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Research Article

Design and Evaluation of Macrolide Antibiotic Ocular Films

Nelluri.K.Durga Devi*[1], P BharghavaBhushan Rao[2], K.Haritha1, K.Mrudula[1], M.Manoj Kumar[1], M.Jigeesha[1]
[1]KVSR Siddhartha College of Pharmaceutical Sciences, Andhra Pradesh, Vijayawada, India
[2]V V Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India

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Abstract

Azithromycin is a semi synthetic macrolide antibiotic mainly obtained from erythromycin. Oral administration of AZT is effective to treat trachoma, topical formulation is difficult to develop because of the hydrophobicity nature. The aim of present work is to formulate in the form of ocular films to treat ocular infections. AZT ocular films are formulated by using hydrophilic polymers HPMC E15, L-HPC, PG or PEG 400, gelatin and sodium alginate in different concentrations eight formulations (F1-F8) are formulated. The prepared formulations are evaluated for its thickness, folding endurance, surface texture, surface pH, moisture absorption, moisture loss, swelling index, content uniformity, in vitro and ex vivo drug release studies. Among eight formulations (F1-F8) F1 is the best formulation, which releases 71.57 % in 6hrs and it can be used to prolong the release.

Keywords: Macrolide Antibiotic, Hydrophilic, ocular films, In-Vitro and Ex-Vivo

INTRODUCTION

Ocular inserts (OC) are sterile preparations, in which a slender, profound, drug-impregnated, solid or semisolid constancy devices positioned into cul-de-sac or conjunctiva sac ready up of polymeric vehicle containing drug (Heller, 1980). The anatomy, physiology and biochemistry of the eye render it exquisitely impervious to foreign materials (Sahane *et al.*, 2010). One of the major barriers of ocular medication is to obtain and maintain a therapeutic level at the site of action for prolonged period of time. Ocular drug delivery is one of the most fascinating and challenging tasks facing the Pharmaceutical researchers (Di Colo *et al.*, 2001).The therapeutic efficacy of an ocular drug can be greatly improved by prolonging its contact with the corneal surface (Gurtler and Gurny, 1995). Polymeric film ocular drug delivery systems/ocular inserts, which are gaining worldwide accolade, release drugs at a pre-programmed rate for a longer period by increasing the pre-corneal residence time.

Ophthalmic inserts are sterile preparations with a solid or a semisolid consistency, and whose size and shape are especially designed for ophthalmic application. The inserts are placed in the lower fornix and less frequently, in the upper fornix or on the cornea. Ocular inserts release drug by controlled, sustained and continuous rate.

The main objective of the ophthalmic inserts is to increase the contact time between the preparation and the conjunctival tissue to ensure a sustained release suited to topical or systemic treatment composing of polymeric support with or without drugs, the latter being incorporated as dispersion or a solution in the polymeric support. AZT is a bacteriostatic drug acts by inhibiting protein synthesis. It binds reversibly to 50S ribosomal subunits of sensitive microorganism. AZT interferes with transpeptidation and translocation thus there is inhibition of protein synthesis and thus inhibition of cell growth.

Materials and Methods

Materials

Pure drug gift sample of Azithromycinwas from *Pvs Laboratories Ltd*., Vijayawada. All other ingredients HPMC E15, PEG 400, Gelatin, L-HPC, Starch,Sodium Alginate used were of pharmaceutical grade.

Manufacturing Procedures

***Corresponding Author:** Nelluri.K.Durga Devi
Address: College of Pharmaceutical Sciences, Vijayawada-520010, Andhra Pradesh, India
Email address: nelluriss@gmail.com

Methods

Preparation of Ocuserts (Sasaki *et al.*, 2003, Macha *et al.*, 2003)**:**

1. Drug is dissolved in the ethanol by stirring.
2. The other ingredients are dissolved in the water by means of magnetic stirrer until clear solution is obtained
3. The contents of step 1 and step 2 were mixed and poured it in to the ring which is placed on the glass plate and dried completely in room temperature or in oven.
4. If necessary magnetic stirrer is used to produce a homogenous mix and it should be free from lumps and air bubbles.
5. The formulae given in **Table 1.**

If air bubbles are seen it is discarded because no uniform distribution takes place

Table 1: Formulation of Azithromycin Ocuserts

Contents	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8
HPMC E15	10	-	50	10	40	40	-	-
LHPC	-	70	-	-	-	-	50	30
PEG 400	-	10	-	50	-	50	-	-
Gelatin	-	-	-	-	60	10	-	10
Starch	-	20		40	-	-	50	60
Sodium Alginate	90	-	50	-	-	-	-	-
Glyerine	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Drug	10	10	10	10	10	10	10	10
Water	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.
Ethanol	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.	Quan.suff.
Total (mg)	112.9	112.9	112.9	112.9	112.9	112.9	112.9	112.9

Evaluation of Ocuserts

Construction of Standard Graph of Azithromycin

Azithromycin powder corresponding to 25 mg of the drug was accurately weighed and it was dissolved in 25ml of ethanol in a 25 ml volumetric flask. From this 1ml of the solution is taken in 100ml of volumetric flask and the volume was made up to the mark with phosphate buffer of pH 7.4. Then the solution of Azithromycin was subsequently diluted with the same buffer and prepared 2, 4, 6, 8 and 10µg/ml concentration and measured the absorbance by spectrophotometer at 210 nm using phosphate buffer of pH 7.4 as blank.

Thickness Measurement

Films of each formulation were taken and the thickness of the film was measured using thickness tester at different places.

The average film thickness are computed.

Folding Endurance (Devi *et al.*, 2010)

Folding endurance was determined by repeated folding of the film at the same place till the strip breaks. The number of times the film is folded without breaking was computed as the folding endurance value.

Surface Texture

The surface texture of the films was evaluated by pressing the film with finger.

Surface pH (Devi *et al.*, 2010)

The surface pH of fast dissolving film was determined in order to investigate the possibility of any side effect in vivo. As an acidic or alkaline pH may cause irritation of the oral mucosa. It was determined to keep the surface pH as close to neutral as

possible. A combined pH electrode was used for this purpose. In order to keep the film wet it was treated with water. The pH was measured by bringing the electrode in contact with the surface of the oral film. The above procedure was repeated thrice and average with standard deviation was noted.

Moisture absorption (Macha *et al.*, 2003)

Ocular inserts were exactly weighed and positioned in a desiccator previously filled with saturated solution of aluminium chloride which generates relative humidity of 79.5%. The ocular inserts were removed from the desiccator after three days and reweighed

%Moisture absorption = Final weight-Initial weight /Initial weight *100

Moisture loss (Macha *et al.*, 2003)

Ocular inserts were measured and placed in a desiccator containing 22g of calcium chloride. The ocular films were reweighed after three days and the percentage moisture loss was determined by the formula

% Moisture Absorbed = Mi.–Mf/ Mi × 100

Swelling Index

The existence of water, polymer concentration and ionic strength help in increasing the swelling of the polymer. To study the phenomena of swelling of prepared ocular inserts (n = 3), initial weight of insert was taken, and then placed in agar gel plate (2% w/v agar in STF pH 7.4) and incubated at 37ºC ± 1ºC 5 h. For insert was removed with help of filter paper, and insert was reweighted. Percent hydration was calculated.

Hydration % or (S_w) % = [$w_t - w_o/w_o$] X100

(S_w) % = equilibrium percent swelling, w_t = weight of swollen insert after time t,w_o= original weight of insert at zero time

Drug Content Determination

Drug content uniformity was resolved byt aking film area of 1.5cm^2 from each the formulation and it was placed in 100 ml of volumetric flask consist of some amou nt of phosphate buffer of pH 7.4 and then the volume is made up to the mark using same buffer. It was kept aside for 6 hours. The content of (drug) AZT was calculated using standard graph.

In-vitro Dissolution Studies

The dissolution study was carried out using modified diffusion cell using semi-permeable membrane of transparent and regenerated cellulose type, which is positioned, between the donor and receptor compartments. Semi permeable membrane was used to mimic in vivo conditions, such as corneal epithelial barrier.The insert was placed in the donor compartment, and 7 μl of STF with pH 7.4 was maintained at the same level throughout the study in the donor compartment to simulate tear volume. The entire surface of the membrane was in contact with the reservoir compartment that contained 25 ml of STF with pH 7.4, which was stirred continuously using a magnetic stirrer at 20 rpm to simulate blinking action.

The release data were analyzed kinetically using kinetic models (zero and first order).

Ex-vivo studies

Goat corneas are used for the studies. The cornea is carefully removed along with a 5-6 mn of surrounding sclera tissue and washed with distilled water. The washed corneas are kept in cold freshly prepared solution of tear buffer og pH 7.4.

The study is carried out by using modified diffusion cell in such a way that cornium side is continuously remained in an intimate contact with formulation in the donor compartment.

The receptor compartment is filled with pH 7.4 at 34ºC ± 0.5ºC.

The receptor medium is stirred on a magnetic stirred. The samples are withdrawn at different time intervals and analyzed for drug content.

Receptor phase is replenished with an eq ual volume of pH at each time interval

Results and Discussion

Pre formulation Studies

Organoleptic Properties: Color: White, Odour: Odourless, Taste: Slightly bitter, Appearance: Amorphous powder. Melting point values of sample was found to be in range of 114^0C. The official melting point range for Azithromycin is between 113-115°C. Hence, results were complied the limits specified in IP.

FT-IR Spectrophotometry

The pure drug and its physical mixture were subjected to IR studies and evaluated for interaction between the drug and the utilized polymer. The IR spectra of pure drug were shown in **Figure 1**. The overlay of pure drug, drug+ HPMC, drug + Methacel, drug + LHPC, drug + Sodium alginate +drug+ gelatin, and drug + PEG are shown in **Figure 2**. The results indicated there was no possible interaction between drug and polymers used. Hence, there was no positive evidence for the interaction between drug and the utilized material. The results showed that the usefulness of the utilized material (HPMC E15, LHPC, PEG 400, Gelatin, Starch and Sodium alginate) for preparation of various ocular films contained Azithromycin.

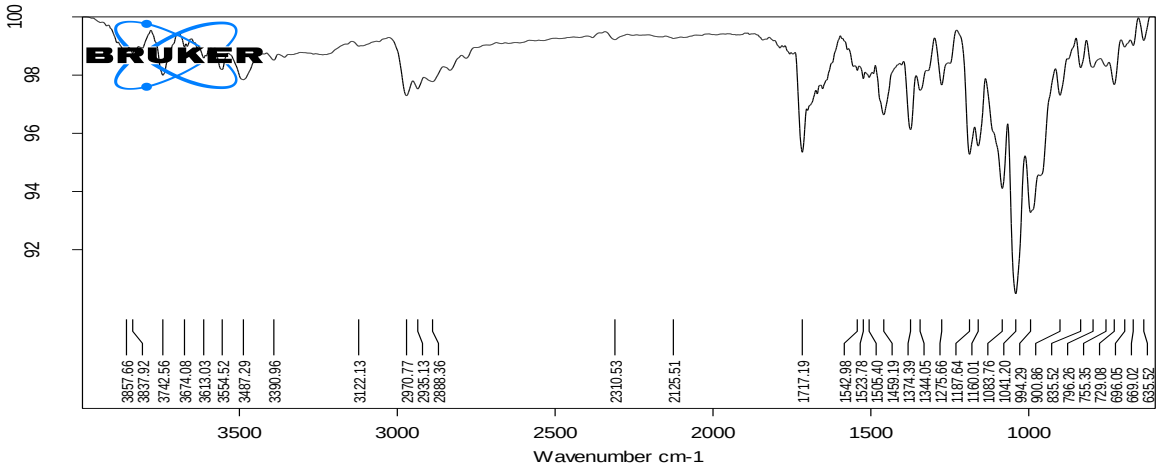


Figure 1: FT-IR Studies of Pure Drug Azithromycin

Overley Of Drug And Polymers

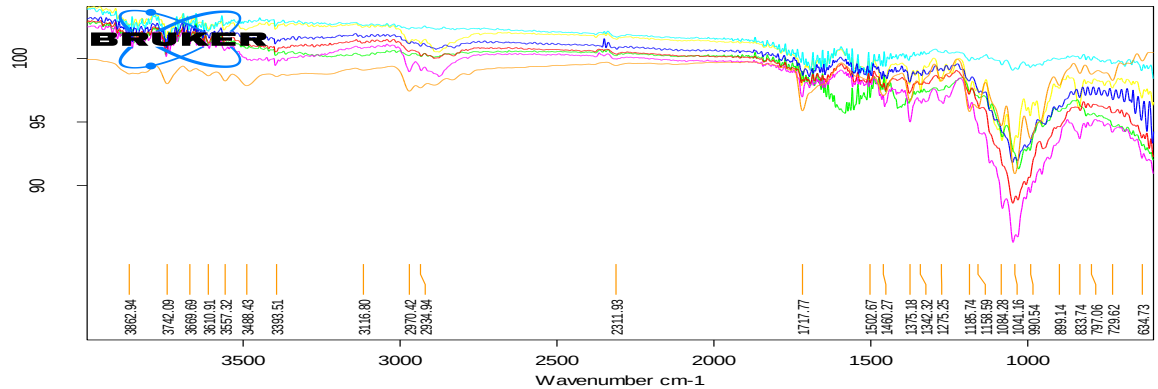


Figure 2: Overlay of Drug and Polymers used

Construction of Standard Graph of Azithromycin

Table 3: Calibration Values ofAzithromycin

Standard graph for Azithromycin:

Concentration	Absorbance
0	0
2	0.035
4	0.065
6	0.095
8	0.123
10	0.159

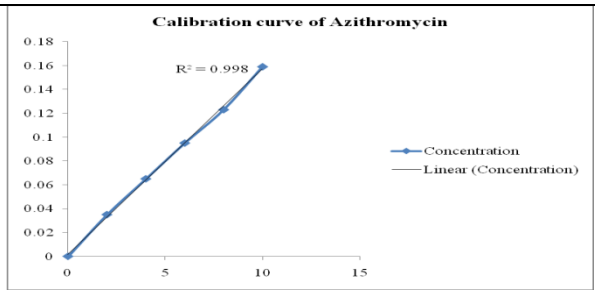


Figure 3: Standard graph for Azithromycin

Thickness Measurement

The thickness of Azithromycin films was measured and results are shown in **Table 4.** The thickness of the various films varies from 239.16 to 252.26 µm due to increase in polymer concentration.

Folding Endurance

The folding endurance of the films was measured manually and they were folded between 212 to 232 times without breaking or cracking. The ranking orders of the folding endurance of various ocular films are as follows: F6 > F7 > F5 > F2 > F1 > F4 > F8 >F3. Among various polymers, PEG 400, Gelatin and HPMC E15 contained films has shown highest folding endurance. The results are shown in **Table 4.**

Table 4: Thickness and Folding Endurance Studies of Formulated Ocuserts

Formulation	Thickness	Folding endurance
AF1	248.1±1.23	287.66±0.94
AF2	247.73±0.32	289±0.81
AF3	244.03±0.24	268±0.81
AF4	243.83±1.08	278.66±1.24
AF5	245.1±0.163	297±0.97
AF6	252.27±0.94	313±0.97
AF7	248.67+0.75	300±0.61
AF8	239.67±0.24	272±0.81

Surface Texture

The surface texture of all the films was examined and they were found to be flexible with smooth surface texture. The results are shown in **Table 5.**

Surface pH

The surface pH of all the films is determined. All the films exhibited almost uniformity in their surface pH values and the following range of pH was observed: 6.72 to 6.81, which indicated its compatibility with ocular pH. The results are shown in **Table 5.**

Moisture absorption

The films were tested for moisture absorption and the results are shown in **Table 5**. The result shows in between 5.66 to 11.8% with low SD.

Moisture loss

The films were tested for moisture loss and the results are shown in **Table 5.** The result shows in between 9.1 to 28.2% with low SD.

Table 5: Surface Texture, Surface pH, Moisture Absorption and Moisture Loss Studies of Formulated Films

Formulation	Texture	Surface pH	% Moisture Absorption	% Moisture Loss
AF1	Smooth	6.79±0.01633	11.82±0.28	9.16±0.25
AF2	Smooth	6.773333±0.016997	8.76±0.26	10.51±0.32
AF3	Smooth	6.813333±0.012472	5.61±0.38	17.82±0.25
AF4	Smooth	6.753333±0.016997	13.66±0.25	21.56±0.32
AF5	Smooth	6.72±0.008165	9.63±0.38	28.23±0.45
AF6	Smooth	6.813333±0.012472	6.17±0.23	17.87±0.21
AF7	Smooth	6.81±0.01633	9.57±1.25	27.22±0.36
AF8	Smooth	6.766667±0.012472	11.23±0.41	20.29±0.32

Swelling Index

The swelling index was determined for all the formulations. The results are between 62.46 to 84.5 %. The results are shown in **Table 6:** The order of swelling index is AF8>AF5>AF3>AF4>AF2>AF6>AF7>AF1.

Formulation	Swelling Index	Drug content
AF1	84.587±0.25	98.82%±0.001247
AF2	75.794±1.24	97.17%±0.000816
AF3	68.905±0.36	98.56%±0.001633
AF4	71.897±0.21	99.30%±0.001247
AF5	65.455±0.35	97.75%±0.000816
AF6	79.364±036	99.32%±0.001247
AF7	82.897±0.19	98.56%±0.0017
AF8	62.696±0.32	96.76%±0.000816

Drug Content Determination

The drug content was estimated for all the formulation using standard method. The drug content of all the films was found to be uniform with low SDvalues, which indicates that the drug was distributed uniformly in all the films. The result are shown in **Table 6.**

Table 6: Swelling Index and Drug Content Studies of Formulated Ocuserts *In-Vitro* Release Studies

The percentage release of drug from all formulation F1 to F8 is as follows:75.57, 92.61, 79.21, 89.88, 86.89, 81.17, 101.46 and 97.69 at the end of 360mins respectively. About 75-100% of drug release was observed from all formulation at the end of 360mins from all the formulation AF1-AF8 respectively **(Table7).**

Solubility Study

Table 2: The Solubility of Azithromycin in various Solvents

Name of solvent	Solubility
Chloroform	Highly Soluble
Ethanol	Soluble
Water	Slightly soluble

Among various formulations AF1has released minimum amount of drug and F7 has released maximum amount of drug within 360mins. The order of retardation time for different films was as follows AF1 >AF3 > AF6 > AF5 > AF4 > AF2 > AF8 > AF7. Hence among 8 formulations AF8 has shown slow release. The cumulative % drug profile was plotted and shows the drug release of 8 formulations (**Figure: 4**).

Table 7: Cumulative % drug release

TIME(min.)	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8
0	0	0	0	0	0	0	0	0
10	14.82	17.04	15.47	16.52	15.86	15.2	18.08	17.43
20	30.95	33.69	29.52	34.21	33.43	31.8	32.78	38.11
30	34.99	43.05	31.34	40.58	39.15	36.5	48.26	46.43
40	44.74	49.56	40.58	46.43	44.87	42.7	53.59	51.91
60	50.6	52.42	48.26	51.25	49.95	46.04	55.28	53.85
90	53.59	57.36	53.2	55.02	53.59	52.4	63.86	59.96
120	59.05	67.77	63.99	58.66	56.58	61.26	68.81	66.6
180	64.13	74.27	66.6	70.5	67.12	64.78	78.69	75.96
240	67.25	79.73	70.63	76.87	74.53	68.16	89.1	81.04
300	73.1	87.67	77.36	84.16	80.78	74.92	92.61	90.79
360	75.57	92.61	79.21	89.88	86.89	81.17	99.46	97.69

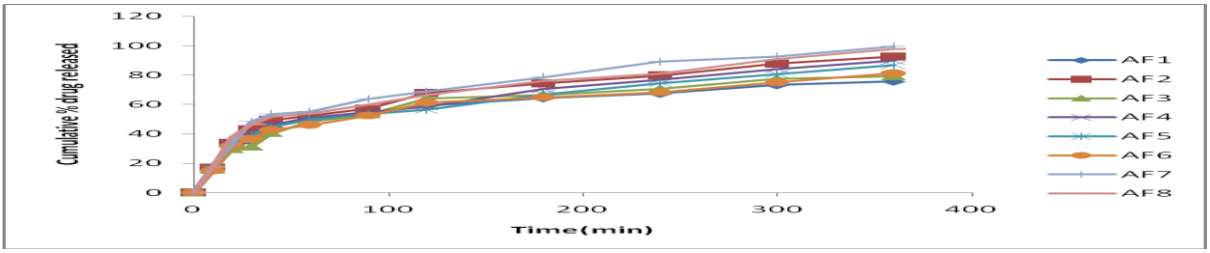


Figure 4: Cumulative % Drug Release Profile of Formulation AF1-AF8

Kinetics of Drug Release

To understand the order and mechanism of the drugs release from ocular films the data was subjected to the various kinetic equations and plotted according to zero order and first order equation. The kinetic values obtained from different plots are listed in **Table 8**. The data was subjected to first order kinetics by plotting cumulative % drug remainedvs. Time in main shown fairly linear plots were obtained for all formulation (AF1 to AF8) with regression value between 0.7173 to 0.9724 indicated that the rate of drug release was followed first order kinetics.

Table 8: Kinetics of Drug Release of Formulations

Code	Zero Order R^2	First Order R^2	Best Fit
AF1	0.7173	0.8767	First Order
AF2	0.7879	0.9316	First Order
AF3	0.7698	0.9169	First Order
AF4	0.8064	0.9669	First Order
AF5	0.8035	0.962	First Order
AF6	0.7715	0.9284	First Order
AF7	0.7917	0.9724	First Order
AF8	0.7914	0.9226	First Order

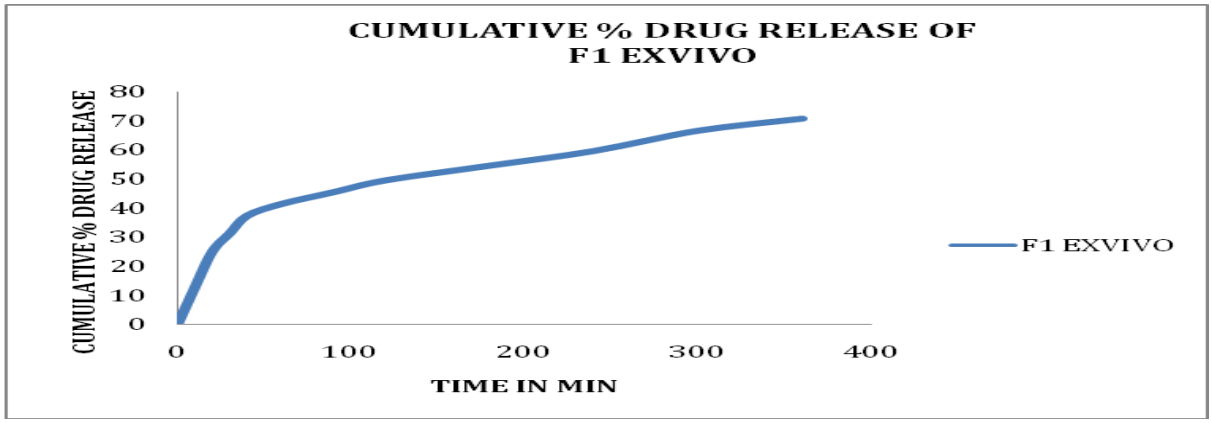
Ex-vivo Permeation Studies

The Ex-vivo permeation studies of formulation AF1 was done and which shows 70.66% drug release in 360mins. The results are shown in **Table 9**. The drug release graph is plotted by time Vs Cumulative % drug release is shown in **Figure 5.**

Table 9: *Ex-vivo* release studies of formulation AF1

TIME	F1 EXVIVO
0	0
10	12.94±0.12
20	25.14±1.34
30	31.293±1.25
40	37.347±0.31
60	41.412±1.22
90	45.454±0.78
120	49.497±0.48
180	54.543±0.25
240	59.579±0.69
300	66.632±0.49
360	70.664±0.25

Figure 5: *Ex-vivo* release studies of formulation AF1



CONCLUSION

Ocuserts of Azithromycin using polymers like HPMC E15, LPHC, PEG 400, Gelatin. Starch and Sodium Alginate in various proportions and combination showed satis factory physic-chemical and drug release characteristics.

The proportional amounts of various hydrophilic polymers in various formulations have influence a drug release from these formulated Azithromycin ocuserts.

These films have appreciable strength and safety and maintained the sustained release, decreased frequency of administration and thus may improve the patient compliance. Hence these films can be used in ophthalmic formulations. Since the formulations showed

ideal release to some extent, it can be selected for future studies. By formulating the drug into ocusert a better therapy can be achieved and the polymers used are hydrophilic, hence, the usage of the formulation is also convenient to the patients.

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