THERMOSTABILITY OF VACCINE

As mentioned in previous chapters, the measles virus has two important envelope proteins, namely the Fusion (F) and the Haemagglutinin (H), which govern the fusion of virus to susceptible cells and its subsequent entry into the susceptible cells. Since this vaccine is a live virus preparation, the intact tertiary structure of these proteins is of great importance in keeping the quality of vaccine to the desired level of infectivity. It is, therefore, important to find out the destabilizing factors which cause the irreversible damages to the viral envelope protein rendering it sensitive to physico-chemical actions. The major importance is given to thermostability of the vaccine. The physico-chemical factors like concentration of various ingredients, rate of freezing during the process of lyophilization, pH, ionic strength etc. could be controlled in the laboratory before the final product reaches to the consumer. However, thermal shocks to the vaccine, after it leaves the manufacturing premises, are many times beyond manufacturer’s control. Such thermal shocks could be because of poor cold chain, bad storage conditions at hospitals and health centres etc. Although attempts have been made to monitor the cold chain maintenance upto the end users of this vaccine, it is extremely difficult to control thousands of health centres. This has led to the development of a thermostable vaccine.

The tertiary and quaternary structure of the protein is a folded structure and is an association of subunits. The forces which govern this structure are hydrogen bonds, ionic and hydrophobic interactions and Van der Wall's forces.

The denaturation (i.e. a loss of tertiary structure) of the protein is mainly because of chemical, enzymatic, microbial and physical reactions and this results in the loss of its activity. The stability of the proteins depends on the physical
forces applied for its preservation like, temperature, freezing pattern, moisture content, aggregation etc.

It is known that some additives significantly improve the thermostability of the proteins. The role of various reagents used so far for the stabilization of various protein preparations is as follows -

1) AMINO ACIDS -

a) Chelating agents - Glutamine and aspartic acid are known to protect insulin against aggregation. The mechanism is believed to involve chelating with zinc.

b) Aggregation agent - Glycine with albumin has been added to human gamma globulin to inhibit aggregation. Arginine and lysine have also been reported to block aggregation.

c) Competitive adsorbents - Some acidic amino acids are known to have this property.

d) Thermostabilizers - Addition of neutral amino acids like glycine or alanine with sugars are known to stabilize factor VIII preparation.

2) LIPIDS -

Some lipids like acetyltryptophosphate are also used to stabilize proteins against thermal degradation. There have been some reports of phosphatidylcholine and ethanolamine used for stabilizing proteins (e.g. factor VIII).

3) POLYOLS -

Sorbitol, mannitol, glycerol, maltose, lactose are some examples of polyols used to stabilize proteins. These substances probably act as bulking agents, cryoprotectants.
When used in lyophilized products, they prevent oxidation and strengthen the conformation.

4] REDUCING AGENTS -

Many thiols can inhibit formation of disulphide and thus protect against aggregation.

5] PROTEIN HYDROLYSATES -

These substances (e.g. gelatin hydrolysate, egg albumin hydrolysate, etc.) are known to protect many viruses. These agents probably act by protecting the protein while freezing and also by imparting important amino acids.
EXPERIMENT-I

To study the effect of time of harvesting on thermostability of measles vaccine.

It has been previously noted that in some cases the thermostability of measles virus is affected by the time of harvesting of the virus. In other words, since the cytopathic effect of infected cells progresses with respect to the time of infection, the stability of multiple harvests is a function of cytopathic effect in cells. To know whether such thing exists in case of MRC-5 cells and measles virus (Edmonston-Zagreb) strain, following experiments were performed.

Materials and Methods -

MEM - Hank's, Gibco Lab.
FBS - Gibco Lab.
Trypsin - 1:250, Difco.
Tissue culture bottles - Nunc, 175 cm² surface area.
Cells used - MRC-5, Human Diploid cells.
Virus used - Edmonston-Zagreb Measles Vaccine Virus.

Ten culture bottles of MRC-5 cells, PDL-28 were infected with measles virus working seed as per the procedure described earlier. After 40 hours of incubation at 37°C these culture bottles were washed twice with MEM without FBS. Then onwards, the incubation of these bottles was continued at 32°C. Multiple harvests were then taken at proper intervals. The harvest collected at each time was stabilized using partially hydrolyzed gelatin (2.5% final concentration) and sorbitol (5% final concentration) and samples were taken out. The harvests
along with individual samples were kept frozen at -70°C. The virus concentration assay of all the samples was carried out using Vero cells as per the procedure described earlier.

The harvests taken at different intervals were dispensed separately in 5 ml glass vials (USP type-1) with a volume of 0.5 ml/vial. These vials were then lyophilized starting from -50°C. After lyophilization, the vials were sealed with aluminum caps. Now that the harvests taken at different intervals were available as lyophilized vaccine, the study on thermostability of these vials was undertaken. These lyophilized vials were titrated for virus content on Vero cells to know the initial titre. Then, about 100 vials from each lot of lyophilized harvests were kept at 37°C for 28 days. After day-7, day-14, day-21 and day-28, 5 out 100 vials of each lot were taken out and titrated on Vero cells to know the residual virus content. The results of the stability of individual harvests were calculated on the basis of the difference between starting titre in the vial and the titre remaining in the vial vis-à-vis the time of incubation at 37°C. A graph was plotted with Difference in potencies before heating (i.e. at +4°C) and after heating at 37°C for 7 days on X axis versus Harvest No. on Y axis (Graph-IV).

Results -

The results of the test are tabulated in Table-XX. It appears from the graph (Graph-IV) that, the first two harvests, i.e. H-1 and H-2, are less stable than those of H-3 and H-4. It was thought that the reason of such difference could be because the progression of cytopathic effect was less at the time of harvesting H-1 and H-2. However, this logic does not hold if we compare H-3 and H-4 with H-5 and H-6. To ascertain this, similar type of experiment was conducted once again and the results of the experiment are given in Table-XXI.

This time also the data could not be brought to any conclusion. It can, therefore, concluded that the effect of extent of cytopathic effect on the stability of measles vaccine (at least in
the present system i.e. MRC-5 cells and Edmonston-Zagreb measles virus) does not seem to have any correlation.
Table - XX

EFFECT OF HARVESTING SEQUENCE ON STABILITY OF MEASLES VACCINE

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Harvest No.</th>
<th>Virus titre at +4°C</th>
<th>Virus titre after 7 Days at 37°C</th>
<th>Difference (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-1</td>
<td>4.575</td>
<td>3.731</td>
<td>0.844</td>
</tr>
<tr>
<td>2</td>
<td>H-2</td>
<td>4.762</td>
<td>3.887</td>
<td>0.875</td>
</tr>
<tr>
<td>3</td>
<td>H-3</td>
<td>4.731</td>
<td>3.356</td>
<td>0.375</td>
</tr>
<tr>
<td>4</td>
<td>H-4</td>
<td>4.606</td>
<td>4.043</td>
<td>0.563</td>
</tr>
<tr>
<td>5</td>
<td>H-5</td>
<td>4.700</td>
<td>4.168</td>
<td>0.532</td>
</tr>
<tr>
<td>6</td>
<td>H-6</td>
<td>4.293</td>
<td>3.637</td>
<td>0.656</td>
</tr>
<tr>
<td>7</td>
<td>H-7</td>
<td>4.231</td>
<td>3.575</td>
<td>0.746</td>
</tr>
<tr>
<td>8</td>
<td>H-8</td>
<td>4.293</td>
<td>3.825</td>
<td>0.468</td>
</tr>
</tbody>
</table>

All titres expressed as Log CCID 50 per 0.5 ml.

(*) - Difference between the titres at +4°C and 37°C (7 Days)
<table>
<thead>
<tr>
<th>Sr. Harvest No.</th>
<th>Virus titre at +4°C</th>
<th>Virus titre after 7 Days at 37°C</th>
<th>Difference (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 H-1</td>
<td>4.731</td>
<td>4.106</td>
<td>0.625</td>
</tr>
<tr>
<td>2 H-2</td>
<td>4.356</td>
<td>4.168</td>
<td>0.188</td>
</tr>
<tr>
<td>3 H-3</td>
<td>4.856</td>
<td>4.137</td>
<td>0.719</td>
</tr>
<tr>
<td>4 H-4</td>
<td>4.762</td>
<td>4.043</td>
<td>0.719</td>
</tr>
<tr>
<td>5 H-5</td>
<td>4.606</td>
<td>4.825</td>
<td>0.781</td>
</tr>
<tr>
<td>6 H-6</td>
<td>4.356</td>
<td>3.793</td>
<td>0.563</td>
</tr>
<tr>
<td>7 H-7</td>
<td>4.356</td>
<td>3.606</td>
<td>0.750</td>
</tr>
<tr>
<td>8 H-8</td>
<td>4.293</td>
<td>3.731</td>
<td>0.562</td>
</tr>
</tbody>
</table>

All titres expressed as Log CCID 50 per 0.5 ml.

(*) - Difference between the titres at +4°C and 37°C (7 Days)
Graph - IV

Effect of Harvesting Sequence on Thermostability of Measles Vaccine

Difference in titer at +4 & 7 days at 37 deg. Cg.

Harvest No.

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EXPERIMENT - II -

To study the effect of use of modified gelatin on thermostability of measles vaccine.

The choice of various additives to the final product depends on the type of product, its intended use, dose, etc. For majority of the available vaccines, partially hydrolyzed gelatin is used along with sorbitol. The term ‘partial hydrolysis’ involves heating of gelatin solution at 115°C for a period of two hours. However, it has been observed that the end product composition of this protein hydrolysis varies with respect to their amino acid composition. Thus, the availability of various amino acids into the virus medium varies from batch to batch. To overcome this problem, a preparation involving controlled gelatin hydrolysis and repolymerization (referred as ‘Experimental gelatin’ hereafter) was assessed in place of the partially hydrolyzed gelatin and its effect on thermostability was studied.

Materials and Methods -

Similar to the earlier experiment, ten culture bottles of MRC-5 cells, PDL-28 were infected with measles virus. After 40 hours of incubation at 37°C, the culture bottles were washed twice with MEM without FBS. Then onwards, the incubation of these bottles was continued at 32°C. Multiple harvests were then taken at proper intervals. The harvest collected at each time was first pooled together, mixed thoroughly and then divided into two equal aliquots. One of the aliquots was stabilized using partially hydrolyzed gelatin (2.5% final concentration and sorbitol (5% final concentration) and designated as control virus pool. To the second aliquot, experimental gelatin preparation was added along with sorbitol. The concentrations of both the components were same as that of control virus pool. The harvests along with individual samples were kept frozen at -25°C. The virus
concentration assay of all the samples was carried out as per the procedure described earlier.

All the multiple harvests stabilized with partially hydrolyzed gelatin (i.e. control) and experimental gelatin were pooled separately to form the bulk vaccine and designated as Control and Experimental vaccine, respectively. Both these vaccines were filled separately in 5 ml USP type-1 glass vials with a volume of 0.5 ml per vial and were lyophilized in the same manner as described in the earlier experiment. The vials were stoppered under vacuum and then sealed with aluminum caps. The vials were then tested for virus concentration in Vero cells as per the procedure described earlier. About 100 vials from each lot of lyophilized harvests were kept at 37°C for 30 days. After day-7, day-14, day-21 and day-30, 5 out 100 vials of each lot were taken out and titrated on Vero cells to know the residual virus content. The results of the stability of individual harvests were calculated on the basis of the difference between starting titre in the vial and the titre remaining in the vial vis-à-vis the time of incubation at 37°C. A graph was plotted with Difference in potencies of both experimental and control gelatin before heating (i.e. at +4°C) and after heating at 37°C for various time periods on X axis versus the duration of incubation at 37°C on Y axis (Graph-V).

Results -

The results of the test are tabulated in Table-XXII. It is evident from the graph (Graph-V) that modified method of preparing gelatin has substantial power of protecting the virus from heat when compared with the use of traditional methodology of preparing gelatin. Thus, the new type of gelatin could be a better replacement for the old one. By using this stabilizer the starting virus titre of bulk and the overages added on account of stability test losses could be reduced to a great extent.
### Table - XII

**EFFECT OF MODIFIED GELATIN ON THERMOSTABILITY OF MEASLES VACCINE.**

<table>
<thead>
<tr>
<th>Details of sample</th>
<th>Control Vaccine</th>
<th>Experimental Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Titre Difference</td>
<td>Titre Difference</td>
</tr>
<tr>
<td>Potency at +4°C</td>
<td>4.900</td>
<td>4.300</td>
</tr>
<tr>
<td>Potency at +37°C after 7 days</td>
<td>4.418 0.482</td>
<td>4.293 0.007</td>
</tr>
<tr>
<td>Potency at +37°C after 14 days</td>
<td>4.168 0.732</td>
<td>4.075 0.225</td>
</tr>
<tr>
<td>Potency at +37°C after 21 days</td>
<td>4.012 0.888</td>
<td>4.106 0.194</td>
</tr>
<tr>
<td>Potency at +37°C after 30 days</td>
<td>3.637 1.263</td>
<td>4.063 0.257</td>
</tr>
</tbody>
</table>

All titres expressed as Log CCID 50 per 0.5 ml.
Graph - V

Effect of Modified Gelatin on Thermostability of Measles Vaccine

- Control Gelatin
- Modified Gelatin

Log difference in Titre

Potency of Samples at

+4 deg. cg.  +37 (7 days)  +37 (14 days)  +37 (21 days)  +37 (30 days)