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Development and Evaluation of Topical Ophthalmic Formulation of Timolol Maleate

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ABSTRACT

The present study deals with the topical formulation of timolol maleate for the treatment of glaucoma. Glaucoma occurs due to the damage of sensitive nerve fibres of optic nerve which carries the images from retina to the brain. This damage results in loss of vision and is permanent. The research is aimed to focus on development of a novel ophthalmic formulation which provides continuous contact of drug with cornea, improved bioavailability and patient compliance due to its combined drug delivery and cosmetic characteristics. Materials and methods: Topically applied formulation of timolol maleate was prepared by using simple and non tedious process. The formulations were evaluated for their grittiness, spreadability, extrudability, drug content. Result and discussions: *In vitro* drug release studies were performed for six hours and it was observed that the formulation F1 had the maximum amount of drug release i.e. 49.61%. The complete study wrap up that this approach of timolol maleate provides an advance and continuous drug delivery to the cornea.

Key words: Glaucoma, timolol maleate, ophthalmic formulation, *in vitro* drug release.

INTRODUCTION

Vision is one of the vital sense organ among the five principal senses. Eye is an intricate structure and it must not be ignored. [1] Topical application of drugs to the eye is the most popular and well accepted route of administration for the treatment of various eye disorders. Drugs are commonly applied to the eye for a localized action, on the surface, or in the interior of the eye. Frequently occurring disorders such as cataract, glaucoma and diabetic retinopathy are chronic and requires continuous treatment. [2, 3]

Glaucoma:

Glaucoma is a condition characterized by an increase in intraocular pressure, which tends in degeneration of the optic nerve which is constituted by retinal ganglion cells and axons resulting in the irreversible loss of vision. [4, 5]

Timolol Maleate:

Timolol maleate belongs to the thiadiazole class of compounds. It is a beta-adrenoreceptor antagonist. It is non-specific of β-1 and β-2 adrenergic receptors. It is efficacious in sinking intraocular pressure. The patients with open angle glaucoma and aphakic glaucoma are given medications using Timolol. Timolol maleate is designated both for the healing hypertension and reduce cardiovascular mortality. [6, 7]

MATERIALS AND METHODS

Timolol maleate, Liquid paraffin, Activated charcoal, Bees wax, Benzalkonium chloride, Carnauba wax, Cetyl alcohol, Cholesterol, Isopropyl myristate, Lanolin, Tocopherol acetate, Yellow petroleum wax were obtained from the laboratory of Bhupal Nobles College of Pharmacy, Udaipur. All chemical solvents were of analytical grade and used without further purifications.

PREFORMULATION STUDIES

Melting point determination:

The melting point of the sample is done to check the purity of the sample. Melting point is defined as the temperature at which a solid substance transits its state from solid to liquid. [8]

Melting point of timolol maleate was found by using the digital melting point apparatus from Remi Instruments.

Partition Coefficient of the Drug:

Partition coefficient is the measure of the lipophilic and hydrophilic nature of a drug substance. It is defined as the extent to which a substance is distributed between two liquid phases, one being the aqueous phase and other being the oily phase. The majorly used phases are water and n-octanol (oil phase) in the ratio 1:1. [9]

$$\mathbf{P}_{o/w} = \frac{\mathbf{C\,(n-octanol)}}{\mathbf{C\,(water)}}$$

Determination of λ_{max} of Timolol maleate in Phosphate buffer saline (pH7.4):

Stock solution was prepared by dissolving 100mg accurately weighed Timolol maleate into a 100ml of phosphate buffer saline (pH 7.4). 100 µg/ml and 10µg/ml concentrations were prepared using the stock solution and buffer. These solutions were filled in the cuvettes and the spectrum was observed for the solutions in the range of 200-400nm using PBS as blank. The spectrum of absorbance versus wavelength was recorded on UV-spectroscopy and analyzed for absorbance maximum and the highest absorbance was noted. [7]

Preparation of Calibration curve:

From the stock solution, 5 µg/ml-50 µg/ml concentrations were prepared using buffer solution (pH 7.4) and the absorbance of each of the solution was measured at 293.6nm in UV-spectrophotometer using phosphate buffer saline as blank and graph of concentration versus absorbance was plotted. [7] The slope, straight line equation and correlation coefficient were obtained from the calibration curve intercept.

PREPARATION OF TOPICAL OPHTHALMIC FORMULATION OF TIMOLOL MALEATE:

Except tocopherol, IPM and benzalkonium chloride all the other ingredients were melted according to their melting point. The drug was dissolved in water (1 part in 15 parts of water). The drug solution is dispersed into the molten waxes by stirring. Charcoal was added and stirred continuously until it distributes uniformly. Then tocopherol, IPM and benzalkonium chloride were added and stirred. The formulation was transferred to well close plastic containers and stored under refrigerator conditions.

Table 1: Composition of Different Timolol Maleate Loaded Ophthalmic Formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Drug	1	1	0.5	0.5	1	1	0.5	0.5
Liquid paraffin	31	31	31	31	29	29	29	29
Carnauba wax	-	-	-	-	5	5	5	5
Bees wax	23	23	23	23	23	21	21	21
Petroleum wax	23	23	23	23	21	21	21	21
Lanolin	10	10	10	10	10	9	9	9
Cetyl alcohol	8	8	8	8	8	8	8	8
IPM	2.5	3	3	3.5	2.5	3	3	3.5
Cholesterol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Tocopherol acetate	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Benzylkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Charcoal	0.5	-	0.5	-	0.5	-	0.5	-

EVALUATION:

Freedom from grittiness:

Around 0.5g of formulation was taken and was spread on the polyethylene sheet. Then the formulation was tested for any abrasive or hard particles by pressing along its length.

Spreadability: [10, 11]

The parallel plate method was used to evaluate spreading behavior using an in-house spreadability testing apparatus that consisted of two smooth glass slides to hold the gel samples tied to a pre weighed volumetric flask. Excess of the gel sample was spread on the surface of the lower slide, then the upper slide was used to cover the sample and a 100 g weight was used to compress the sample between the two slides for 5min to obtain a layer of uniform thickness.

The time (in seconds) required for the upper slide to move away to the edge of the lower slide and the weight required was recorded and spreadability was calculated using to the following equation-

$$S = \frac{M}{T} x L$$

Where, S is the spreadability (gm.cm/sec), M is the mass placed on the pan, L is the length of the slide (cm), and T is the time (in seconds) required to move the upper slide.

Extrudability: [10]

The extrudability is defined as the amount of formulation that can be ejected from a collapsible tube. The test was performed using a clean aluminum collapsible tube (20 g capacity) with a tip opening diameter of 1 cm. The extrudability was evaluated in by measuring the weight of gel sample ejected from the tube opening upon pressing with fingers, while holding the tube in hands.

Uniformity drug content:

Each formulation of 0.5gm was accurately weighed and transferred to beaker containing 50 ml of ethanol. Then the solution was filtered using Whattman filter paper and the filtrate was diluted 10 times with ethanol. The absorbance values were determined using UV- Spectrophometer at 298nm.

***In Vitro* Drug Permeation studies:**

In vitro permeation studies were performed using a cellophane membrane of molecular weight of 12000D (Glycerin treated). The cellophane membrane was clamped to hollow open end glass tube and was dipped inside the media (100ml) taken in a beaker (receptor). Exactly 1gm of formulation was weighed and placed on the membrane in donor compartment (hollow glass tube). The PBS solution in receptor compartment is stirred continuously using magnetic stirrer. Both the compartments should be in contact with each other and maintained at 37±2˚C. At pre-determined intervals, samples of 5ml were collected and replaced with fresh buffer solutions to maintain the sink conditions. The samples were analyzed and the concentrations of drug in the samples were determined using UV-Spectrophotometer at 293.60nm for PBS.

RESULTS AND DISCUSSION

Identification of pure drug

Melting point determination:

Melting point of timolol maleate was determined to be 202.2°C. It was observed that timolol maleate melting point falls in line with standard therefore, it confirms that the drug is free of any impurities.

Partition coefficient:

The Log P of timolol maleate was found to be around 1.5 indicating that the drug is lipophilic in nature.

Determination of λ_{max}

The absorbance spectrum of pure timolol maleate was scanned using a double beam UV spectrophotometer (Shimadzu 1900) in the range 400nm-200nm with concentration of 100µg/ml in PBS (pH 7.4). The maximum absorption was obtained at 293.6 nm.

Standard Calibration Curve of Timolol Maleate

The calibration curve of timolol maleate was obtained by using the 5-50 µg/ml concentrations using buffer solution (pH 7.4) and the absorbance of each of the solution was measured at 293.6 nm. The calibration curve which was plotted was linear in the range of 5-50 µg/ml ml at λ_{max} 293.6 nm. The correlation coefficient was found to be 0.998. The calibration curve is depicted in Figure 1 and Table 2.

Table 2: Timolol Maleate Calibration Curve (λ_{max}=293.6 nm)

Concentration (µg/ml)	Mean Absorbance ± S.D.
5	0.113±0.002
10	0.218±0.009
15	0.32±0.001
20	0.421±0.006
25	0.528±0.007
30	0.649±0.031
35	0.761±0.0218
40	0.856±0.0224
45	0.999±0.034

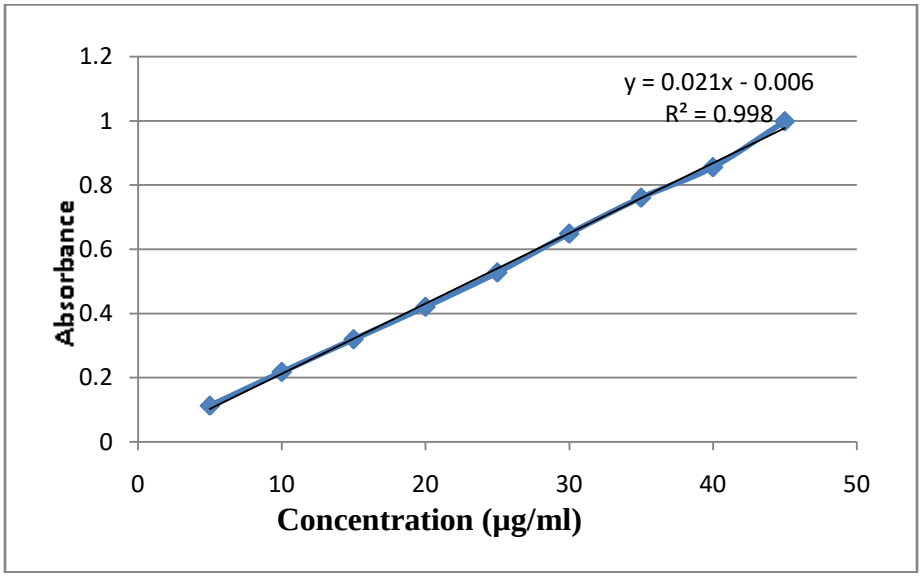

Figure 1: Calibration Curve of Timolol Maleate in PBS (7.4)

Evaluation of Formulation:

As the formulations are intended to be applied on to most sensitive sense organ, they should be free from hard and abrasive foreign. So the test for grit particles confirmed that the formulations were free from rigid particles which prevent various irritation reactions.

Among all the formulations, F1, F5, F6, F7 and F8 exhibited more spreadability than that of the formulations F2, F3 and F4. This indicates that the formulations have good spreading ability at the site of application to deliver the standard dose.

Table 3: Evaluation Parameters of Formulations

Formulation	Freedom from grits	Spreadability (gm.cm/sec)	Extrudability (gm)	Uniformity content (%)
F1	Passed	21.11±1.154	3.33±0.577	99.47±0.0458

F2	Passed	18.66±0.456	4.33±0.577	96.84±0.5597
F3	Passed	18.71±1.19	3.6±0.577	97.89±0.01
F4	Passed	18.95±0.825	4.3±0.577	98.94±0.6359
F5	Passed	22.96±2.7306	5.4 ± 0.11	91.05±0.1058
F6	Passed	24.39±1.2104	9.0 ± 0.15	87.89±0.3464
F7	Passed	22.15±0.6639	6.8 ± 0.21	85.26±0.0458
F8	Passed	20.45±1.1431	4.4 ± 0.3	89.47±0.1458

***In vitro* drug release:**

Release of timolol maleate from different formulations was estimated by using UV spectrophotometer at 293.6 nm using PBS at pH 7.4. The drug release at different time intervals was recorded. The study revealed that the drug release from timolol maleate was maximum in formulation F1.

Table 4: % Drug release in PBS (pH 7.4)

Formulation	**% Drug release in PBS**
F1	49.61±0.105
F2	40.05±0.425
F3	36.91±0.144
F4	36.19±0.454
F5	35.11±0.087
F6	34.07±0.42
F7	34.63±0.045
F8	30.95±0.269

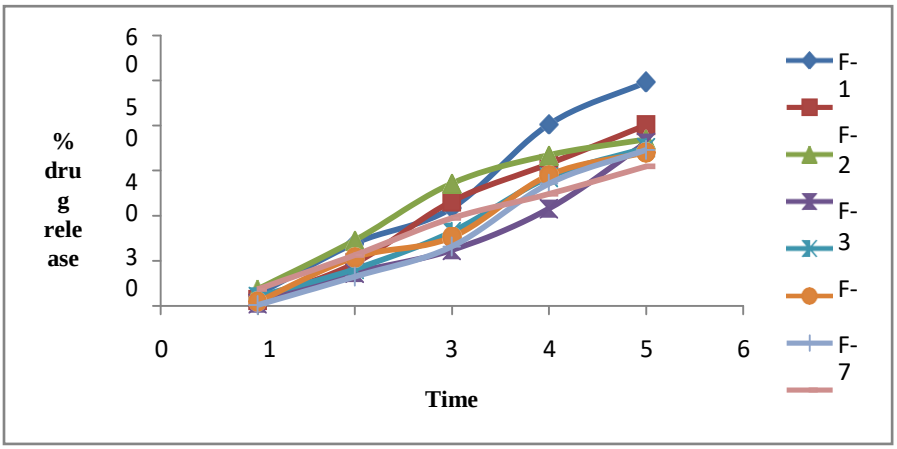

Figure 2: % Drug Release *vs.* Time Plot for F1-F8 in PBS

SUMMARY AND CONCLUSION:

Glaucoma is an intraocular disease which is the vital reason of blindness worldwide specifically in elder people. It is caused due to the damage of sensitive nerve fibres of optic nerve which carries the images from retina to the brain. The damage of optic nerve and loss of vision is permanent. The major problem associated with ophthalmic formulations is precorneal elimination of the drugs by drainage and lacrimal washing leading to their incomplete absorption and bioavailability. Hence, there is need of novel formulation and strategies which can overcome the problems.

The present research focuses on development of a novel ophthalmic formulation which provides continuous contact of drug with cornea, improved bioavailability and patient compliance due to its combined drug delivery and cosmetic characteristics.

In pre-formulation studies, different suitable components were selected. Formulations were developed using a simple process and they were evaluated for the parameters such as grittiness, spreadability, extrudability and in-vitro permeation study. From the first trial of eight formulations, formulation F1 was selected based on percent drug release and physical characteristics.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVATIONS

IOP: Intra Ocular Pressure; IPM: Isopropyl myristate; PBS: Phosphate Buffer Saline

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