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# Preparation and Evaluation of Biodegradable Ocular Inserts of Timolol Maleate

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**Abstract:** Ocular inserts are sterile, thin, multilayered, drug-impregnated solid or semi-solid devices, made of polymeric materials. Bio-erodible ocular inserts are formed by bio-erodible polymers (e.g., cross-linked gelatine derivatives, polyester derivatives) which undergo hydrolysis of chemical bonds and hence dissolution. Bio-erodible ocular inserts are formed by bio-erodible polymers (e.g., cross-linked gelatine derivatives, polyester derivatives) which undergo hydrolysis of chemical bonds and hence dissolution. All prepared ocular films have good appearance with smooth surface. A good weight uniformity of all formulation indicates an equal distribution of polymers in the inserts film prepared by solvent evaporation technique.

The surface pH of the prepared inserts were found in between 6.3 to 7.1 indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range. Good uniformity in the drug content among the batches was observed for all the formulations. All formulations prepared were subjected to *in vitro* release study to ensure the effect of polymer concentration.

**Keywords:** Ocular inserts, Ocimum Basilicum, Timolol maleate, Sterility.

## Introduction

Ocular inserts are sterile, thin, multilayered, drug-impregnated solid or semi-solid devices, made of polymeric materials, to be placed in the conjunctival sac or cul-de-sac to deliver drugs to the ocular surface [1].The advantages of inserts are the increased ocular residence time, accurate dosing, better patient compliance due to reduced frequency of administration, reduction in systemic side effects and possibility of drugs releasing at a constant and slow rate as well as increase stability and shelf life. These advantages lead to effective therapy in eye diseases [2].

 

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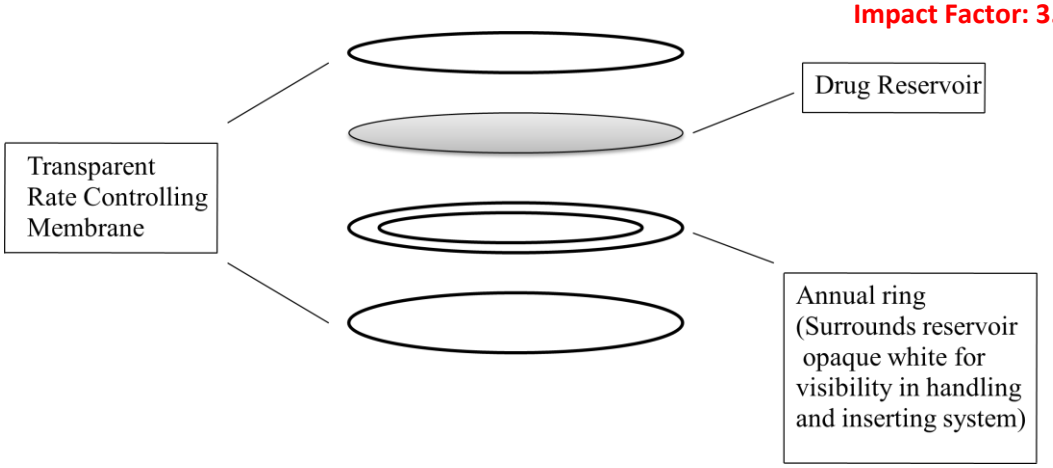


**Fig. 1: Schematic Diagram of Ocular Inserts** [3]

***Types of ocular inserts*** *[1]*

**I. Insoluble Ocular Inserts**

A. Reservoir systems,

B. Matrix systems.

**II. Soluble Ocular Inserts**

A. Based on Natural polymers

B. Based on Synthetic and semi-synthetic polymer

**III. Bio-Erodible Ocular Inserts**

**I. Insoluble Ocular Inserts**

Insoluble polymer based ocular inserts can be classified into two categories:-

A. Reservoir systems,

B. Matrix systems.

**A. Reservoir systems**

The reservoir systems can release drug by diffusion or by an osmotic process. It contains respectively, a colloid, a liquid, a gel, a solid matrix, a semisolid, or a carrier which containing drug. These Carriers are made of hydrophilic, hydrophobic, organic, natural or synthetic polymers.

Reservoir systems have been sub-classified a follows:

1. Diffusional inserts e.g. Ocuserts,
2. Osmotic inserts.

**1. Diffusional insert or Ocuserts**

Ocusert system is a novel ocular drug delivery system based on porous membrane. Release of drug from diffusional inserts or Ocusert is based on a diffusional release mechanism. Diffusional insert consists of a central reservoir of drug enclosed in specially designed microporous membrane allowing the drug to diffuse from the reservoir at a precisely determined rate.

 

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2. **Osmotic insert**

   The osmotic inserts are generally composed of two distinct compartments. In osmotic inserts one compartment contains drug and other contains osmotic solute, which is sandwiched between the rate controlling membrane and tears diffuse into osmotic compartment inducing an osmotic pressure due to which drug diffuses [3].

**B. Matrix systems**

Matrix system is a group of insoluble ophthalmic devices mainly represented by contact lenses. Matrix system comprises of covalently cross-linked hydrophilic or hydrophobic polymer that forms a three dimensional network or matrix capable of retaining water, solid components or aqueous drug solution. The hydrophobic or hydrophilic polymer swells by absorbing water.

**Contact lenses**

Contact lenses initially used for vision correction. Their use has been extended as potential drug delivery devices by pre-soaking them in drug solutions. Main advantage of this system is the possibility of correcting vision and releasing drug simultaneously. According to Refojo contact lenses subdivided into 5 groups.

a) Rigid,
b) Semi-rigid,
c) Elastomeric,
d) Soft hydrophilic,
e) Bio-polymeric.

**II. Soluble Ocular Inserts**

These types of inserts are entirely soluble so that they do not need to be removed from their site of application. Soluble Ocular Inserts can be broadly divided into two types based on:

A. Natural polymers,
B. Synthetic and semi-synthetic polymer.

**A. Natural polymers**

The soluble inserts offer the additional advantage of being of a generally simple design, of being based on products well adapted for ophthalmic use and easily processed by conventional methods. The main advantage is decreased release rate, but still controlled by diffusion.

**B. Synthetic and semi-synthetic polymer**

The second type of soluble insert is usually based on semi-synthetic polymers (e.g., cellulose derivatives) or on synthetic polymers such as polyvinyl alcohol. A decrease of release rate can be obtained by using Eudragit, a polymer normally used for enteric coating, as a coating agent of the insert.

**II. Bio-erodible ocular inserts**

Bio-erodible ocular inserts are formed by bio-erodible polymers (e.g., cross-linked gelatine derivatives, polyester derivatives) which undergo hydrolysis of chemical bonds and hence dissolution. The advantage

 

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of these bio-erodible polymers is the possibility of modulating their erosion rate by modifying their final structure during synthesis and by addition of anionic or cationic surfactants [4].

***Types of polymer used in ocular drug delivery***

**Classification of polymer**

**a. According to their charge**

- **Anionic Polysaccharides**

  **Natural**: Alginic acid, pectin, Xanthan gum, Hyaluronic acid, Chondroitin sulfate, Gum Arabic, Gum Karaya, Gum Tragacanth

  **Semi-Natural**: Carboxymethyl, Chitin, Cellulose gum

- **Cationic Polysaccharides**

  **Natural**: Chitosan

  **Semi-Natural**: Cationic Guar gum.

  **Cationic:** Hydroxyethylcellulose (HEC).

- **Nonionic Polysaccharide**

  **Natura**l: Starch, Dextrins, Guar gum.

  **Semi-Natural**: Cellulose Ethers (e.g. hydroxyethyl cellulose, Methylcellulose, Nitrocellulose).

- **Amphoteric Polysaccharides**

  **Semi-Natural**: Carboxymethylchitosan, N-hydroxyl-Dicarboxyethylchitosan, Modified Potato starch.

- **Hydrophobic Polysaccharides**

  **Semi-Natural**: Cetylhydroxyethylcellulose, Polyquaternium.

**b. According to their Sources**

- **Marine origin:** Agar, Carrageenans, Alginic acid, Laminarin.
- **Plant origin**

  **Shrubs/tree exudates**—Gum Arabica, Ggum Ghatti, Gum Karaya, Gum Tragacanth, Khaya and Albizia gums;

  **Seed gums** Ocimum Basilicum mucilage, Locust bean Gum, Guar Gum, and Starch.

  **Extracts -** Larch gum, Pectin;

  **Tuber and roots**- Potato starch.

- **Animal origin:** Chitin and chitosan, Hyaluronic acid, Chondroitin sulfate.
- **Microbial origin (fungal and bacterial):** Xanthan, Dextrin, Curdian, Pullulan.
- **Prepared gums**

  Biosynthetic gums Xanthan, scleroglucan, dextrins.

  Starch and its derivatives, dextrins.

 

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Cellulose derivatives.

- **Semi-synthetic**

  **Starch derivatives** - Heta starch, Starch acetate, Sarch phosphates.

  **Cellulose derivatives** - Carboxymethyl cellulose (CMC), Hydroxyethyl cellulose, Hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), microcrystalline cellulose (MC).

**c. According to their shape**

- **Linear**: Algins, Amylose, Cellulose, pectins.
- **Branched**

  **Short branches** - Xanthan, Xylan, Galactomannans**;**

  **Branch-on-branch** - Amylopectin, Gum Arabic, Tragacanth.

**d. According to Manomeric units in chemical structure**

- Homoglycans - Amylose, Arabinanas, Cellulose;
- Diheteroglycans - Algins, Carragennans, Galactomannans;
- Tri-heteroglycans - Arabinoxylans, Gellan, Xanthan;
- Tetra-heteroglycans - Gum Arabic, Psyllium seed gum;
- Penta-heteroglycans - Ghatti gum, Tragacanth [5].

### *Novel Approach in the Development of Ocular Inserts*

Ocimum Basilicum Mucilage is used as a polymer in the formulation of ocular insert for treatment of Glaucoma. Ocimum Basilicum obtained from seeds of *Ocimum basilicum L.* belonging to **Family** Lamiaceae.

Ocimum Basilicum L. is also known as Kali Tulsi or Krishna Tulsi. Due to its holistic properties Tulsi is widely used in many ayurvedic and naturopathic medicines. Not only its leaves but also its flower and seeds have the properties to fight a number of diseases.

A Tulsi plant can be used in Eye problems: Black basil juice plays a very important role for eyes diseases. A few drops of black basil juice in the eyes can cure sore eyes [6, 7].

## Formulation of ocular inserts

Ocular inserts of Timolol maleate and Natural Polymer (Ocimum Basilicum Mucilage) were prepared by solvent casting method. Distilled water was used as Solvent.

The composition of ocular insert of Timolol maleate is shown in Table 1.

 

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***Table 1: Formulation compositions for ocular inserts of Timolol maleate***

| Formulation | Timolol Maleate (mg) | Natural Polymer (in %) | HPMC (mg) | Plasticizer (mg) | Solvent (ml) |
|---|---|---|---|---|---|
| A1 | 30 | 1 | 250 | 150 | 10 |
| A2 | 30 | 2 | 250 | 150 | 10 |
| A3 | 30 | 3 | 250 | 150 | 10 |
| A4 | 30 | 3 | 300 | 150 | 10 |
| A5 | 30 | 2 | 300 | 150 | 10 |
| A6 | 30 | 1 | 300 | 150 | 10 |
| A7 | 30 | 1 | 350 | 150 | 10 |
| A8 | 30 | 2 | 350 | 150 | 10 |
| A9 | 30 | 3 | 350 | 150 | 10 |

**Natural Polymer**: Ocimum Basilicum mucilage,

**Plasticizer**: Glycerin,

**Solvent**: Distilled Water.

The polymers Ocimum Basilicum mucilage and HPMC were accurately weighed in requisite ratios and dissolved in distilled water to form a Polymer solution. Timolol maleate was then added in to the polymeric solution under mild agitation until it was dissolved. Plasticizer Glycerin was added to the above solution under mild agitation for complete mixing of the solution. The mixture was set for mixing on magnetic stirrer for 15 min to ensure uniform distribution of ingredients. Polymeric solution was sonicated for 30 Sec to remove the air bubbles.

Polymeric solution was then poured into a petridish placed on a flat even surface. The rate of evaporation was controlled by inverting the funnel over the petridish. After drying at room temperature for 24 hr, circular ocular inserts of diameter 5 mm were cut using fabricated mould, sterilized under UV for 10 min and 60 min and packed in aluminum foil and stored in desiccators until further use.

 

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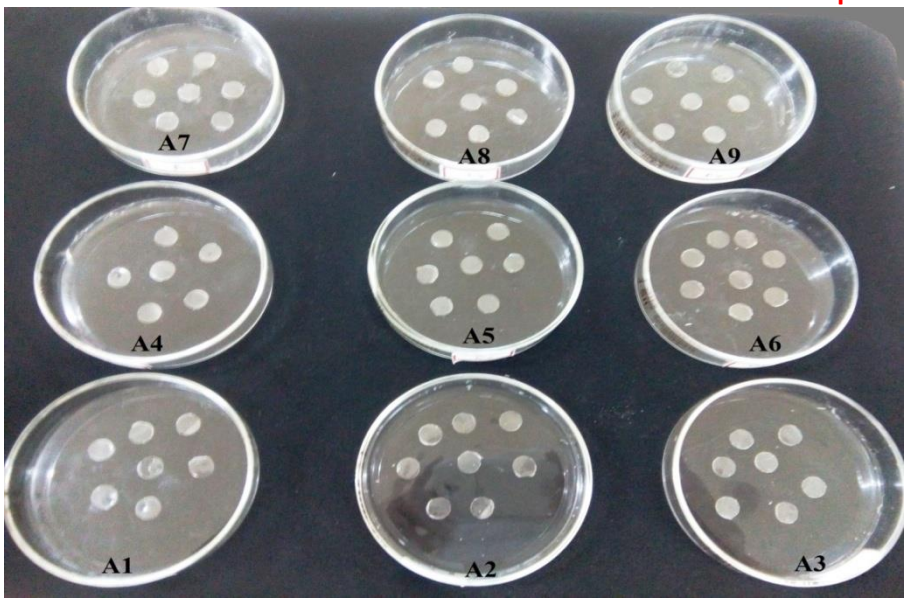


***Fig. 2: Prepared ocular inserts***

**Amount of Drug Loaded on Single Insert**

Diameter of the prtridish = 7.5 cm

Radius = 7.5/2 = 3.75 cm

**Area of Film = $\pi r^2$**

Area of Film = 3.24× $(3.75)^2$

**Area of Film = 44.156 $cm^2$**

Diameter of single insert = 0.8 cm

Radius of single insert =0.4 cm

Area of single insert = 3.14× $(0.4)^2$

**Area of single insert = 0.5024 $cm^2$**

44.156 $cm^2$ contain 30 mg drug

0.5024 $cm^2$ area of insert contain = 30×0.5024/44.156

= 0.341 mg

= 341 µg per insert

Single insert contain 341 µg or 0.341 mg of Timolol maleate.

## Evaluation of Formulations [8-12]

### *Physical Appearance*

All the ocular films were visually inspected for colour, clarity and smoothness.

### *Thickness uniformity*

Insert thickness was measured at five different points using Vernier calliper and mean insert thickness was noted ($n$ = 3).

 

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***Uniformity of weight***

The weight variation test was done by weighing three patches cut from different places of the same formulation and their individual weights were determined by using the digital balance. Next, their mean value was calculated. The standard deviation of weight variation was computed from the mean value.

***Folding endurance***

The flexibility of polymeric films can be measured quantitatively in terms of folding endurance. The ocular inserts film was folded at center, between the fingers and the thumb and then opened. This termed as one folding.

The process was repeated until the film showed cracks in center or breakage. The total folding operations known as folding endurance value. This test was done on three inserts of each formulation.

***Surface pH determination***

For the determination of surface pH, the ocular insert was allowed to swell in the Petri dish at room temperature for 30 min in 0.1 ml of distilled water. The pH paper was kept on the surface and after1 min the colour that developed was compared with the standard colour scale.

***Drug content uniformity***

Content uniformity of the drug in the circular films was determined using three inserts punched out from each film. Each insert was then dissolved in 10 ml of simulated tear fluid at pH 7.4. The absorbance of the solution was measured by Double Beam spectrophotometer at 294 nm against blank solution which was prepared by dissolving a placebo insert in the simulated tear fluid and the same volume used with medicated insert to prevent polymer or plasticizer interference.

$$\%\ \textbf{drug content drug} = \frac{As}{Ar} X \frac{Dr}{ds}$$

Where;

As = Absorbance of sample solution.

Ar =Absorbance of standard solution.

Dr = standard dilution.

Ds = Sample dilution.

The same procedure was adopted for all batches of films in triplicate and the mean drug content and standard deviation of were calculated.

***Swelling index determination***

Three inserts were weighed and placed separately in beakers containing 4 ml of distilled water. At regular time intervals, inserts were removed and the excess water on their surface was wiped using a blotting paper and again weighed. The procedure was continued till there was no increase in the weight of inserts. Swelling index was calculated by dividing the increase in weight by the original weight and was expressed as percentage.

 

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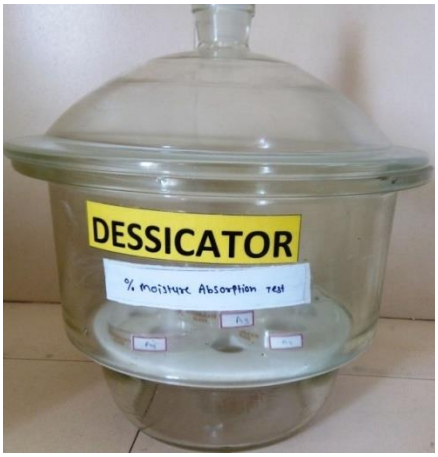
$$\% \textbf{ Swelling index} = \frac{\textbf{Increase in weight}}{\textbf{Original weight}} \boldsymbol{X100}$$

***% Moisture absorption study***

Percentage moisture absorption test was carried out to check physical stability and integrity of the films withstanding high humidity. The films were placed in a dessicator containing silica gel for 24 hours to make sure that no moisture was absorbed by the film under normal conditions. The films were weighed individually. Then the films were placed in a dessicator which maintained at high Relative Humidity (RH) at about 75±5% RH using saturated sodium chloride solution. During three days the films were taken each day out and reweighed.




The percentage moisture was calculated according to the following equation:

$$\% \textbf{ Moisture absorption } = \frac{\textbf{Final weight} - \textbf{Initial weight}}{\textbf{Initial weight}} \times \textbf{100}$$

***%Moisture loss study***

The percentage moisture loss is done to check out the integrity of the film in dry conditions. The ocular inserts are weighed and kept in desiccators containing anhydrous calcium chloride. After three days, the ocular inserts are taken out and reweighed.

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The percentage of moisture loss is then calculated by using the formula.

$$\% \textbf{ Moisture loss} = \frac{\textbf{Initial weight} - \textbf{Final weight}}{\textbf{Initial weight}} \times \textbf{100}$$

***Drug permeation study***

*In vitro* drug permeation studies were carried out by putting insert on a Millipore membrane filter (0.80 $\mu$m), which was fixed between donor and receptor compartment of an all-glass modified Franz diffusion cell. The Millipore membrane filter was used to simulate corneal epithelial barrier as isolated cornea will not remain viable beyond 4 h. The required amount i.e. 10 ml of ringer solution was placed within the receptor compartment and then temperature of ringer solution was adjusted to 37 ± 0.5°C. The ringer solution was stirred at a low speed using magnetic stirrer. At specific time intervals, 1 ml aliquot of solution was withdrawn from the receptor compartment and replaced with fresh bicarbonate ringer solution. The aliquot was analyzed for the drug content using a UV-VIS spectrophotometer at 294 nm after appropriate dilution against reference using a bicarbonate ringer solution as a blank.

***Release kinetics***

The mechanism of drug release was investigated by fitting the drug release data into zero order, first order, Higuchi kinetics, and Korsmeyer–Peppas equations. The goodness of fit of drug release was evaluated by the regression coefficient ($R^2$) value.

***In Vitro Drug Release***

The in vitro diffusion of drug from the inserts was studied using the classical biochemical donor - receptor compartment model comprising a cylindrical tube (15 mm internal diameter and 80 mm height) and a glass beaker fabricated in the laboratory. The dialysis membrane No.50 was tied to one end of open cylinder. This acted as a donor compartment. The insert was placed inside this compartment. The dialysis membrane acted as conjunctival epithelium. The entire surface was in contact with the receptor compartment containing 25 ml of STF pH 7.4 in 100 ml beaker. The content of receptor compartment was stirred continuously at low speed maintaining a temperature of 37 0C ± 1 $^0$C. At specific time intervals samples were withdrawn from the receptor compartment and replaced with fresh STF pH 7.4. The samples were analyzed using UV spectrophotometer at 294 nm.

 

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### *Sterilization and Sterility testing*

Sterilization of ocular inserts was carried out by placing inserts under ultra violet light and exposed both sides for 15 min at a 10 cm height from UV lamp. **Manju.**

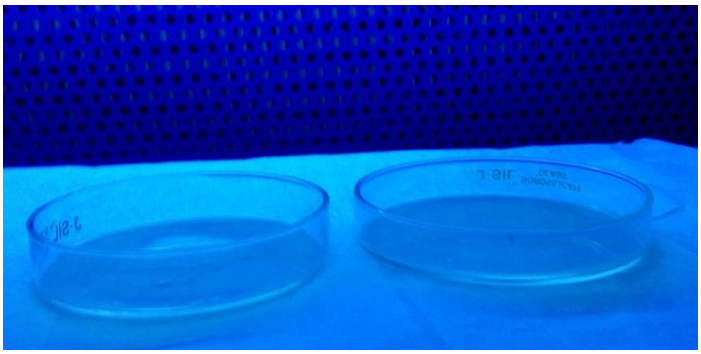

***Fig. 3: Sterilization of Ocular Inserts***

Sterility is one of the most required testing for an ophthalmic preparation. Sterility tests are used for detecting the presence of viable forms of microorganisms in ocular preparations. The principle governing these tests is that if the microorganisms are placed in a medium which provides nutritive material and water, kept at a favourable temperature, the organisms will grow and their presence can be indicated by turbidity in the originally clear medium. In the present study, two media namely, alternate thioglycolate medium (ATGM) and soyabean-casein digest medium (SBCD) were used to investigate the presence/absence of aerobic bacteria and aerobic fungi, in the formulated sterilized ocular inserts.

### *Preparation of culture medium*

**a) Fluid Thioglycolate medium** FTGM was used to detect the growth of aerobic and anaerobic bacteria. 7.25 Gms of readymade was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in an autoclave at 121°C for 20 minute. The medium was freshly prepared and allowed to cool just prior to use.

**b) Soyabean-casein digest medium** was used to detect the growth of aerobic fungi. 7.25 Gms of readymade SBCD was dissolved in 250 mL of purified water and the pH was adjusted to 7.1±0.2 with 1M NaOH. This was sterilized in 121°C for 30 min. The medium was freshly prepared and allowed to cool just prior to use.

### *Test procedure*

Sterilized ocular inserts were directly inoculated in above medium aseptically and labelled as 'Test'. Similarly, positive and negative controls were also prepared and all the three tubes were incubated at specified condition as given in table. [IP]

 

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***Table 2: Condition of incubation of different micro-organism***

| Medium | Test micro-organism | Incubation | | |
|---|---|---|---|---|
| | | Temp (°C) | Duration | Type of microorganism |
| Fluid Thioglycollate | *Bacillus subtilis* | 30 to 35 | 3 days | Aerobic |
| Soyabean-Case.in Digest | *Candida albicans* | 20 to 25 | 5 days | Aerobic |

## Result

### *Physical Appearance*

In the present investigation solvent evaporation technique is adopted and it was found to be giving thin uniform films. All prepared ocular films have good appearance with smooth surface. Films prepared were semitransparent and surface texture was smooth and uniform.

### *Uniformity of weight*

Measurement of films was carried out and low standard deviation values in film weight measurements ensure the uniformity of weight in each film. A good weight uniformity of all formulation indicates an equal distribution of polymers in the inserts film prepared by solvent evaporation technique. Formulation A3 shows lower weight 5.333 mg and formulation A9 shows higher weight11.33 mg. Uniformity of Weight data of ocular inserts is shown in Table 3.

### *Uniformity of Thickness*

Thickness values of inserts were found in range of 0.073±0.0047 to 0.16±0.0163 mm. Measurements of films thickness were carried out and low standard deviation values in each film thickness measurements which indicate the uniformity of thickness in each film. Good thickness uniformity of all formulation indicates an even distribution of drug and the polymers in the matrix film prepared by solvent evaporation technique. Uniformity of thickness data of ocular inserts is shown in Table 3.

### *Folding endurance*

Folding endurance of inserts was found in range of 140±7.4833 to 186.333±4.988. Formulation A6 shows highest folding endurance. Higher folding endurance indicates good flexibility of the inserts. Folding endurance values revealed that maximum folding endurance was found at low concentration of Natural polymer. Decrease in folding endurance at higher concentration of Natural polymer, may be due to increase in thickness of films. Folding endurance data of ocular inserts is shown in Table 3.

 

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***Surface pH***

The surface pH of the prepared inserts were found in between 6.3 to 7.1 indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range. Surface pH data of ocular inserts is shown in Table 3.

***Table 3: Uniformity of weight, thickness, folding endurance and pH***

| Formulation | Weight (mg) | Thickness (nm) | Folding endurance | pH |
|---|---|---|---|---|
| A1 | 6.333±0.471 | 0.16±0.016 | 168.333±13.424 | 6.3±0.235 |
| A2 | 6.0±1.414 | 0.11±0.008 | 153.333±8.806 | 6.5±0.492 |
| A3 | 5.333±0.471 | 0.113±0.012 | 147±13.063 | 6.5±0.355 |
| A4 | 5.666±0.471 | 0.073±0.004 | 141±2.943 | 6.4±0.286 |
| A5 | 6.0±0.816 | 0.08±0.008 | 159.573±1.426 | 6.6±0.169 |
| A6 | 6.333±0.471 | 0.14±0.016 | 186.333±4.988 | 6.9±0.294 |
| A7 | 6.666±0.471 | 0.123±0.012 | 166.666±8.379 | 7.1±0.509 |
| A8 | 8.0±1.414 | 0.116±0.004 | 154±14.165 | 6.8±0.509 |
| A9 | 11.33±1.247 | 0.14±0.021 | 140±7.483 | 7.066±0.579 |

Mean±SD (n=3)

***Drug content uniformity***

Good uniformity in the drug content among the batches was observed for all the formulations. Drug content was found in the range of 96.080±0.3137 to 99.467±0.2464 %. The drug content analysis of the prepared formulations have shown that the process employed to prepare ocular insert films in this study was capable of giving films with uniform drug content and minimum batch variability. Drug content data of ocular inserts is shown in Table 4.

***% Moisture absorption study***

Moisture absorption values of inserts were found in range of 4.259±0.6930 to 6.22±0.3113. Formulation A3 shows highest moisture absorption. It was also noticed that moisture absorption of formulation also depends on Natural polymer concentration. Natural polymer concentration increase then increase in % moisture absorption. % Moisture absorption data of ocular inserts is shown in Table 4.

***%Moisture loss***

% Moisture loss values of inserts were found in range of 2.920±1.0898 to 6.92±0.6159. Formulation A8 shows highest moisture loss. % Moisture loss data of ocular inserts is shown in Table 4.

 

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***Table 4: %Moisture absorption, moisture loss and swelling index of diff. formulation***

| Formulation | % Drug Content | % Moisture absorption | %Moisture loss | % Swelling index |
|---|---|---|---|---|
| A1 | 97.145±0.840 | 5.238±0.336 | 6.0±0.424 | 3.912±0.188 |
| A2 | 98.79±0.181 | 5.333±0.471 | 4.761±0.337 | 3.446±0.243 |
| A3 | 98.499±0.840 | 6.22±0.311 | 5.666±0.471 | 4.786±0.299 |
| A4 | 96.080±0.313 | 5.8866±0.682 | 4.996±0.583 | 4.252±0.080 |
| A5 | 99.467±0.246 | 5.095±.0703 | 2.920±1.089 | 4.802±0.603 |
| A6 | 97.725±0.652 | 5.238±0.336 | 4.523±0.337 | 4.231±0.139 |
| A7 | 97.048±0.789 | 4.999±0.583 | 4.413±0.938 | 5.184±0.179 |
| A8 | 97.048±0.478 | 4.259±0.693 | 6.92±0.615 | 6.276±0.342 |
| A9 | 97.870±1.168 | 5.838±0.599 | 5.181±0.607 | 6.690±0.165 |

Mean±SD (n=3)

### *% Swelling index*

% swelling index values of inserts were found in range of 3.912±0.1886 to 6.690±0.1652. Formulation A9 shows highest swelling properties. When Increase in the HPMC and Natural polymer concentration increase the swelling index properties. % swelling index data of ocular inserts is shown in Table 4.

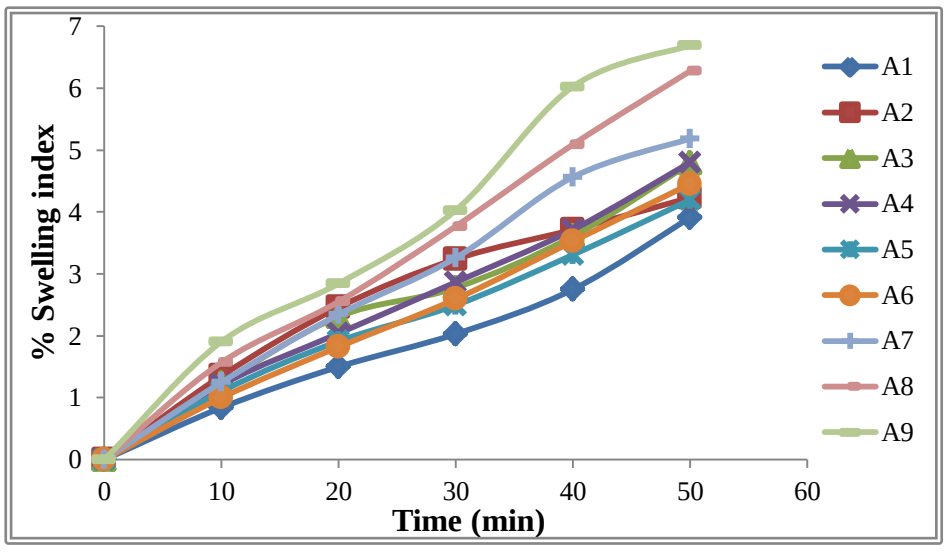


***Fig. 4: % Swelling index of diff. formulation***

 

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### *In vitro drug release study*

All formulations prepared were subjected to *in vitro* release study to ensure the effect of polymer concentration. The data obtained for *in- vitro* study were tabulated and represented graphically. Table shows percentage cumulative drug release profile (average of 3) for all formulations F1 to F9. The Maximum drug release was found at medium polymer concentration and as the polymer amount was increased from 1% to 3% w/v, the release was found to be decreased. Ocular insert A5 shows maximum drug release % in 9 hr.

From *in vitro* release results, insert A5 was selected as optimized formulation and subjected to further studies.

**Table 5: I*n vitro* cumulative % drug release**

| Time (hrs) | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 |
|---|---|---|---|---|---|---|---|---|---|
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1** | 14.276±0.475 | 15.048±0.378 | 17.055±0.288 | 25.389±0.663 | 11.19±1.139 | 12.887±0.787 | 13.659±0.756 | 17.132±0.756 | 26.238±0.475 |
| **2** | 28.754±0.232 | 25.230±0.525 | 37.844±0.248 | 46.284±0.751 | 30.736±0.628 | 28.570±0.668 | 22.807±0.863 | 32.607±0.779 | 46.474±0.640 |
| **3** | 44.396±0.594 | 38.588±0.351 | 54.507±0.403 | 64.203±0.562 | 47.415±0.765 | 45.785±0.651 | 37.442±0.865 | 51.649±0.753 | 60.277±0.840 |
| **4** | 64.312±0.167 | 47.552±0.415 | 76.615±0.626 | 78.547±0.638 | 64.131±0.844 | 70.670±1.076 | 46.590±0.861 | 66.681±0.940 | 73.933±0.856 |
| **5** | 72.424±0.214 | 62.726±0.154 | 84.629±0.549 | 86.275±0.642 | 73.201±0.764 | 89.143±0.398 | 58.601±0.856 | 76.401±0.964 | 82.644±0.764 |
| **6** | 86.303±0.240 | 78.738±0.403 | 91.666±0.537 | 91.022±0.571 | 82.712±0.940 | 92.353±0.636 | 76.429±0.764 | 87.578±0.869 | 86.135±0.752 |
| **7** | 90.209±0.164 | 93.812±0.831 | 96.443±0.452 | 94.709±0.540 | 91.182±0.712 | 96.134±0.665 | 86.939±0.850 | 92.395±0.856 | 90.950±0.466 |
| **8** | 95.204±0.388 | | | 96.828±1.115 | 94.936±0.368 | | 92.755±0.782 | 94.709±0.779 | 94.246±0.762 |
| **9** | | | | | 97.852±0.988 | | 96.237±0.848 | | |

### *Sterility testing*

The sterility testing of ocular inserts A5 was performed for aerobic bacteria and fungi by using Fluid thioglycolate medium and soyabean casein digest medium.

### *Test for aerobic bacteria*

*Bacillus subtilis* was used as a test organism. As shown in Table 6.17, there was no evidence of growth found in the ‘test’ and ‘negative control’ tubes and there was visual inspection of microbial growth in ‘positive control’ tube. The results suggest that the ocular inserts tested for aerobic bacteria were passed the test for sterility.

 

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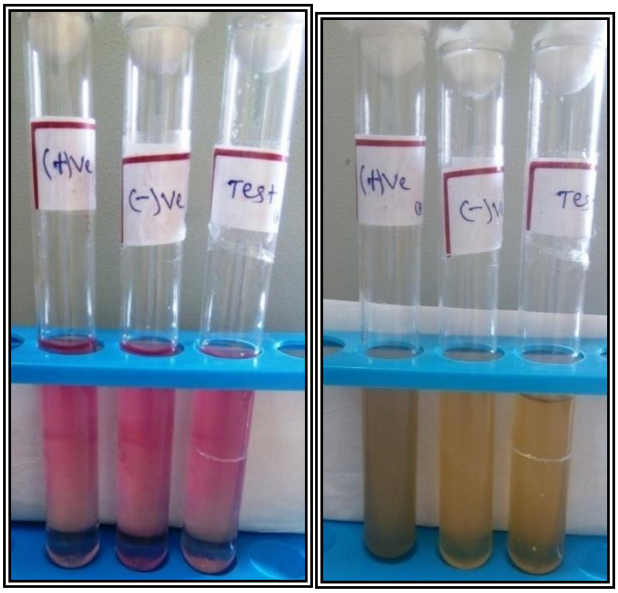


***Fig. 5: Sterility test observations***

***Table 6: Sterility test observations in FTGM***

| S. No. | Sample | Day | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 7 |
| 1. | Positive control | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2. | Negative control | _ | _ | _ | _ | _ |
| 3. | Test | _ | _ | _ | _ | _ |

### *Test for fungi*

*Candida albicans* was used as test organisms. As shown in Table , there was no evidence of growth found in the 'test' and 'negative control' tubes and there was visual inspection of microbial growth in 'positive control' test tube. The results suggest that the ocular inserts tested for fungi were passed the test for sterility.

 

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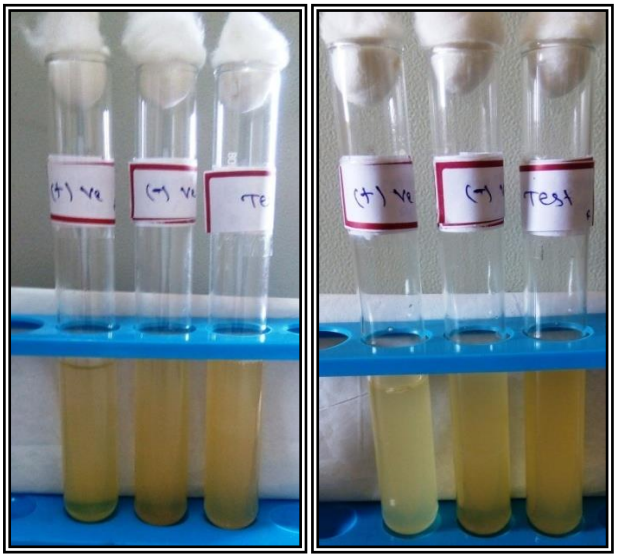

***Fig. 6: Sterility test observations***

***Table 7: Sterility test observations in SBCD medium***

| S. No. | Sample | Day | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 7 |
| 1. | Positive control | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2. | Negative control | – | – | – | – | – |
| 3. | Test | – | – | – | – | – |

## Conclusion

In this investigation development of an ideal and bio-compatible polymer free from toxic and allergic manifestation. In the present investigation solvent evaporation technique is adopted and it was found to be giving thin uniform films. All prepared ocular films have good appearance with smooth surface. Measurement of films was carried out and low standard deviation values in film weight measurements ensure the uniformity of weight in each film. A good weight uniformity of all formulation indicates an equal distribution of polymers in the inserts film prepared by solvent evaporation technique. Thickness values of inserts were found in range of 0.073±0.0047 to 0.16±0.0163 mm. Folding endurance of inserts was found in range of 140±7.4833 to 186.333±4.988.

The surface pH of the prepared inserts were found in between 6.3 to 7.1 indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range. Surface pH data of ocular inserts is shown in Table 3. Good uniformity in the drug content among the batches was observed for all the formulations. Drug content was found in the range of 96.080±0.3137 to 99.467±0.2464 %. Moisture absorption values of inserts were found in range of 4.259±0.6930 to 6.22±0.3113. Formulation A3 shows highest moisture absorption. % Moisture loss values of inserts were found in range of 2.920±1.0898 to 6.92±0.6159. When Increase in the

 

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HPMC and Natural polymer concentration increase the swelling index properties. % swelling index data of ocular inserts is shown in Table 4. All formulations prepared were subjected to *in vitro* release study to ensure the effect of polymer concentration. The sterility testing of ocular inserts A5 was performed for aerobic bacteria and fungi by using Fluid thioglycolate medium and soyabean casein digest medium.

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