 – Plot illustrating relative significance of each factor investigated. The further a factor is away from the line, the more significant the factor.

Figure 3 – Modelled 3-D plot of the interaction of pH and formaldehyde concentration at higher concentrations of glycine and sorbitol.

• The model is considered statistically significant, as F = 45.972, > 0.0001, and therefore useful for prediction, as illustrated by Figure 4, which plots predicted versus actual results using the equation:

$$\log_{10}(y) = 6.17 - 290.43A - 0.65B + 1.02D + 0.16F + 0.41AB$$

Where y = antigen recovery, A = formaldehyde concentration, B = pH, D = glycine concentration and F = sorbitol concentration.

Figure 4 – Plot of predicted vs. actual data. The closer the points to the line, the better the model is at predicting results.

Conclusions & optimisation potential

• Using an increase in pH to mitigate against antigen loss at higher formaldehyde concentrations has implications for inactivation times as more rapid inactivation of JEV particles can be achieved (Darwish et al. 1966). Furthermore, temperature can also be used to decrease inactivation times as it is shown to have little effect on antigen integrity.

• Figure 5 illustrates the potential improvement over the starting conditions certain modifications may have, including the use of stabilisers, which could have used elsewhere in a process.

• The potential for optimisation would include investigations into the effect on other responses, such as product aggregation, inactivation kinetics and stability. This, in conjunction with increasing the observed ranges of significant factors, would allow for the optimisation of the method of operation for the given set of factors.

Figure 5 – Potential increase in antigen recovery over starting conditions.

References


