CHAPTER 8
PACKAGING, STERILIZATION & INTERACTION STUDIES
PACKAGING, STERILIZATION AND INTERACTION STUDIES

The selected optimized ocular inserts and sol to gel systems were subjected for packaging, sterilization and interaction studies. These were

(I) Ocular inserts:
   1. For ACZ: III-D
   2. For ACZ-HPβcd complex: IX-C
   3. For LVB HCl: XI-L

(II) For Poloxamer based sol to gel systems
   1. For ACZ: P3-C
   2. For ACZ-HPβcd complex: P3-CC
   3. For LVB HCl: P4-L

(III) For Gelrite based sol to gel systems
   1. For ACZ: G5-D
   2. For ACZ-HPβcd complex: G4-CC
   3. For LVB HCl: G4-L

Packaging, sterilization and test for sterility on ocular inserts and sol to gel systems.

(A) Packaging

(i) For ocular inserts:
The selected ocular inserts were packaged in laminated aluminium foils by strip packaging machine.

The ocular insert were placed in between two pieces of papers and then it was placed in between two aluminum foils. The papers were used to prevent the sticking of the ocular insert to the aluminium surface. The aluminum foils were then sealed. The packaging system were evaluated for leak test, water vapour transmission and for removability.

Leak Test: Three strip packs were immersed in 100 ml solution of methylene blue (0.1%) in water in Buchner flask. Vacuum was created for 15 minutes and released instantly. The process was repeated three times. Packs were washed with running...
water, dried and opened. Entry of colored solution in the pack was checked. The package passed the test as no colored solution entered into the package.

**Water Vapour Transmission test:** Three strip packs were weighed separately and marked and kept at 75% of relative humidity and 25°C temperature. The packs were removed after 24 hrs and weight was taken again. The package system passed the test as no significant differences in weights were observed.

**Removability test:** The ocular inserts could be easily removed from the strip package without any breakage of inserts, so the package system passed the test.

(ii) For ocular sol to gel system: The selected ocular sol to gel systems were packaged in 10 ml capacity plastic eye drops bottles made of high density polyethylene (HDPE).

This packaged system for gel was evaluated for resistance to autoclaving, closure efficiency and pourability of sol to gel.

**Resistance to autoclaving:** In the packaged system the teats did not become sticky or less resilient and neither teats nor caps changed in size or shape when autoclaved at 121°C for 20 minutes thus, the packaged system passed the test.

**Closure efficiency:** The cap was closed to a specified torque and the packaged bottle was immersed in a beaker containing dye solution (0.1% methylene blue in water). The whole assembly was autoclaved at 121°C for 20 min. The bottle was removed, cooled and dye was rinsed away from the surface and the bottle and then these are inspected. There was no alteration in the volume or color of the contents. Thus the packed system passed the test as there was no leakage.

**Pourability:** The ocular sol to gel system was poured by pressing the dropper. As it flows down easily in the form of drops, the packaged system was found ideal for packaging the sol to gel system.

**(B) Sterilization and test for sterility**

(i) **Ocular inserts:** The selected optimized ocular inserts after packaging were sterilized by gamma radiation, a method recommended for sterilization of polymeric devices. The packages were exposed to a total dose of 2.5M rad. The total dose was given in 24 hours (Courtesy: INMAS, Delhi).
The sterilized ocular inserts were also evaluated for physical stability for color of packaging material, color and intactness of ocular inserts to observe the effect of gamma radiation. The assay of drug before and after sterilization and other parameters are given in Table 81.

Table 81: Evaluation of physical characterization of optimized ocular inserts and sol to gel systems after sterilization

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Assay</th>
<th>Physical parameters (colour, consistency and clarity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before stability</td>
<td>After stability</td>
</tr>
<tr>
<td>Ocular inserts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III D</td>
<td>99.86</td>
<td>99.10</td>
</tr>
<tr>
<td>IX C</td>
<td>99.92</td>
<td>99.38</td>
</tr>
<tr>
<td>XI L</td>
<td>99.85</td>
<td>99.00</td>
</tr>
<tr>
<td>Sol to gel (Polaxamer based)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3 C</td>
<td>99.13</td>
<td>98.23</td>
</tr>
<tr>
<td>P3 CC</td>
<td>99.53</td>
<td>99.06</td>
</tr>
<tr>
<td>P4 L</td>
<td>99.64</td>
<td>98.92</td>
</tr>
<tr>
<td>Sol to gel (Gelrite based)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G5 D</td>
<td>99.36</td>
<td>97.89</td>
</tr>
<tr>
<td>G4 CC</td>
<td>99.48</td>
<td>99.01</td>
</tr>
<tr>
<td>G4 L</td>
<td>99.87</td>
<td>98.65</td>
</tr>
</tbody>
</table>

(ii) Ocular Sol to gel systems:

Polaxamer based sol to gel system: The Polaxamer based sol to gel system could not be sterilized by autoclaving because during autoclaving, sol was converted to gel and after removing only a part of gel was converted back to sol. Therefore, the purpose of delivery system was defeated. This might occurred due to the polymers, i.e. Polaxamer 407 sensitive towards change in temperature. Therefore, the sol to gel P1-A was
packaged in plastic eye drop bottles and sterilized by gamma radiation. A total dose of 1.5 M rad was given for 24 hours (Courtesy: INMAS, Delhi). The assay of sol to gel preparation before and after sterilization and other parameters are shown in Table 81.

**Gelrite based sol to gel system:** In order to avoid degradation of drug at higher temperature of the autoclaving, an aseptic technique was used for getting a sterilized packaged system for formulation. The method followed is given as under.

The pre sterilized glass containers with dropper and closures were obtained from Dr. R. P. centre for ophthalmic Sciences, AIIMS, New Delhi. The drug solution in boric acid buffer was sterilized by passing through membrane filter under aseptic condition (0.22µm). Other components of the formula i.e. polymers (gelrite), preservative (methyl and propyl paraben) in boric acid buffer was sterilized by autoclaving at 121°C and 15lb/inch² for 20 minutes. The two components were mixed under aseptic conditions and packaged in presterilized bottles under aseptic conditions. This method was found successful as the formulation remained in sol form in the packaged system.

**Test for Sterility**

(i) **Test for sterility for ocular inserts III-D, IX-C & XI-L:** The test for sterility on the sterilized ocular inserts was carried out as per I.P. 1996. The test was carried out in the following manner:

a. **Minimum number of items recommended:** For ophthalmic and other non-injectable preparations, the minimum number of sample size recommended to be tested is 5% or two containers whichever is greater if batch size contains not more than 200 containers (I.P. 1997). In the present case less than 100 packs for each preparation were prepared. Therefore, 5 ocular inserts packs were used for the test.

b. **Minimum quantity of product:** For solids, if the content is less than 50 mg, the total contents are used for the test and minimum volume of culture medium should be 40 ml. In the present case, weight of the ocular insert was less than 50 mg and therefore whole ocular inserts were used for the test.

c. **Volume of the culture medium:** 40 ml was taken in boiling test tube, properly plugged with cotton and sterilized by autoclaving (Volume was selected as per I.P.: for solid less than 50mg, total content for inoculation and 40ml of culture medium).
d. **Culture media.**

1. **For bacteria (aerobic/anaerobic):** Fluid thioglycolate medium was used.

2. **For fungi:** Soybean casein digest medium was used.

Mediawere prepared according to I.P. 1996 and sterilized in the test tubes by autoclaving at 121°C at 15lb/inch² gauge pressure for 20 minutes.

e. **Inoculation:** 10 ocular inserts were taken for the test and 5 were inoculated into fluid thioglycolate medium and five into soyabean casein digest medium. Both media were shaken vigorously; the process was repeated for other ocular inserts also.

f. **Incubation:** The inoculated culture media for bacteria and fungi were incubated at 32±1°C and 25±1°C respectively in a BOD incubator.

g. **Controls:** Sterile media without inoculation was also incubated.

h. **Interpretation of Results:** Results were based on visual observations for appearance of turbidity in culture media after every day for a period of 14 days.

i. **Result:** The ocular inserts III-D, IX-C & XI-L passed the test for sterility as no turbidity was obtained in the test tubes.

(ii) **On ocular sol to gel systems:** Sterility test was performed on gels which were sterilized by

(a) Gamma radiation (for Polaxamer based sol to gel formulation P3-C, P3-CC, P4-L)

(b) Autoclaving and aseptic processing (for gelrite based sol to gel formulations G5-D, G4-CC and G4-L)

Test for sterility on the sterilized gels were performed according to I.P. 1996: The test was carried out in the following manner.

(1) **Number of Items recommended:** For ophthalmic preparations, 2 containers or 5%, whichever is greater used for the test if total number of containers are not more than 200. In the present case, 10 containers of each gels were prepared and sterilized, therefore 2 containers of each gel were used for the test.
(2) Minimum quantity taken: For liquid I.P. recommended 2ml if container content is 4ml or more but less than 20ml. In the present case each container content was 10ml. Therefore 2ml of preparation was inoculated.

(3) Volume of the culture medium: 20ml was taken in boiling test tube, properly plugged and sterilized by autoclaving (I.P. 1996).

(4) Culture media: As given under sterility test for ocular insert.

(5) Inoculation: Half quantity of each container was added to the fluid thioglycollate remaining half was added to the soyabean casein digest medium.

(6) Incubation: Same as in the case of ocular inserts.

(7) Controls: Sterile media without inoculation was also incubated.

(8) Interpretation of results: Results were based on visual observations for appearance of turbidity in culture media after every day for the period of 14 days.

(9) Results: The gels in containers passed the test, for sterility in all cases, as no turbidity was obtained in any test tube for bacteria or fungi.

(2) Interaction studies on ocular insert and sol to gel preparations

The interaction studies were done in order to investigate any interaction between the drug and the polymers.

(A) Assay

The ocular insert III-D, IX-C and X-L and sol to gel formulations P3-C, P3-CC, P4-L, G5-D, G4-CC and G4-L were assayed using the assay method reported in analytical methodology. Drug contents were calculated to estimate the percentage recovery of the loaded drug. Results are given in Table no. 81.

(B) Physical appearance: The preparation was observed for physical appearance (Table 81).

(C) UV Scanning:

The ocular inserts were triturated in a mortar with IPB of pH 7.4. Also ocular sol to gel P3-C, P3-CC, P4-L, G5-D, G4-CC and G4-L were dissolved in STF of pH 7.4.

The solutions were filtered through a Whatman filter paper no. 42 and filtered solutions were scanned for absorption between 200 to 400 nm. The spectra recorded were taken as qualitative in order to assess the change in peaks, pattern of curve etc. if any.
(D) TLC studies

TLC studies on the sterilized and unsterilized ocular insert and sol to gel formulations were done as per the method reported in analytical methodology.

Inference:

The selected optimized ocular inserts were packaged in aluminium foils using a strip packing machine and these were evaluated for leak test, water vapour transmission test and removability test. The packaging was found satisfactory as all test were passed. These ocular inserts were sterilized by gamma radiation at 2.5 mm rad dose and further evaluated for assay and other parameters before and after sterilization. It was found that same assay results obtained and there was no change in color etc. Therefore it was concluded that gamma radiation affect the drug or other excipients chemically. In the test for sterility the test was passed as per I.P. procedure which indicated that sufficient gamma radiation dose was achieved and hence products were sterilized effectively.

Poloxamer based sol to gel preparations were packed in plastic eye drop bottles and sterilized by gamma radiation as autoclaving was not found suitable due to sensitivity of poloxamer towards temperature. The test before and after sterilization i.e. assay and physical appearance revealed that there was no any effect of radiation on the assay of the drug and an stability of sol to gel formulations. The preparation were found sterile as the test for sterility passed as per I.P.

In case of gelrite based sol to gel systems aseptic procedure was adopted i.e. gelrite and preservatives in buffer was sterilized by autoclaving and drug in buffer was passed through bacteria proof membrane filter then these two portions were mixed together and packed in already sterilized eye bottles. This procedure was adopted to avoid exposure of the drug towards the heat which occurred in autoclaving process. The test for sterility was performed as per I.P. and it was passed.

The interaction studies were performed on the samples stored for 1 month in order to ascertain any chemical interaction of the drug with excipients in the formulations. It was performed by observing assay, physical appearance, UV scanning, IR and TLC studies. In the assay almost the whole amount of drug which was added to formulation was noticed in ocular inserts and sol to gel systems. The preparations did not change in physical appearance. The pattern of peaks and UV absorption of the drug extracted from the formulation was almost found matching with the spectrum of
pure drug as evident from UV spectra. Similarly IR spectra were also matching in terms of peaks and patterns for the extracted drug from the formulation with that of pure drug. In TLC studies no extra peaks were seen on the plates for the drug extracted from the formulations and single spot only for drug at Rf value of pure drug was seen. On the basis of the above observations it was revealed that the preparation were not having any interaction between the drug and excipients in case of ocular inserts and sol to gel preparations.